INTERACTIVE EFFECTS OF ELEVATED CARBON DIOXIDE AND TEMPERATURE ON HELICOVERPA ARMIGERA HUB. AND SPODOPTERA LITURA FAB. ON BT COTTON

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M. Sc. (Ag.)

DOCTOR OF PHILOSOPHY IN AGRICULTURE
(ENTOMOLOGY)

ANGRAU

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BY

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M. Sc. (Ag.)

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2022

DECLARATION

I, **Mr. D. V. Sravan Kumar**, hereby declare that the thesis entitled **"INTERACTIVE EFFECTS OF ELEVATED CARBON DIOXIDE AND TEMPERATURE ON HELICOVERPA ARMIGERA HUB. AND SPODOPTERA LITURA FAB. ON BT COTTON"**, submitted to the Acharya N. G. Ranga Agricultural University for the degree of Doctor of Philosophy in Agriculture in the major field of **Entomology** is the result of original research work done by me. Part of the thesis has been published by me as "Sravan Kumar, D.V., Krishnayya, P. V., Srinivasa Rao, M., Anil Kumar, P and Srinivasa Rao, V**.** 2021. Impact of Elevated CO_2 and Temperature on Growth and Development of *Helicoverpa armigera* Hubner. *Journal of Experimental Agriculture International*. 43(3): 42-54".

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Mr. D. V. Sravan Kumar has satisfactorily prosecuted the course of research and that the thesis entitled **"INTERACTIVE EFFECTS OF ELEVATED CARBON DIOXIDE AND TEMPERATURE ON HELICOVERPA ARMIGERA HUB. AND SPODOPTERA LITURA FAB. ON BT COTTON"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor has its part thereof not been previously submitted by him for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled **"INTERACTIVE EFFECTS OF ELEVATED CARBON DIOXIDE AND TEMPERATURE ON HELICOVERPA ARMIGERA HUB. AND SPODOPTERA LITURA FAB. ON BT COTTON"** submitted in partial fulfilment of the requirements for the degree of 'Doctor of Philosophy in Agriculture' in the major field of **Entomology** of the Acharya N. G. Ranga Agricultural University is a record of the bonafide original research work carried out by **Mr. D. V. Sravan Kumar** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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Place *:* Bapatla **(*D.V. Sravan Kumar*)**
Date *:*

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LIST OF SYMBOLS AND ABBREVIATIONS

@	:	At the rate of
%	:	Per cent
ºC	:	Degree Celsius
/	:	Per
&	:	And
>	:	Greater than
<	:	Less than
µg	:	Microgram
aCO_2	:	Ambient Carbon dioxide
AD	:	Approximate Digestibility
ANOVA	:	Analysis of Variance
CD (P = 0.05 %)	:	Critical difference at 5 per cent level
*a*Temp	:	Ambient Temperature
cm	:	Centimeter
CO_2	:	Carbon dioxide
RCR	:	Relative Consumption Rate
CRIDA	:	Central Research Institute for Dryland Agriculture
CRD	:	Completely Randomized Design
CTGC	:	Carbon dioxide and Temperature Gradient Chamber
DAS	:	Days after Sowing
et al	:	And others
etc.	:	*et cetera*
eCO_2	:	Elevated Carbon dioxide
ECD	:	Efficiency of Conversion of Digested Food
ECI	:	Efficiency of Conversion of Ingested Food
*e*Temp	:	Elevated Temperature

Ltd	:	Limited
Fig	:	Figure
g	:	Gram
IPCC	:	Intergovermental Panel on Climate Change
Kg	:	Kilogram
kg ha^{-1}	:	Kilogram per hectare
M t	:	Million tone
M ha	:	Million hectare
mg	:	Milligram
mg g^{-1}	:	Milligram per gram
ml	:	Millilitre
No.	:	Number
NS	:	Non significant
ppm	:	Parts per Million
RCR		Relative Consumption Rate
RGR	:	Relative Growth Rate
SEm	:	Standard Error of Mean
viz	:	Namely

ABSTRACT

Name of the Author	:	**D.V. SRAVAN KUMAR**
Title of the Thesis	:	**"Interactive Effects of Elevated Carbon Dioxide and Temperature on *Helicoverpa armigera* Hub. and *Spodoptera litura* Fab. on Bt Cotton"**
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The present investigations titled **'Interactive effects of elevated Carbon dioxide and Temperature on *Helicoverpa armigera* Hub. and *Spodoptera litura* Fab. on Bt cotton'** of Department of entomology, Agricultural college, Bapatla, Acharya N. G. Ranga Agricultural university (ANGRAU) were conducted during 2018-2020 at ICAR - Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad, Telangana. The two test insects, *H. armigera* and *S. litura* are major polyphagous cotton pests, causing heavy yield losses. In the context of global warming, largely driven by elevated carbon dioxide (eCO$_2$) and elevated temperatures (eTemp), understanding the future status of these pests is an important task. Hence, growth, development and feeding indices of *H. armigera* and *S. litura* for three successive generations on cotton and also the biochemical constituents of cotton foliage and the larvae were elaborately studied under ambient carbon dioxide (aCO$_2$: 380 ± 25 ppm) and elevated carbon dioxide (eCO$_2$: 550 ± 25 ppm), at five temperatures (28, 29, 31, 33 and 35 ± 1°C).

Growth parameters in test insects, *H. armigera* and *S. litura*, *viz.*, food ingestion, larval weight and larval excretion increased under eCO$_2$ (550 ppm) + eTemp (35 °C) whereas, pupal weight and fecundity decreased. Food ingestion capacity of the larvae increased significantly in every next generation, thus indicating greater adaptation of the test insects to climate change with advancement of generations. Notably, larval and pupal weights in the three successive generations were relatively higher for *S. litura*, probably due to good food ingestion. The durations of larvae, pre-pupae and pupae increased significantly under eCO$_2$ + eTemp. The major reproductive parameter fecundity decreased under eCO$_2$ + eTemp. In Bt cotton, mean fecundity of *H. armigera* under eCO$_2$ was reduced by 7.29, 9.03, 9.58, 9.93 and 6.39 % in fiber comparison to 1.90, 3.54, 5.18, 1.62 and 1.53 % in *S. litura*.

Among feeding indices of both the test insects, with increase in CO_2 and temperature, Approximate Digestibility (AD) and Relative Consumption Rate (RCR) increased whereas Efficiency of Conversion of Ingested food (ECI), Efficiency of Conversion of Digested food (ECD) and Relative Growth Rate (RGR) decreased. In Bt cotton, under *e*CO_2, the mean decrease of RGR of *H. armigera* was higher by 14.79, 9.80, 10.36, 7.11 and 7.81 % compared to 11.50, 13.08, 14.39, 13.65 and 13.75 % in *S. litura* compared to that of *a*CO_2. At elevated conditions, *H. armigera* has higher ECI in Bt cotton and high RGR in both non-Bt and Bt cotton, compared to *S. litura.* It implies that *H. armigera* could be a potential key pest in future climate stress scenarios even in Bt cotton.

The biochemical constituents of non-Bt and Bt cotton were down regulated by *e*CO_2 + *e*Temp i.e., nitrogen, proteins, Cry1Ac and Cry2Ab toxin contents were reduced, there by the future cotton production may challenged by these polyphagous insect pests under stressed environmental conditions. Commensurate with the decline in leaf Bt endotoxins, the larval mortality of both the test insects also reduced. Among the biochemical components in test insects, carbohydrate content in *H. armigera* increased at elevated conditions, whereas the protein content in *H. armigera* and *S. litura* decreased.

Succinctly, anticipated *e*CO_2 necessitates the test insects to attain more nitrogen from the under nutritious foliage by huge consumption as evident from the enchanced foliage ingestion and AD and RCR. Apart from the other growth and feeding indices, higher foliage consumption itself is a major threat of future crop production. Further, increased larval and pupal durations may play a key role in gaining biomass and sufficient energy for transforming into adults with improved fitness. Besides, they could adapt to changing climate as evident from the higher values of all the parameters in the third generation under *e*CO_2 +*e*Temp. Another intriguing issue is the decrease in Bt toxin production in Bt cotton leaves under *e*CO_2 +*e*Temp, thus cotton crop can become more vulnerable for these pests, especially for *H. armigera*.

Chapter – I

Introduction

Chapter I

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the most important fiber crop grown throughout the world and is one of the key economic commodities in India's economy. Cotton is used as a raw material in textile as well as in oil industries. Several compounds from cotton have pharmacological value *viz*., anti-microbial, anti-inflammatory, cancer therapy and contraceptive roles in both humans and animals. India occupies a prominent position in cotton production in terms of area (12.61 m ha), production (28.04 million bales) and productivity (378 kg/ ha) partly due to the introduction of Bt cotton (Ministry of Agriculture & Farmers Welfare, GOI, 2018-19).

Cotton cultivation is affected by various biotic stresses imposed by major insect pests such as American bollworm, *Helicoverpa armigera* (Hubner), tobacco caterpillar, *Spodoptera litura* (Fabricius), pink bollworm, *Pectinophora gossypiella* (Saunders), spiny bollworm, *Earias vittella* (Fabricius), whitefly, *Bemisia tabaci* (Gennadius), leaf hoppers, *Amrasca biguttula biguttula* (Ishida) etc. These pests have a potential to reduce yields by 20-80 %. By the mid 1990s, Indian farmers used to spend more than 43 % of the variable costs of cotton production on insecticides, around 80 % of that against american bollworm. *H. armigera* and *S. litura* are the major polyphagous pests attacking more than 150 different host species and considered as the most economically important insect pests in many countries including India, Japan, China and Southeast Asia (Arasu *et al*., 2013).

Transgenic cotton is a potential biotechnological tool for the management of polyphagous and major insect pests *viz*., *H. armigera* and *S. litura*. The first and foremost genetically modified *Bacillus thuringiensis* (Bt) cotton 'Bollgard I' comprising of cry1Ac gene acts selectively against *H. armigera*, was not effective against *S. litura* (Arshad and Suhail, 2011). 'Bollgard II' containing both cry1Ac and cry2Ab genes in single cotton hybrid resulted in effective management of *S. litura* too. In Bt cotton, particularly the younger leaves have the highest insecticidal efficacy, and squares had higher insect resistance than flowers and bolls (Stone, 2011 and Supriya *et al.* 2018).The expression of the Bt protein in transgenic cotton is regulated by the growth and development of the plant (Torres *et al*., 2006), which is dependent on

weather and hence climate change too. The Bt toxin expression was found to be affected by unfavourable temperatures and carbon dioxide (CO_2) concentrations (Chen *et al*. 2005a and Wu *et al*. 2007) that in turn might affect bollworm and leaf caterpillar survival.

Climate change is multi-faceted caused by changing levels of greenhouse gases in the atmosphere, rising temperatures, changes in precipitation patterns, and increasing frequency of extreme weather events. Over the past 250 years a 30 and 150 % rise in the concentration of the CO_2 and methane has been observed by Lal (2004) and Friedlingstein *et al*. (2010). Studies show that the current global atmospheric concentration of CO_2 is about 380 ppm which is 100 ppm more over pre-industrialisation period (1850-1900). The Intergovernmental Panel on Climate Change (IPCC) and the Indian Institute of Science had projected a figure of 575 ppm of CO_2 in the atmosphere by 2085. Recent reports showed that greenhouse gas emissions have increased by 2.2 % per year between 2000 and 2010, compared with 1.3 % per year from 1970 to 2000 (Kranthi, 2014). Climate change affects all crops by decreased root elongation, perturbation of root growth angle and reduced seed yield in response to drought, or an increase in root biomass in shallow soil in response to elevated CO_2 (eCO_2) early onset of reproductive stages, etc., and altered metabolism to compensate for the energy to survive through stress ultimately compromising with yield. Korres *et al*. (2016) proposed that C3 crops may offset the competition with C4 weeds while C3 weeds may threaten the survival of either crops in tropical areas. They may also widely impact damage due to herbivores and subsequent effects in higher trophic levels.

Climate change is likely to affect cotton production both positively and negatively. Under eCO_2, cotton plant might produce more number of branches, more vegetation and more bolls. But, higher temperatures (~40 °C) can cause boll shedding irrespective of the CO_2 levels. Hot weather may increase the evaporative demand leading to more intense water stress (Hall, 2000). However, the vegetative and reproductive vigour of plants exposed to eCO_2 levels may create higher demand for irrigation, pesticides and fertilizers, in the absence of which yields would decline (Kranthi, 2014). It is well known that both elevated temperature (eTemp) and eCO_2 affect the plant biochemical constituents also.

Agricultural crop insect pests may progress in environment made conducive with *e*CO_2 levels in the atmosphere and tropical regions will face more pest damage and more new pests. Under-nutritious host plants will induce both lengthened larval developmental times and greater mortality. Due to lower availability of nitrogen in foliage, insect herbivores will have increased consumption rate, developmental times, decreased fitness and fecundity. Insects grown under *e*CO_2 have lower efficiency of conversion of ingested food (ECI) to body mass (Fajer *et al*. 1989., Coviella and Trumble, 1999). Reduced efficiency of conversion of food into biomass leads to lower growth rate of larvae (Lindroth *et al.* 1993 and Masters *et al*. 1998). Whittaker (1999) showed that Bt cotton and *e*CO_2 slows the development of bollworms and consequently reduce larval weight gain, relative growth rate (RGR) and mean relative growth rate (MRGR).

Climate-smart agriculture is the way forward to lower the negative impact of climate variations on crop adaptation, before it might affect global crop production. While exposure of plants to a single climate change factor is more tractable, it also limits our ability to make inferences about plant responses to realistic climate change scenarios. Studies on interactive climate change factors have often demonstrated responses that differ strongly from the independent effects of climate change factors. FAO (2015) appeals greater attention for experiments like Free-Air CO_2 Enrichment (FACE) and complex interactions among Carbon, Temperature, Water and Nitrogen (CTWN).

In anticipation of drastic global climate change, the ensuing effects have to be studied with emphasis on the key factors *viz*., temperature and CO_2 in order to organize pest management strategies for producing fairly appreciable cotton yields with least damage from the polyphagous pests. With this background studies entitled '**Interactive Effects of Elevated Carbon Dioxide and Temperature on *Helicoverpa armigera* Hub. and *Spodoptera litura* Fab. on Bt Cotton'** were planned with the following objectives:

1. Effect of *e*CO_2 and *e*Temp on growth parameters and feeding indices of *H. armigera* on Bt and non-Bt cotton.

2. Effect of *e*CO_2 and *e*Temp on growth parameters and feeding indices of *S. litura* on Bt and non-Bt cotton.

3. Characterization and quantification of plant biochemical constituents *viz.*, carbon, nitrogen, C:N ratio, leaf protein estimation, polyphenol and Bt endotoxin, at *e*CO_2 and *e*Temp conditions.

4. Studies on the biochemical parameters of the test insects *viz*., protein, carbohydrate and Bt endotoxin contents at *e*CO_2 and *e*Temp conditions.

Chapter – II

Review of Literature

Chapter II

REVIEW OF LITERATURE

Studies on **'Interactive Effects of Elevated Carbon Dioxide and Temperature on *Helicoverpa armigera* Hub. and *Spodoptera litura* Fab. on Bt Cotton**' of Department of Entomology, Agricultural college, Bapatla, Acharya N. G. Ranga Agricultural University (ANGRAU) were conducted at ICAR-Central Research Institute for Dryland Agriculture (ICAR-CRIDA), Hyderabad, Telangana during 2018-20. The literature on similar lines of research carried out elsewhere has been reviewed in this chapter under different sub-headings.

The constant emission of Green house gases (GHGs), their frequency, intensity and duration will bring notable changes in the annual average temperatures (2.5-4.3 °C) in the cultivable areas of the world by 2080 (Christensen *et al*., 2007). Climate change may increase the productivity of crops and forest trees, through positive responses to higher CO_2 concentrations, but also cause yield losses from pests and pathogens (Grunig *et al*., 2020). Elevated carbon dioxide (eCO_2) and temperature (*e*Temp) are considered to be principal dimensions of climate change that influence crops and insect herbivores both directly and indirectly (Srinivasa Rao *et al*., 2015). Temperature plays a vital role in determining the distribution range, food consumption and multiplication of insects (Fand *et al*., 2015). It has been reported that, global warming may lead to altitude wise expansion of the geographic range of insect pests at the mean rate of 6.1 km per decade (Hill and Dymock, 1989., Parry and Carter, 1989., Elphinstone and Toth, 2008 and Parmesan, 2006). Shifts in insect-pest distribution and range due to changing climate may alter regional structure, diversity and functioning of ecosystems (Timoney, 2003). Tropical insect species abundance may increase (Cannon, 1998., Patterson *et al*. 1999., Bale *et al*. 2002 and Diffenbaugh *et al*. 2008) and sudden outbreaks of insect-pests can wipe out certain crop species (Kannan and James, 2009 and Stireman *et al*. 2005). *H. armigera* (Hubner), a major pest of cotton, pulses and vegetables in North India (Sharma *et al*., 2010) has extended its range nationwide with global climate warming as predicted. Paramasiva *et al*. (2004) apprehended that *H. armigera* might develop resistance to transgenic crops in future, keeping in view, their resistance against almost all the synthetic insecticides.

Climate driven pest infestation may intensify the yield losses and subsequently threaten global food security (Chahal *et al*., 2008). Complex physiological effects exerted by increasing temperature and CO_2 may affect the crop pest interaction (Hare, 1992., Yamamura and Kiritani, 1998., Rao *et al*. 2006., Deka *et al*. 2009., Petzoldt and Seaman, 2010 and Fand *et al.* 2015). Polyphagous insects feeding on diverse diet may attain certain fitness advantages that outweigh diet specialization (Muller *et al*. 2014), through getting adapted to those plant defensive substances by evolving physiological detoxification mechanisms (Raffa, 1987) and biochemical detoxiofication (Gould, 1984). The major dimensions of climate change *viz.*, *e*Temp and *e*CO_2 influence the growth and development of insect pests directly or indirectly and in turn affect their population dynamics (Veteli *et al*. 2002., Srinivasa Rao *et al*. 2015., Stips *et al*. 2016 and Deekshita *et al*. 2019).

2.1 EFFECT OF *e*CO_2 and *e*Temp ON GROWTH AND DEVELOPMENT OF INSECTS

Temperature and CO_2 have been found to exert both direct and indirect effects on the tri-trophic interaction among crops, insects and natural enemies by means of certain physiological changes related to host suitability and nutritional status (Gutierrez *et al*, 2008). They together alter the quantity and quality of host plant foliage, which in turn influence the growth and development of insect herbivores indirectly, termed as host mediated effects (Bauerfeind and Fischer, 2013., Dyer *et al.* 2013 and Kumar *et al*. 2020). Insects especially lepidopterans raised on foliage from *e*CO_2 grew slowly and experience greater mortality, especially in the early instars due to the reduced foliar water and nitrogen concentrations (Fajer *et al*. 1989., Stange 1997 and Berzitis 2013).

2.1.1 Effect of *e*CO_2 on Growth and Development of Insects

Caulfield and Bunce (1994) reported that beet army worm, *Spodoptera exigua* Hubner (Noctuidae: Lepidoptera) exhibited significantly longer development time (14.2 days) under *e*CO_2 (700 µl L^{-1}) than at ambient conditions (350 µl L^{-1}) with 12.4 days. Stange (1997) observed that at *e*CO_2, females of cactus moth, *Cactoblastis cactorum* Berg (Pyralidae: Lepidoptera) tend to find host plants more slowly and laid few eggs on their host plant *Opuntia stricta* Haw.

Insects raised on plants grown in *e*CO_2 grew significantly slower than insects on plants grown in *a*CO_2 based on review of insect-plant interactions (Coviella and Trumble, 1999). Coviella *et al*. (2000) observed that *S. exigua* on Bt cotton experienced lower larval weights and growth rate at *e*CO_2 (900 µmol mol^{-1}) and particularly in Bt cotton (0.16 with non-Bt and 0.1 mg mg^{-1} day^{-1} with Bt cotton). Goverde and Erhardt (2003) found that *e*CO_2 prolonged the developmental time of small heath butterfly, *Coenonympha pamphilus* (Satyridae: Lepidoptera) by two days when fed on four C_3 grass species (*Agrostis stolonifera* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., *Poa pratensis* L.) grown at higher CO_2 concentration compared to ambient conditions. The larvae of *S. litura* consumed significantly higher quantity of groundnut, *Arachis hypogea* foliage under *e*CO_2 (4.24 g per larva in 550 ppm and 4.29 g per larva in 700 ppm) than *a*CO_2 conditions (2.4 g per larva in 350 ppm) with increase in larval duration by two days (Reddy *et al*., 2004).

Chen *et al.* (2004) reported significant differences in *H. armigera* development on milky grains of wheat, *Triticum aestivum* among *a*CO_2 (382 µl L^{-1}) and *e*CO_2 (738 µl L^{-1}) conditions. Food ingested was 559.8 mg in *a*CO_2 and 817.5 mg in *e*CO_2. Likewise, frass production was 139.1 and 191.7 mg at respective conditions. Pupal weight decreased from *a*CO_2 (193 in female and 174 mg in male) to *e*CO_2 (186 in female and 166 mg in male). Pupation rate was also observed to decrease from 82.2 to 77.8 %. Fecundity reduced from 595 to 518 eggs per female in *e*CO_2. On the contrary, adult longevity increased from *a*CO_2 (17.9 in female and 8.2 days in male) to *e*CO_2 (20.5 in female and 11.4 days in male). Chen *et al*. (2005b) reported that in Bt cotton, *e*CO_2 (754 ± 33 µl L^{-1}) resulted in decreased body weight and fecundity in *H. armigera*. In Bt cotton larval, pupal, and adult moth weights were significantly less than those over non-Bt cotton, irrespective of CO_2. Soybeans, *Glycine max* grown in *e*CO_2 atmosphere had 57 % more damage from insects (Hamilton *et al*., 2005).

Wu *et al*. (2006) reported that CO_2 caused increase in larval duration from 11.3-11.7 days in *a*CO_2 (370 ppm) to 12-12.4 in *e*CO_2 (750 ppm) and decrease in pupal weight of *H. armigera* on spring wheat from 190-200 mg (*a*CO_2) to 170-180 mg (*e*CO_2) among the three generations studied. Decrease in fecundity was observed from 446-453 eggs per female (*a*CO_2) to 369-413 eggs per female (*e*CO_2). Chen *et al*. (2007) observed that *H. armigera* reared on transgenic cotton grown under *e*CO_2 (750 µl L^{-1}) exhibited higher consumption, longer larval duration and lower frass output, mean

relative growth rate, pupal weight, survival rate and fecundity in three successive generations. Decreased frass output by 9.9 % in Bt cotton under *e*CO_2. Further, the damage inflicted by cotton bollworm on cotton, regardless of the presence or absence of insecticidal genes, is predicted to be more serious under *e*CO_2 conditions because of the individual compensatory feeding on host plants caused by nitrogen deficiency.

Stiling and Cornelissen (2007) demonstrated that *e*CO_2 conditions can result in decline in insect abundance by almost 22.0 %. An increase of almost 17.0 % in consumption rates, 4.0 % in development time and a decrease of 9.0 % in relative growth rate and 5.0 % in pupal weight were estimated. The impact of *e*CO_2 on growth and development of castor semilooper, *Achaea janata* Linnaeus (Notuidae: Lepidoptera) was evaluated by Srinivasa Rao *et al*. (2007) and reported gain in larval weight at 550 ppm (0.87 g) followed by 700 ppm (0.86 g) as against ambient conditions (0.78 g). The larval developmental time extended by two days under *e*CO_2 compared to *a*CO_2. The life span of Japanese beetle, *Popillia japonica* Newman (Scarabaeidae: Coleoptera) is prolonged by 8.25 % when fed on foliage grown under *e*CO_2 in soybean. Also females laid approximately twice as many eggs as compared to females fed on foliage grown under normal ambient conditions (O'Neill *et al*., 2008). Yin *et al.* (2009) reported that CO_2 enrichment (750 μl L^{-1}) significantly delayed the larval stage of *H. armigera* in spring wheat. In the first generation, longer developmental durations were observed in all stages except the first instar (DF = (1, 137), F= 2.845, P= 0.094) and sixth instar (DF = (1, 78), F= 0.831, P= 0.365). However, in the second generation, only the duration of sixth instar increased by 31.5 %.

Wu *et al*. (2009) recorded development of *S. exigua* in transgenic (cv. GK-12) and non-transgenic cotton (cv. Simian 3). It was observed that larval food consumption and frass output were significantly lower in Bt over non-Bt cotton. Significantly longer larval duration in first (F = 47.44, d.f. = 1, 6, P = 0.0005), second (F = 38.27, d.f. = 1, 6, P = 0.0008) and third (F = 105.10, d.f. = 1, 6, P = 0.0001) generations of *S. exigua* on cotton. Pupal weight was less and did not significantly vary among three generations on non-transgenic Bt cotton and transgenic Bt cotton. However, pupal weight was less in the first (F = 17.1, d.f. = 1, 6, P = 0.061), second (F = 22.59, d.f. = 1, 6, P = 0.0031) and third (F = 90.0, d.f. = 1, 6, P = 0.0001) generations on Bt cotton compared with non-Bt cotton. Higher survival rate and fecundity in three successive generations of *S. exigua* was observed on Bt cotton compared with non-Bt cotton. Fecundity was 566 eggs per

female in the first, 583 eggs per female in the second and 587 eggs per female in the third generations on non-Bt cotton that were comparatively higher than those of Bt cotton. Fecundity was significantly reduced in the first (F = 97.79, d.f. = 1, 6, P = 0.0001), second (F = 123.45, d.f. = 1, 6, P = 0.0001) and third (F = 480.14, d.f. = 1, 6, P = 0.0001) generations. Fecundity was significantly high in the third generation over the first on Bt cotton. Abdul *et al*. (2014) reported that larval duration of *H. armigera* increased by 10.60-12.45 % in four successive generations under *e*CO_2 compared with *a*CO_2. Larval life span was 16.37, 17.15, 17.27 and 16.77 days in first, second, third and fourth generations, respectively under *e*CO_2 over *a*CO_2 (14.80, 15.25, 15.50 and 15.12 days). An increase of additional one to two days in larval duration was observed due to *e*CO_2. It was also observed that there was significant reduction in fecundity of *H. armigera* under *e*CO_2 conditions compared with ambient. Abdul *et al*. (2014) revealed that larval weight was more under *e*CO_2 (381.25- 384.16 mg) compared to *a*CO_2 conditions (367.91 mg). Similarly, Shrikant and Bandeppa, (2014) reported that larvae fed with castor foliage of *e*CO_2 conditions recorded more weight (0.78 g) over those fed with foliage of *a*CO_2 conditions (0.73 g). *S. litura* consumed 30 % more leaves and exhibited high fecundity on plants exposed to *e*CO_2 (Kranthi, 2014).

Srinivasa Rao *et al.* (2014b) reported that larval duration of *S. litura* increased by 6-8 % under *e*CO_2 across four successive generations compared with ambient CO_2, where it was 10 % higher in the fourth generation over the first. Foliage consumption and larval weights were significantly higher in fourth generation than preceding three generations under all three CO_2 conditions. Faeces output also increased along the generations and was commensurate with consumption. Accentuating effect of CO_2 on generations indicate that larvae consume more and gain more weight over a prolonged time period. Akbar *et al*. (2016) recorded that larval period and larval weights of *H. armigera* were significantly lower under *e*CO_2 750 ppm (10.67 days and 340 mg) compared to *a*CO_2 350 ppm (13.67 days and 420 mg). On the contrary, pupal period and pupal weights were significantly higher under *e*CO_2 (11.33 days and 340 mg) compared to *a*CO_2 conditions (10.67 days and 320 mg). Larval survival, pupation per cent and adult emergence were also lower in *e*CO_2 (40, 32 and 20.67 %) compared to *a*CO_2 conditions (50, 38.67 and 26 %). With increase in CO_2, fecundity increased from 831 to 1293 eggs per female, i.e. increase by 1.5 fold.

Sharma *et al.* (2016) observed that *e*CO_2 adversely affected the larval survival, mean larval weight, larval period, pupation, pupal weight and adult emergence of *H. armigera* in chickpea, *Cicer arietinum* L. Abdul (2017) recorded that pre-oviposition period of *H. armigera* ranged from 2.57-3.14 days across the CO_2 and generations and was highest in the third generation on semi-synthetic diet. Decreased oviposition period was recorded in first, second, third and fourth generations (6.71, 6.5, 6.35 and 6.28 days) under *e*CO_2 condition compared to *a*CO_2 (7.64, 7.35, 7.64 and 7.35 days). Post-oviposition period ranged from 2.71 to 3.28 days across the CO_2 conditions. Divya *et al.* (2019) reported significantly higher larval food consumption in *S. exigua* under *e*CO_2 over *a*CO_2 conditions in first (9.29 %) and second (11.56 %) generations over third and fourth generations on semi-synthetic diet. Fecundity was also lower under *e*CO_2 (409, 447, 409 and 374 eggs) in first, second, third and fourth generations compared to *a*CO_2 (423, 508, 432 and 406 eggs), respectively.

2.1.2 Effect of *e*Temp on Growth and Development of Insects

Global warming within certain favourable range may accelerate the rates of development, reproduction and survival in tropical and subtropical insects. It has been estimated that with an increase of 2 °C temperature insects might experience one to five additional life cycles per season (Yamamura and Kiritani, 1998), even resistant biotypes may develop (Kambrekar *et al*., 2015). Ranga Rao *et al*. (1989) reported the results of six generations of *S. litura* on groundnut in field and laboratory conditions. Predicting the seasonal occurrence and abundance of any pest is essential for the accurate scheduling of control tactics. Developmental threshold for eggs was 8.2 °C, for larvae 10 °C and for pupae 10.2 °C. There was no difference in either the development period or the pupal weight between males and females, the data for both sexes were pooled. The adult during the pre-oviposition period had slightly higher threshold of 10.8 °C. There was no oviposition at 35 and 37 °C. Under laboratory conditions, the first, second, third, fourth, fifth and sixth instars require 14, 11, 12, 13, 15 and 35 % of the total larval development time. Larval duration decreased from 19.9 days (25 °C) to 12.4 days (35 °C). Pupal duration decreased from 10.9 (25 °C) to 6.3 days (35 °C). Pre-oviposition duration decreased from 3.3 (20 °C) to 1.6 days (30 °C) and experienced death at 35 °C.

Pre-oviposition period is very important for any insect population, where lack of fertilization leads to a few sterile eggs. Jiang *et al.* (1998) reported that high temperature reduces the development, survival, longevity and fecundity of *Mythimna separata* (Walker) (Noctuidae: Lepidoptera). Saethre and Hofsvang (2002) reported that pre-ovipositional period was strongly affected by temperature, and that with favorable temperatures, apple codling moth, *Cydia pomonella* (L.) (Tortricidae: Lepidoptera) usually start depositing eggs two days after emergence. It was also found that the pre-ovipositional period is much longer when colder weather prevails and decreases with increase in temperature.

Population outbreak and widening of distribution range of rice stem borer, *Chilo suppressalis* Walker (Crambidae: Lepidoptera) was attributed to enhanced fecundity under warm condition in Hokkaido Island of Japan (Kiritani and Morimoto, 2004). Calvo and Molina (2005) reported that fecundity is an important life history trait in understanding population dynamics, for determining population changes along with environmental factors. It has been found in a range of insects that the fecundity of females is proportional to their weight. In lappet moth *Streblote panda* Hubner (Lasiocampidae: Lepidoptera) determinants of larval development such as temperature and food plant may influence fecundity through their effects on pupal weight and adult body size. Larvae were able to compensate for the lower nutritional status of some host plants by extended larval development, observed at 25 °C temperatures, and it may be viewed as a way to minimize adverse effects on fecundity. Larvae developing at higher temperature (28 °C was considered higher in temperate climates) were heavier at pupation. Any biotic or abiotic determinant reducing larval growth will reduce pupal weight and hence the reproductive performance of *S. panda*. Pupal weight was correlated with the total number of eggs per female (egg load) and was the better estimator of female egg load (54.2 % of variance).

Insect hosts may pass though vulnerable life stages more quickly at higher temperatures, reducing the window of opportunity for parasitism based on a review by Petzoldt and Seaman (2010). Regniere *et al*. (2012) stated that optimal environmental temperature allows rapid development and reproduction of insects, while temperatures above or below this range can have adverse effects. Fand *et al*. (2015) reported that extreme temperatures (38 °C) were lethal to larval and pupal stages of *S. litura* and that they failed to grow further. Using pest modeling studies Srinivasa Rao *et al.* (2015) predicted 1-2 additional generations of *S. litura* during distant (year 2050) and very

distant (2080) future scenarios due to higher future temperatures through CNRM-CM3, ECHams5 & CSIRO-Mk 3.5 models in peanut. Temperature projections indicated that generation time would decrease by 18-22 % over baseline year, 1975. Akbar *et al*. (2016) reported that that high temperature above 35 °C exhibited a negative effect on larval survival, larval period, pupal weights and pupal period, but a positive effect on larval weight and growth on semi-synthetic diet. Larval, pupal periods and pupal weight and pupation per cent decreased with increase in temperatures from 25 °C (12 and 10.33 days; 350 mg and 27.33 %) to 35 °C (10 and 8.33 days; 330 mg and 23.33 %). Larval survival and adult emergence also decreased from 25 °C (62.67 and 20 %) to 35 °C (46.67 and 12.67 %). Surprisingly larval weight increased from 320 mg (25 °C) to 370 mg (35 °C).

Zhang *et al.* (2016) reported that, when newly-emerged adults of the predatory mite, *Neoseiulus barkeri* Hughes (Phytoseiidae: Acari) were exposed to 42 ºC for 4 hours, females had a markedly extended pre-oviposition period, shortened oviposition period, reduced fecundity and longevity. Heat stress had a detrimental effect on reproduction, particularly by delaying the onset of oviposition and reducing reproductive output and thereby influencing the population dynamics of *N. barkeri*. with high temperature spermatophores and oocytes were most likely to be damaged, which might cause a significantly reduced fecundity, especially when females and males were stressed together.

Khafagi *et al*. (2016) attributed enormous weight gain in 5^{th} or 6^{th} larval instars of cotton leaf worm, *S. littoralis* (Boisd.) (Noctuidae: Lepidoptera) at 15 or 20 °C is apparently due to the prolongation of the larval duration and concomitant prolonged feeding. Nanditha *et al*. (2017) reported that the larval (27.45 to 13.50 days) and pupal duration (15.33 to 6.00 days) of *S. litura* feeding on jute, *Corchorus olitorius* L. decreased with increase in temperature from 18 to 33 ± 1 °C. The per cent adult emergence of *S. litura* increased from 76 to 91 % with increase in temperatures from 18 to 27 °C, further decreased with increase in temperature to 33 °C. Similar report on reduced pupal duration (from 7.68 to 3.12 days) with increase in temperature from 20 to 38 °C was reported by Dai *et al.* (2017) in *S. exigua*. Tejinder and Sharma (2017) examined that *S. litura* larval and pupal durations were higher at 25:11 °C (23.4 ± 0.8 and 9.3 ± 0.3 days, respectively) than at 25:14 °C (21.2 ± 0.9 and 8.7 ± 0.4 days, respectively) on cauliflower, *Brassica oleracea* var. botrytis. The per cent adult emergence was more at 25:14 °C (91.8 %) compared to that at 25:11 °C (82.0 %).

The larval (40.20 days) and pupal periods (6.80 days) of *S. litura* on sunflower, *Helianthus annuus* L. foliage decreased (15.00 and 4.80 days) with increase in temperature from 20 to 30 °C (Rama Devi and Jha, 2017). The development rate of *S. litura* immature stages in cotton increased linearly as a function of temperature until approximately 34–36 °C, after which it became nonlinear (El-Sayed *et al*., 2018). Plessis *et al*. (2020) recorded that *Spodoptera frugiperda* (J.E.Smith) (Noctuidae: Lepidoptera) larval duration on sweet corn decreased from 14.86 days (26 °C) to 11.38 (30 °C) and 10.45 days (32 °C). Pupal duration also decreased from 11.43 to 9.00 and 7.82 days at 30 and 32 °C, respectively.

Ghazanafar *et al.* (2020) reported that at constant temperature of 35 °C, survival to the pupal stage was significantly reduced to 34 % for tobacco budworm, *Heliothis virescens* (Fabricius) and 6 % for *S. littoralis*. Pupal weight reduced to 40-50 % compared to that at *a*Temp. In the *H. virescens* assay, 72-84 % of the larvae managed to pupate with a mean pupal weight of 270-289 mg across the three temperature regimes. Time to pupation was on average 15-16 days. In the *S. littoralis* assay, survival to pupation was 92-98 %, the mean pupal weight was 363-389 mg, and the mean time to pupation was 14 days for the 3 temperature regimes. When pulverized leaf powder from Bt-cotton was mixed into the diet of *H. virescens*, larval weight (8 days after hatching) was approximately 10 % of the weight of that in non-Bt treatments, development time to pupation doubled, and pupal weight was reduced significantly by 20-35 %. Larval weight of *S. littoralis* after 8 days in the Bt treatment was reduced to 30-40 % of that in the non-Bt treatment and larvae needed 2-3 more days to pupation. Pupal weight at constant 25 °C was 16 % lower in the Bt compared to the non-Bt treatment.

2.1.3 Interaction Effect of *e*CO_2 + *e*Temp on Growth and Development of Insects

Feed quality is the main factor for determining the utilization of food in many plant feeding insects (Hodar *et al*., 2002), because host plant components directly affect the plant feeders' intake (Awmack and Leather, 2002). Hence, alteration in plant chemistry due to changes in CO_2 and temperature levels have resulted in lower growth rate, slower larval developmental and increased compensatory feeding in herbivorous insects. This is in close agreement with the reports of Fajer *et al*. 1989., Lincoln *et al*. 1993., Hattenschwiler and Schafellner, 2004., Chen *et al*. 2007., Wu *et al*. 2007., Srinivasa Rao *et al*. 2009 and Abdul *et al*. 2014.

Sailaja *et al*. (2005) corroborated that interactions between climate stress factors exacerbate the rate and direction of individual climate stress factors and their effects on terrestrial ecosystems. Cumulative stress response index (CSRI) was positive for soybean genotypes under *e*CO_2, but when plants were grown under CO_2 + temperature + UV-B radiation, the responses were negative in all the genotypes. Similar phenomenon was reported earlier by Wheeler *et al*. (2000) and Prasad *et al*. (2003) in peanuts.

Akbar *et al.* (2016) reported that *e*CO_2 and *e*Temp increased the consumption and metabolism of *H. armigera* fed on semi-synthetic diet by increasing protease activity and carbohydrates in the midgut. Shwetha *et al*. (2017) reported significantly higher leaf consumption (3758.07 mg) and faecal matter (893.72 mg per larva) of *S. litura* when fed with groundnut foliage grown under *e*CO_2 + *e*Temp (550 ppm + 2 °C) compared to the reference plot (3341.28 mg; 884.38 mg per larva, respectively). The larval and pupal duration were prolonged by two days under *e*CO_2 over that of ambient. Fecundity was 485 under *e*CO_2 and 521 eggs per female with a decrease of 6.86 % over reference ambient conditions.

Divya *et al.* (2018) reported that durations of *S. exigua* larvae (24.60 to 9.45 days), pupae (15.45 to 5.60 days), adults (11.50 to 5.40 days) and total developmental period (56.75 to 22.45 days) decreased from 20 to 35 °C temperature under *e*CO_2. Larval and pupal periods of *S. exigua* decreased with increase in temperature from 20 to 35 °C. The mean developmental time of larva (29.8 to 12.87 days) and pupa (16.46 to 7.93 days) decreased at 35 °C under *e*CO_2 conditions. Similarly, larval (27.60 to 13.13 days) and pupal (16.80 to 6.66 days) developmental period decreased under *a*CO_2 with increase in temperature.

Mounica *et al.* (2020) reported that the duration of different growth stages of maize aphid, *Rhopalosiphum maidis* Fitch (Aphididae: Hemiptera) reduced with an increase of temperature from 20 to 35 °C under both *a*CO_2 and *e*CO_2 conditions. Divya *et al*. (2020) recorded that fecundity of *S. exigua* was lower with 208 eggs per female under *e*CO_2 (550 ppm) than *a*CO_2 conditions with 391 eggs per female. At *e*CO_2, mean generation time also decreased from 49.52 days at 20 °C to 20.20 days at 35 °C. Mallikarjuna *et al*. (2020) observed that larval and pupal weights of *H. armigera* reduced from reference ambient conditions (364 and 231 mg) to *e*CO_2 +*e*Temp (242

and 169 mg) with 33 and 26.83 % reductions, respectively. Larval survival also decreased from 49 to 32 % at *e*CO_2. Fecundity also reduced by 37.8 % from 238 eggs per female at ambient to 148 eggs per female at elevated condition.

Viswajyothi *et al.* (2020) studied the interactive effects of CO_2 and temperature on pink stem borer, *Sesamia inferens* Walker (Noctuidae: Lepidoptera) in maize, *Zea mays* L. Larval duration reduced by 19.0 % at *e*Temp (32 °C) and increased by 5.1 % at *e*CO_2 (450 ppm). Pupal duration was significantly reduced by 46.8 % at *e*Temp and increased by 8.3 % at *e*CO_2. Fecundity of *S. inferens* increased by 10.8 % at *e*Temp and got reduced by 14.5 % at *e*CO_2. Temperature had predominant influence on the larval and pupal duration, whereas CO_2 has a major influence on fecundity where interaction treatment had non-significant influence.

2.2 EFFECT OF *e*CO_2 AND *e*Temp ON FEEDING INDICES OF INSECTS

Coviella and Trumble (1999) reported that *e*CO_2 causes compensatory increase in food consumption, reduced digestive efficiency, survival rates and population abundance of herbivore insects due to lower nitrogen availability in the crop foliage. Under nutritious host plants will induce both lengthened larval developmental times and greater mortality. Insects grown under *e*CO_2 have lower efficiency of conversion of ingested food to body mass (Fajer *et al*., 1989). Elevated carbon dioxide did not affect the developmental time, growth rate and larval weight of gypsy moth, *Limantria dispar* Linnaeus (Erebidae: Lepidoptera), however it caused increased consumption and decreased conversion efficiencies (Kinney *et al.*, 1997). Reduced efficiency of conversion of food into biomass due to low nitrogen leads to lower growth rate of larvae (Lindroth *et al.* 1993 and Masters *et al.* 1998). Higher approximate digestibility (AD) under *e*CO_2 has been attributed to accumulation of starch content (Lindroth *et al*., 1993) and lower N content (Wang *et al*., 2008). Whittaker (1999) showed that Bt cotton and *e*CO_2 slows the development of bollworms and consequently reduce larval weight gain, relative growth rate (RGR) and mean relative growth rate (MRGR).

Chen *et al.* (2004) reported that imposition of high levels of CO_2 (738 µl L^{-1}), caused a decrease in Efficiency of Conversion of Ingested Food (ECI) in the range of 44.4 to 32.3 % and Efficiency of Conversion of Digested Food (ECD) in the range of 57.5 to 42.9 % parameters of *H. armigera* accounting to reduction of 27.2 and 25.4 %, respectively over *a*CO_2 (382 µl L^{-1}). Relative Consumption Rate (RCR) increased

significantly by 58.8 % from 318 to 505 mg g^{-1} day^{-1} compared to *a*CO_2. RGR decreased slightly from 138 to 130 mg g^{-1} day^{-1}. Chen *et al*. (2005b, 2007) reported that with *e*CO_2 (754 ± 33 µl L^{-1}) RGR, MRGR, ECI and ECD in *H. armigera* significantly reduced and AD improved on transgenic Bt cotton, but without a significant CO_2 or CO_2 × cotton cultivar interaction.

Wu *et al*. (2006) reported that RCR of *H. armigera* on spring wheat increased from *a*CO_2 (459.9, 485, 488.5 mg mg^{-1} day^{-1} at F1, F2 and F3 generations) to *e*CO_2 (504.2, 567.1, 594.3 mg mg^{-1} day^{-1} at respective generations). ECI reduced from *a*CO_2 (19.23, 17.87, 17.42 at F1, F2, F3 generations) to *e*CO_2 (16.32, 14.48, 13.41 respectively). RGR decreased with increase in CO_2 from 17.4 – 19.2 (*a*CO_2) to 13.41-16.32 mg mg^{-1} day^{-1} (*e*CO_2). Wu *et al*. (2009) recorded feeding indices of *S. exigua* in transgenic (cv. GK-12) and non transgenic cotton (cv. Simian 3). Significantly lower consumption and RGR were observed in three successive generations of *S. exigua* fed on transgenic Bt cotton compared with non-transgenic Bt cotton.

Srinivasa Rao *et al.* (2009) while examining the host mediated effects of *e*CO_2 on feeding indices of *S. litura* inferred that AD and RCR of larvae increased (from 58.61 and 37.12 @ *a*CO_2 to 74.51 % and 48.27 mg mg^{-1} day^{-1} @ *e*CO_2), while the efficiency parameters ECI and ECD significantly decreased from 380 ppm *a*CO_2 (19.83 and 34.09 %) to 700 ppm *e*CO_2 (15.93 and 24.49 %). Similarly Srinivasa Rao *et al*. (2012) reported increase of RCR (19 - 24 %) with decrease of ECD (35-32 %) and ECI (25 %) of *S. litura* on peanut foliage at *e*CO_2 over ambient. Srinivasa Rao *et al*. (2013) quantified the effects of *e*CO_2 on the feeding indices of *A. janata* larvae on castor foliage and observed significant increase in AD (0.32 - 12.26 %) and RCR (6-22 %) and decrease in ECD (23-34 %) and RGR (12-15 %) in all four generations over *a*CO_2 conditions.

Abdul *et al*. (2014) reported that chickpea foliage grown under *e*CO_2 condition stimulated an increase in approximate digestibility and relative consumption rate of *H. armigera*. The other parameters *viz.*, ECI and ECD were decreased by 35.88 and 37.88 % under *e*CO_2 compared to *a*CO_2 and attributed it to alteration in the phytochemistry of foliage. Chen *et al.* (2004) reported that ECI and ECD parameters of larvae decreased by 27.2 % and 25.4 %, respectively and significant increase in RCR by 58.8 % compared with ambient conditions. Srinivasa Rao *et al.* (2014b) reported an increase of AD (9 %) and RCR (7 %) and decrease of ECI (13 %), ECD (19 %) and RGR (9 %) was

observed in all four generations under *e*CO_2 over ambient on peanut foliage. Larvae consumed more and assimilated better (higher values of RCR and AD) but grew slower (lower RGR) and took longer time (one day more than ambient) to pupate.

Akbar *et al.* (2016) reported an increase in RGR from 0.073 to 0.093 g g^{-1} day^{-1} for *H. armigera* reared on chickpea based semi synthetic diet. In the same lines, Dalal and Arora (2016) reported that the relative growth rate (RGR) of *H. armigera* increased from 0.139 ± 0.002 at 25:10 °C to 0.174 ± 0.003 at 30:10 °C. Investigations of Zhang *et al*. (2017) on the effect of *e*CO_2 on the food utilization of *S. litura* on soybean cultivars revealed that RGR and ECD decreased significantly by 16.75 and 33.10 % respectively and RCR and AD increased by 19.34 and 12.90 %, respectively. Karmakar and Pal (2017) tested the effect of 5 °C rise in temperature on sixth instar larvae of *S. litura* and found that RCR, ECD, ECI and RGR increased while AD decreased.

There was an increase in RCR (1.27-1.41 % in castor and 1.07-1.09 in tomato, *Lycopersicon esculentum* Mill.); ECI(13.21-22.19 % in castor and 12.71-19.32 % in tomato); ECD (25.11-46.56 % in castor and 20.65-38 % in tomato) and RGR increased from 0.22 to 0.31 g g^{-1} day^{-1} in castor and 0.13 to 0.22 g $g^{-1}day^{-1}$ in tomato, whereas there is a decrease in AD (46.89 - 28.98 % in castor and 47.54 - 32.84 % in tomato). Shwetha *et al*. (2017) reported RGR of *S. litura* larva when fed with groundnut foliage decreased by 9.74 %, from 128.50 (*a*CO_2 + *a*Temp) to 118.76 g g^{-1} day^{-1} (*e*CO_2 + *e*Temp). Luo *et al*. (2018) observed that under 150 mmol L^{-1} NaCl stress, food consumption of *H. armigera* decreased by 25.88 % (p = 0.002) and faeces production decreased by 23.06 % (p = 0.021), respectively in GK19 Bt cotton. It also resulted in significant decreases in the MRGR, RGR, AD, ECI and ECD of the 5^{th} instar bollworm larvae. AD of 5^{th} instar bollworm larvae feeding on Bt cotton was much higher than that of 5^{th} instar larvae feeding on non-transgenic cotton.

Sharma and Brar (2018) studied the effects of minimum rise in temperature (5 °C) and CO_2 (400 and 500 ppm) concentrations on the nutritional indices of *S. litura* on cauliflower. The feeding indices *viz.,* food consumption, CI and AD showed significant increase by 2.0, 8.9 and 4.7 % with *e*CO_2, while the ECD, ECI and RCR was decreased by 8.50, 27 and 26.6 % respectively, at 500 ppm of CO_2 compared to 400 ppm. Similarly with minimum increase in temperature by 5 °C, the food consumption, CI, AD, values increased by 43.23, 50.83 and 22.0 %, whereas ECD, ECI and RCR decreased by 20.28 and 35.89 %, respectively.

2.3 EFFECT OF *e*CO_2 AND *e*Temp ON BIOCHEMICAL CONSTITUENTS OF PLANT FOLIAGE AND INSECTS

There are only few studies related to biochemistry of cotton under elevated levels of CO_2 and temperatures. Thus, the information on annual and biennial crops was also summarized.

2.3.1 Effect of *e*CO_2 and *e*Temp on Biochemical Constituents of Plant Foliage

Plants grown at high CO_2 levels have higher plant growth rate and significant changes in the physical and chemical composition of their tissues (Ramakrishna and Ravishankar, 2011 and Sudderth *et al.* 2005). Yan *et al*. (2002) reported that Bt cotton contained higher concentration of certain monoterpenes e.g., α and β- pinene and lesser concentrations of myrcene and ocimene when compared to non-transgenic cotton. Nutritional evaluation of Bt cotton relative to non-transgenic cotton by Mohanta *et al*. (2011) revealed that they differ slightly in the general composition of proximate constituents (carbohydrate, protein, crude fat and total ash), fiber, minerals and secondary metabolites.

2.3.1.1 Nitrogen, carbon and C: N ratio in foliage: Nitrogen is the single most important limiting resource for herbivorous insects (Mattson, 1980). Lincoln *et al*. (1984) examined the effect of *e*CO_2 on the foliar chemistry of soybean plants at CO_2 levels of 350, 500 and 650 µl L^{-1}. The results indicated that leaf nitrogen was significantly reduced by 5 and 11 % (70.9, 67.2 and 62.9 mg g^{-1}, respectively) and C: N ratio was enhanced by 5 and 9 % (6.24, 6.61 and 6.82 mg g^{-1}, respectively) over ambient. Higher foliar C: N ratios stimulate the herbivores to consume about 80 % more vegetation to compensate for the little available nitrogen (Lincoln *et al*., 1986 and Marks and Lincoln, 1996). Brooks and Whittaker (1999) reported that decrease in the foliar N of host plants could limit insect growth and development and decrease the survival rates of phytophagous insects. Coviella *et al*. (2000) attributed the reason for lower C: N ratio solely to the decrease in N level, and has no obvious increase in C content. Coviella *et al*. (2002) reported 16 % decrease in N content in cotton grown in *e*CO_2 (900 µmol mol^{-1}) over *a*CO_2 (300 µmol mol^{-1}) conditions and projected higher C: N ratios and carbon defensive compounds that affected *S. exigua* adversely.

Legumes like soybean capable of N fixation also get affected during early growth stages under *e*CO_2 (Rogers *et al*. 2006). Maintaining an appropriate balance of C and N nutrients is critical from the metabolic point of view (Zheng, 2009). Decrease in nitrogen content and increase in carbon and C: N ratio was reported by Wang *et al*. (2009). Srinivasa Rao *et al*. (2009) reported that leaf nitrogen content in castor was significantly lower in *e*CO_2 compared to *a*CO_2. In contrast, carbon content increased under *e*CO_2 conditions and as a consequence the relative proportion of carbon to nitrogen also increased. Lourdes *et al*. (2012) reported that sunflower foliage grown under *e*CO_2 (800 ppm) exhibited higher contents of starch, soluble sugars, carbon and C: N ratio over ambient (400 ppm). Srinivasa Rao *et al*. (2012) reported that groundnut leaf nitrogen content was significantly lower under *e*CO_2 (2.95 % @ 700 and 550 ppm) than ambient conditions (3.20 % @ 380 ppm). Though the carbon content was not significantly influenced by CO_2 conditions an increase in C: N ratio was observed under *e*CO_2 (13.5 % and 13.6 %) than in ambient (12.0 %).

Abdul *et al*. (2014) examined the influence of *e*CO_2 (550 and 700 ppm) on chickpea foliage and reported that leaf nitrogen content was significantly lower (2.92 and 3.21 % in 550 and 700 ppm conditions, respectively) compared to ambient (3.88 %). However there is significant increase in carbon content under elevated conditions 43.13 (700 ppm) and 40.46 (550 ppm) compared to the ambient (33.99 %) and resulted in significant increase of C: N ratio. Manimanjari *et al.* (2014) reported that there is a significant variation in the biochemical parameters of sunflower grown under 380 ppm and 550 ppm CO_2 levels. Per cent leaf nitrogen was significantly lower in *e*CO_2 (2.67 %) than *a*CO_2 (2.81 %). In contrast, there is a significant increase in carbon content of the foliage (41.63 %) over *a*CO_2 (38.63 %) and increase in C: N ratio (15.60) compared with *a*CO_2 (13.76). Similarly, Lakshmi *et al.* (2017) reported that nitrogen per cent was significantly high in the sunflower seed grown at 700 ppm (4.95 %), over 550 ppm (5.15 %) and ambient CO_2 (5.37 %). Meena *et al*. (2017) noticed significantly less nitrogen content under *e*CO_2 (4.08 %), followed by *e*CO_2 + *e*Temp (4.39 %), *a*CO_2 + *e*Temp (5.16 %), and *a*CO_2 (5.73 %) in bell peppers.

Shwetha *et al*. (2017) reported that groundnut grown under *e*CO_2 (550 ppm) recorded highest per cent of carbon and C: N ratio (46.95 % and 14.29 %, respectively) compared with *a*CO_2. In contrast, nitrogen was significantly less in *e*CO_2 condition (3.18 %) than at *a*CO_2 condition. Mallikarjuna *et al*. (2020) recorded significantly high carbon content of 42.21 % and 35.94 % for ICCL 86111 and ICC 3137 chickpea varieties, in *e*CO_2 + *e*Temp compared to ambient conditions.

2.3.1.2 Carbohydrate content in foliage: Srivastava *et al*. (2002) studied the effect of long-term CO_2 enrichment on the foliar chemistry of mungbean (*Vigna radiata* L. Wilczek). Under enriched CO_2 (650 µl L^{-1}), level of leaf nitrogen and protein declined significantly, whereas levels of starch and total soluble sugars (reducing and non-reducing) increased. Hattenschwiler and Schafellner (2004) revealed that leaf quality of oak tree, *Quercus* sp. grown at *e*CO_2 condition had higher tannins, starch, sugars and non-structural carbohydrates compared to the *a*CO_2 condition. Wu *et al*. (2006) reported that with increase in CO_2 to 750 ppm, N content reduced from 12.71 mg g^{-1} to 11.8 mg g^{-1} and protein content decreases from 1.53 to 1.34 g L^{-1}; total non-structural carbohydrates (TNC) increased from 223.98 mg g^{-1} to 237.75 mg g^{-1} level; C: N ratio increased from 17.63 (*a*CO_2) to 20.16 (*e*CO_2) in spring wheat.

Yin *et al*. (2010) reported that *e*CO_2 increases the TNC content in maize plants and decreases the N content, causing insects to consume more plant tissue to obtain enough N-based nutrients, and extends their development time. Li *et al*. (2013) recorded that the starch, total soluble sugar and sucrose concentrations increased significantly in tomato plants grown under *e*CO_2 condition (800 µmol mol^{-1}) by 90, 60 and 44 % respectively. Xin *et al*. (2013) showed that the starch, total soluble sugar, and sucrose concentrations increased significantly in tomato plants grown under *e*CO_2 condition (800 µmol mol^{-1}) wherein, the concentrations of the three carbohydrates increased by 90, 60 and 44 %, respectively In C3 plants, *e*CO_2 can contribute to increase of the total non-structural carbohydrates (Loladze, 2014). Haicui *et al*. (2015) reported that there was 8.3 % increase in starch in maize leaves grown under *e*CO_2 condition. Wheat leaf carbohydrate concentration increased 3-fold at *e*CO_2 (Yilmaz *et al*., 2017) implying decreased carbohydrate transport from source to sink tissues due to *e*CO_2 (Soares *et al*., 2019). Elevated CO_2 decreased N content and increased TNC content and the C:N ratio in maize leaves (Xie *et al*., 2018).

2.3.1.3 Protein content in foliage: Proteins have significant role in growth, development, morphogenesis and many other metabolic pathways of insects (Kar *et al*., 1994). Idso and Idso (2000) corroborated that decrease of the nitrogen concentration in vegetative parts as well as seeds result in the decrease of the protein levels. The results are in conformity with Srivastava *et al*. (2002) that protein and non-protein nitrogen declined significantly, under *e*CO_2 (650 µl L^{-1}) in mungbean leaves. Higher temperature influences the nutritional composition of leaves i.e., reduction in the

nitrogen content (Wollenweber *et al.,* 2003) and protein content (Haba *et al.*, 2014) ultimately affecting the growth and development of herbivores. Chen *et al*. (2004) recorded reduction in gross protein content in milky grains of wheat from *a*CO_2 (8.1%) to *e*CO_2 (7.05 %) and increase in soluble protein content from 1.22 to 1.36 % at the respective conditions.

The leaf soluble protein content decreased to 70 % in sunflower foliage grown under higher temperature (33: 29 °C) compared to 45 % in control 23: 19 °C day/night (Haba *et al*., 2014). Temperature above 38 °C brought about increase in square amino acid content and marked decrease in the soluble protein levels in cotton (Wang *et al*., 2015). Adishesha *et al*. (2017) noticed lesser soluble protein content in maize leaf under *e*CO_2 (0.64 mg g^{-1}) compared with *a*CO_2 (0.72 mg g^{-1}). Lavanya *et al*. (2017) reported that protein content in mulberry, *Morus alba* L. was less in *e*CO_2 (5.39 mg g^{-1}) over *a*CO_2 (6.12 mg g^{-1}). Shwetha *et al*. (2017) reported significantly less protein content in *e*CO_2 (3.45 mg g^{-1}) than at *a*CO_2 in groundnut.

2.3.1.4 Phenol content in foliage: Bryant *et al*. (1983) hypothesized that when plants are stressed, an exchange occurs between carbon to biomass production and results in formation of defensive secondary compounds. Anthocyanin accumulation is stimulated by various environmental stresses. Dury *et al*. (1998) reported that larval development of winter moth, *Operophtera brumata* Linnaeus was detrimentally affected by increased phenolic content and decreased nitrogen content of leaves of oak tree due to higher CO_2 levels. Phenolic compounds and terpenoid aldehydes increased with increase in CO_2 in cotton (Coviella *et al*. 2002 and Bazin *et al*. 2002). Agrell *et al*. (2000) studied the effect of normal (387 ± 8 µl L^{-1}) and *e*CO_2 content (696 ± 2 µl L^{-1}) on white-marked tussock moth, *Orgyia leucostigma* (Erebidae: Lepidoptera) larvae on three tree species among different light conditions. Elevated CO_2 caused reduced water and nitrogen content with higher starch, phenolic glycoside and tannin content. The survival rate of larvae decreased by 62 %, development time increased and larval mass significantly decreased. Sudderth *et al.* (2005) also reported increase in polyphenols in groundnut leaves under *e*CO_2 condition.

Chen *et al*. (2005b) reported that in Bt cotton, irrespective of CO_2 there was significant (p<0.05) increase in TNC: Nitrogen ratio, condensed tannin and gossypol, and decrease in Bt toxin protein of young bolls. Wu *et al*. (2007) reported a significant

increase in condensed tannins, and a significant decrease in nitrogen based compounds (proteins) in the leaves of transgenic Bt cotton under *e*CO_2 conditions. Yin *et al*. (2010) stated that phenolic compounds have a negative influence on the development and fitness of chewing herbivore insects. Srinivasa Rao *et al.* (2014b) also recorded higher polyphenol content in *e*CO_2 (1.90 % and 1.69 %) over ambient (1.66 %) in groundnut. Lavanya *et al*. (2017) reported significant increase in tannins (1.91 mg g^{-1}) and phenols (4.19 mg g^{-1}) over *a*CO_2 (1.07 and 3.26 mg g^{-1} respectively) in mulberry. Xu *et al*. (2019) reported that total phenolics of maize increased by 5.13 % under *e*CO_2.

2.3.1.5 Bt toxin content in cotton foliage: Naturally Bt cotton leaf Cry1Ac toxin significantly decreased as the crop approached maturation (Wu *et al*., 2003), while toxins of both Cry1Ac and Cry2Ab, would be higher during the early growth stages and drop significantly from anthesis onwards (Liu *et al*., 2019). Supriya *et al.* (2018) stated that major causes for variation in performance of Bt transgenic could be either insect related (resistance), crop performance (decline in expression) and environment related. In various Bt cotton hybrids at 90-120 DAS, quantity of Cry1Ac was highest in the leaves over squares and bolls (4.44, 2.09 and 1.09 ppm in leaf, square and boll of NCS - 207 Bt I hybrid). The quantity of Cry2Ab toxin is relatively lesser in the leaves (126.20, 210.71 ppm) than that in squares (213.24, 318.14 ppm) and bolls (456.36, 767.08 ppm) in Bt-II hybrids NCS-207 and RCH-134 respectively during 90-120 DAS.

Kaiser (1996) reported that cotton bollworm, *Heliothis virescens* destroyed Bt cotton due to high temperatures in Texas, USA. Greenplate *et al*. (1998) reported significant declines in Bt efficacy against *Helicoverpa* sp. during flowering stage, coinciding with high temperatures. Expression of Bt toxins in transgenic plants is greatly influenced by environmental factors like high temperature, soil moisture, *e*CO_2 levels and drought, (Dhaliwal and Dilawari, 1993 and Kranti *et al*. 2005) leading to decreased resistance to insect pests (Chen *et al*. 2005a and Dong and Li, 2007). The reason for decline in toxin content was explained by Coviella *et al*. (2000) that *e*CO_2 causes lower nitrogen and Bt toxin production also. Hence Bt cotton pest resistance is maintained only for 110 days, after which the toxin level dropped below the lethal level of 1.9 μg g^{-1}, and there is every chance for damage from bollworms (Guo *et al*. 2001 and Kranthi *et al.* 2005).

Chen *et al.* (2005a) reported significant decrease in Bt toxin when exposed to 37 °C for 48 h during peak boll development phase. It decreased from 288.8 to 77.8 ng g^{-1} fresh weight in Kumian No.1 (73.1 % decrease) and from 159.2 to 59.7 ng g^{-1} in Xinyang 822 (62.5 % decrease). A significant positive correlation between the leaf insecticidal protein and soluble protein content ($r = 0.75^{*}$), and negative correlation between the leaf insecticidal protein and free amino acid content at the boll period ($r = -0.79^{*}$) was observed. Hence high temperature stimulated higher protease activity that resulted in degradation of leaf soluble protein along with leaf insecticidal protein i.e. Bt toxin during peak boll development phase. Leaf nitrogen deficits could also develop as a result of competition between growth of fruiting and vegetative organs during boll period (Wadleigh, 1944 and Radin and Mauney, 1986). It was reiterated that high temperature reduced nitrogen metabolism that cannot recover again because of leaf senescence. Because GPT (Glutamic-pyruvic transaminase) activity decreased sharply after 24 h, the protease activity increased, and free amino acid content increased significantly coupled with decrease of soluble protein content.

Wu *et al*. (2007) reported decrease in Bt toxin content by 1.5 % and 1.4 % in transgenic Bt cotton after injecting CO_2 for 2 months over 1 month treatment under *e*CO_2. Wang *et al*. (2015) reported that temperature above 38 °C caused significant decrease of square Bt protein concentration. The expression of Bt proteins in cotton has been shown to be influenced by temperature (Rana *et al*., 2015). The highest expression levels were observed at 31-35 °C, while concentrations declined rapidly at temperatures higher than 40 °C. At 36 and 18 °C, Bt toxin content reduced by 12.5 and 17.5 % at 24 h, by 24.1 and 32.9 % at 48 hours after treatment respectively (Abidallha *et al*., 2017).

The concentration of Bt protein in plant tissues was also significantly positively correlated with total soluble protein and total nitrogen (Oosterhuis and Brown 2004., Wang *et al*. 2012 and Chen *et al*. 2018). To maintain optimum Bt toxin, nitrogen fertilization was resorted to by many researchers. Chen *et al*. (2019) recorded that high dose of N fertilizer elevated the Bt endotoxin expression by 14 % relative to the low-dose treatments, and that leaf Bt endotoxin levels in cotton planted early were 12 % lower than levels in late planted crop.

2.3.2 Effect of *e*CO_2 and *e*Temp on Biochemical Constituents of Insects

Insect metabolic rates may double with an increase of 10 °C across the full range of regular temperatures (Berggren *et al*., 2009). Krishnayya (1993) reported decrease in haemolymph protein and trehalose content in *H. armigera* upon stress imposition by the application of plumbagin. Bale and Hayward (2009) noted that insect adaptation is not only related to adjustment in their body temperatures, but also adaptation to changed dietary environments. Elevated CO_2 in the atmosphere could result in increased ratio of carbon to nitrogen in leaf tissues which could decrease the nutritional value for insects. Willmer *et al*. (2004) reported that high temperature affected all biological process including the structure of proteins and biological members and rates of biochemical and physiological reactions.

2.3.3.1 Carbohydrate content in insects: Effect of high temperature on haemolymph sugar levels in the three selected races of mulberry silk worm was reported by Malik and Reddy (2008). They reared the fifth instar larvae and pupae of NB4D2 and CSR2 (bivoltine races) and pure Mysore (univoltine race) at two selected temperatures i.e. 31 and 36 °C. At higher temperatures, an increase in blood sugar level and trehalase activity was observed in the larvae. Kamel *et al*. (2018) reported significant decrease in the carbohydrate content of *S. littoralis* by 50 % which is one of the insects' important energy sources. Ismail (2021) reported that carbohydrate content of *S. littoralis* in ambient temperature (25 °C) was 28.3 mg g^{-1} body weight, which reduced by 75 % under elevated temperature (35 °C).

2.3.3.2 Protein content in insects: Proteins may have enzymatic, structural, receptive and molecular transportation and storage purposes (Chapman *et al.*, 2013). Protein synthesis in late juvenile stages of holometabolic insects and adult females is completed mainly in the fat body (Gillott, 2005). Gullan and Cranston (2005) concluded that high temperature tend to kill insect's cells by denaturing proteins, altering membrane, enzyme structures and properties, by the loss of water. Hachiya *et al*. (2007) reported that proteins denature more rapidly at higher temperatures, which, in turn, requires greater rates of protein synthesis and repair to maintain basic cellular function. At high temperatures (36 °C) the protein level decreased significantly in mulberry silkworm, *Bombyx mori* Linnaeus (Bombycidae: Lepidoptera) larvae as well as in pupae (Malik and Malik, 2009). Zeng *et al*. (2012) reported that nymphs of *Nilaparvata lugens* Stal (Delphacidae: Hemiptera) had significantly lower protein and higher glucose content in the *e*CO_2 treatment compared to the *a*CO_2.

Lemoine and Shantz (2016) reported that *S. exigua* protein decreases at 30 °C due to decline in nitrogen digestibility by almost 25 % compared with 25 °C. Increased cellular division and somatic growth rates may also be responsible for the observed protein limitation at high temperatures. Kamel *et al*. (2018) reported significant decrease in the total protein content of *S. littoralis* by 63 % (62.3 at 25 °C and 22.67 mg g^{-1} body weight at 35 °C). Ismail (2021) found that temperature can impose severe stress on *S. littoralis* as evident from decrease in their protein and carbohydrate level. Protein level in ambient temperature (25 °C) was 59.6 mg g^{-1} body weight which reduced by 64 % under elevated temperature (35 °C).

2.3.3.3 Bt endotoxin content in insects: Meissle and Romeis (2018) conducted bitrophic and tritrophic interaction experiments on Bt and non-Bt cotton plants with different chewing and sucking pests. They found that *H. virescens* was more susceptible to the plant produced Bt proteins than *S. littoralis*. While *H. virescens* caterpillars lost weight during the two days of feeding on Bt cotton, *S. littoralis* gained weight. Consequently, *H. virescens* contained lower concentrations of both Bt toxins than *S. littoralis*, likely because the caterpillars reduced feeding after being poisoned by the Bt proteins. *S. littoralis* contained relatively high concentrations of Bt protein, reaching almost half the levels of the plant tissue. They observed that concentrations of Cry2Ab were proportionally lower in herbivores and predators than concentrations of Cry1Ac when compared to the levels in leaves, indicating that Cry2Ab might be less stable than Cry1Ac. It was also expressed that Bt protein production in plants grown in the climate chamber might differ from those grown in the field, because in reality, arthropods are exposed to different growth stages over the season and some may prefer reproductive structures containing lesser Bt protein content over leaves.

Zhao *et al*. (2016) reported that Cry toxins were detectable only in larval stage of *H. armigera* which might get expelled from body before pupation. This also suggests that natural enemies that target *H. armigera* eggs, pupae and adults might not get affected by endotoxins. ELISA results showed only trace amounts of the Cry1Ac toxin in the midgut, peritrophic membrane and its contents, while no Cry1Ac was detected in the hemolymph, fat body or integument. Cry1Ac could be detected in midgut and peritrophic membrane, when diet had 5 μg $g^{-1}$of toxins. They were detectable in the haemolymph, when the diet had 10 μg g^{-1} and to be seen in the fatbody, the semi-synthetic diet should have had 30 μg g^{-1} of toxins. Irrespective of the tissue, Cry toxins degraded rapidly in fat bodies, haemolymph and peritrophic membrane of the larvae within 4 to 48 hours.

Chapter – III

Material and Methods

Chapter III

MATERIAL AND METHODS

The present investigation titled '**Interactive Effects of Elevated Carbon Dioxide and Temperature on *Helicoverpa armigera* Hub. and *Spodoptera litura* Fab. on Bt Cotton**' of Department of entomology, Agricultural college, Bapatla, Acharya N. G. Ranga Agricultural university (ANGRAU) was conducted during 2018-2020 at ICAR- Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad, Telangana. The research was carried out using facilities like Carbon dioxide and Temperature Gradient Chambers (CTGC) and insect growth chambers with carbon dioxide ($CO_{2)}$ and temperature regulation system. Research materials utilized in conducting various experiments and methods employed during the course of inquiry are given in this chapter.

3.1 CARBON DIOXIDE AND TEMPERATURE GRADIENT AND CARBON DIOXIDE GROWTH CHAMBERS

CTG chambers were realistically designed to study the cumulative impact of elevated CO_2 (eCO_2) and elevated temperature (*e*Temp), the prime drivers of climate change (Plate: 3.1). The chambers simulate eCO_2 and *e*Temp conditions, which may certainly impact the growth of crop plants and insect pests (Srinivasa Rao *et al*., 2018). The facility has eight chambers of 30 meters length, 6 meters width and 4 meters height at centre.

Plate 3.1. Carbon dioxide and Temperature Gradient Chamber (CTGC)

Among the eight CTG chambers,

- Two chambers maintain ambient concentrations of CO_2 (aCO_2) *i.e.*, 380 ± 25 ppm at ambient temperature (*a*Temp) 28 °C.
- Two chambers maintain only *e*Temp *i.e.*, 29, 31, 33 and 35 ± 1 °C at aCO_2 (380 ± 25 ppm)
- Two chambers maintain eCO_2 (550 ± 25 ppm) at *e*Temp (29, 31, 33 and 35 °C).
- Two chambers maintain only eCO_2 (550 ± 25 ppm) at *a*Temp 28 °C.

All eight chambers were made up of high quality, light weight, rigid, polycarbonate sheet of lexan with excellent impact and weather resistance, and have more than 90 per cent light diffusion and above 85 per cent PAR (Photosynthetically Active Radiation) transmission. CO_2 concentrations were maintained in the chambers using gas regulators, pressure pipelines and solenoid valves. The CO_2 levels were monitored through PC linked PLC (Program Logic Control) and SCADA (Supervisory Control and Data Acquisition).

The CO_2 growth chambers (M/s Percival I-36LL) were used for conducting growth and development experiments of insects on cotton foliage under eCO_2 conditions. (Srinivasa Rao *et al*., 2014a). Light is provided inside the chamber through two fluorescent lamps, horizontally mounted above each shelf and intensity programmed to 65 μmoles m^{-2} s^{-1}, light irradiance measured @ 6" from lamps on 2 on/ off light events (Plate: 3.2). Air circulation was maintained inside the chamber from a specifically designed air diffuser. The CO_2 was supplied into the chamber in the range of 0 - 3000 ppm, and provided with appropriate regulatory system attached to CO_2 cylinder. The programming and control of the lighting, CO_2 and temperature was automatically monitored and controlled using Intellus Ultra Controller.

Plate 3.2. Carbon dioxide and temperature growth chambers

3.2 MAINTENANCE OF COTTON CROP

Popular Bt and non-Bt cotton variety (RCH-659) was sown in CTG chambers at set conditions during 2018-2020. They were maintained at ambient (380 ± 25 ppm CO_2 with 28 °C), *e*Temp (29, 31, 33 and 35 ± 1 °C with 380 ppm CO_2), *e*CO_2 (550 ± 25 ppm CO_2 with 28 °C) and *e*CO_2 + *e*Temp (550 ppm CO_2 with 29, 31, 33 and 35 ± 1 °C). Each one forms a treatment and in total ten treatments were maintained. The cotton crop was maintained (Plate: 3.3) under insecticide free conditions.

Plate 3.3. Cotton plants inside CTGC

3.3 LABORATORY REARING OF TEST INSECTS

3.3.1 Maintenance of Test Insect – *Helicoverpa armigera* Hub.

Larval population of *H. armigera* (Noctuidae, Lepidoptera) was collected from farmer's fields and culture is maintained in the Entomology laboratory of CRIDA under normal conditions (27 ± 1 °C temperature and 60 % RH). The cages/ iron racks/ plastic tubs/ glass jars used for mass rearing were cleaned with Labklin reagent, sun dried and sterilized with four per cent formaldehyde before use. The larvae of *H. armigera* were reared on standard semi-synthetic diet (Armes *et al*., 1992) individually in 6-cell well plates (3.5 cm diameter × 2.0 cm depth) to obtain bulk larval population for experiments. The larvae were allowed to pupate in the individual cells of rearing trays (Plate: 3.4) and were collected after 1-2 days of pupation and transferred to a plastic jar with moist cotton and filter paper at the

bottom. In each jar about five pupae were kept and covered with muslin cloth. Immediately after emergence, four to five pairs of virgin adult moths were transferred to oviposition cages (Plate: 3.5) where butter papers were provided for egg laying. Cotton swab soaked in 10 per cent honey solution was provided inside the cage to feed the adult moths.

Plate 3.4. Mass rearing of test insects in the laboratory conditions

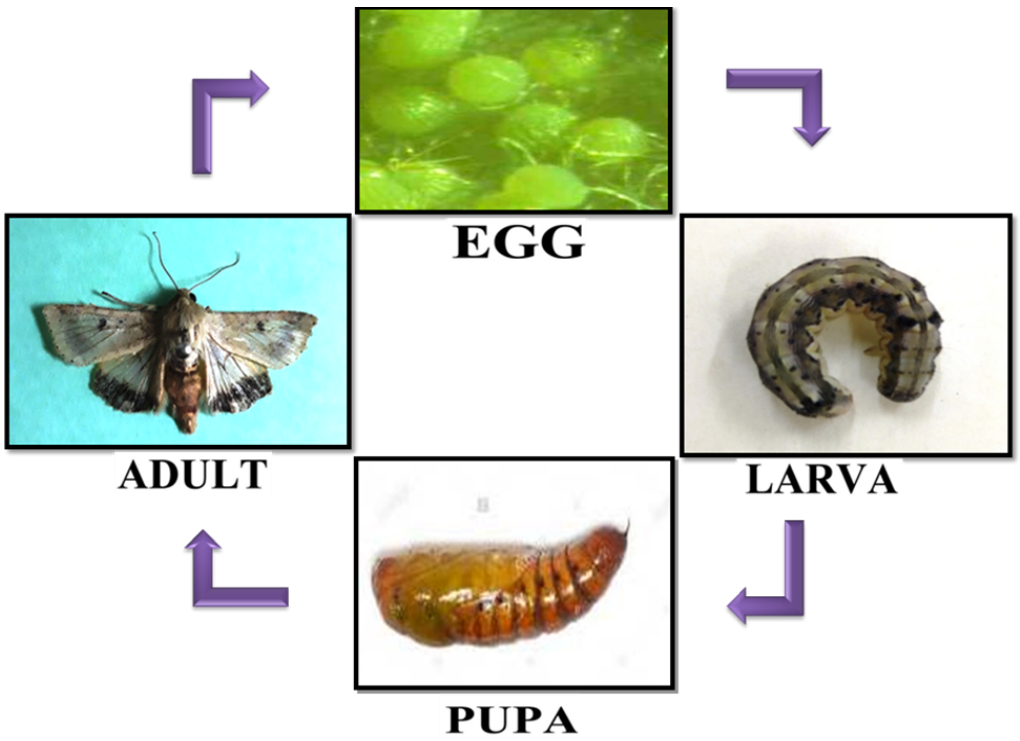


Plate 3.5. Life cycle of *Helicoverpa armigera*

After oviposition, butter papers with eggs were collected periodically and placed on a moist filter paper in petri plates. After hatching, the neonate larvae were transferred to six cell-well plates with semi-synthetic diet and were reared individually till pupation.

Plate 3.6. Ingredients of semi-synthetic diet for *H. armigera*

3.3.1.1 Preparation of semi-synthetic diet (Plate: 3.6)

Part – A

1. Bengal gram flour : 55.00 g
2. Yeast extract : 10.00 g
3. Casein : 5.00 g
4. L- Ascorbic acid : 1.30 g
5. Methyl parahydroxy benzoate: 1.00 g
6. Sorbic acid : 0.25 g
7. Cholesterol : 0.05 g
8. Distilled water : 100 ml

After adding, the contents were properly mixed by using mixer grinder

Part – B

1. Agar agar type- I : 6.50 g
2. Distilled water : 260 ml

The contents were boiled for 2 minutes

Part – C

1. Vitamin – E : 1 capsule
2. Multivitamin capsules : 2 capsule
3. Streptomycin : 0.1 mg
4. Formaldehyde : 0.5 ml

First, Part-C was added to Part-A, later Part –B was added and mixed thoroughly and then poured into petri plates before the solidification of agar agar. Contents were allowed to cool and then placed in refrigerator.

3.3.2 Maintenance of Test Insect - *Spodoptera litura* Fab.

Egg masses and larvae of *Spodoptera litura* (Noctuidae, Lepidoptera) were collected from the crop fields of Hayathnagar Research Farm (HRF) of CRIDA. The initial population was maintained in the Entomology laboratory under ambient conditions (27 ± 1 ºC temperature and 60 % RH). The shelves, counters and workstations used for mass rearing of *S. litura* were cleaned with Labklin reagent and sterilized with four per cent formaldehyde before use. The collected egg masses were kept between two tender cotton leaves to prevent mortality of the young larvae on hatching due to starvation. A moist filter paper is kept beneath the leaves to maintain turgidity and freshness of the leaves. The freshly hatched neonate larvae (Plate: 3.7) were changed to new plastic tubs with fresh leaves and the ends of the petioles were tied with wet cotton swab to maintain the turgidity of the leaf. The open sides of the plastic tubs were closed with white muslin cloth. As the larvae grew, enough caution was taken in providing adequate space (only 10-15 larvae/ tub) to avoid overcrowding. They were allowed to pupate in the tub and after 2 days, five to six pupae were transferred to a plastic jar with moist cotton and filter paper at the bottom.

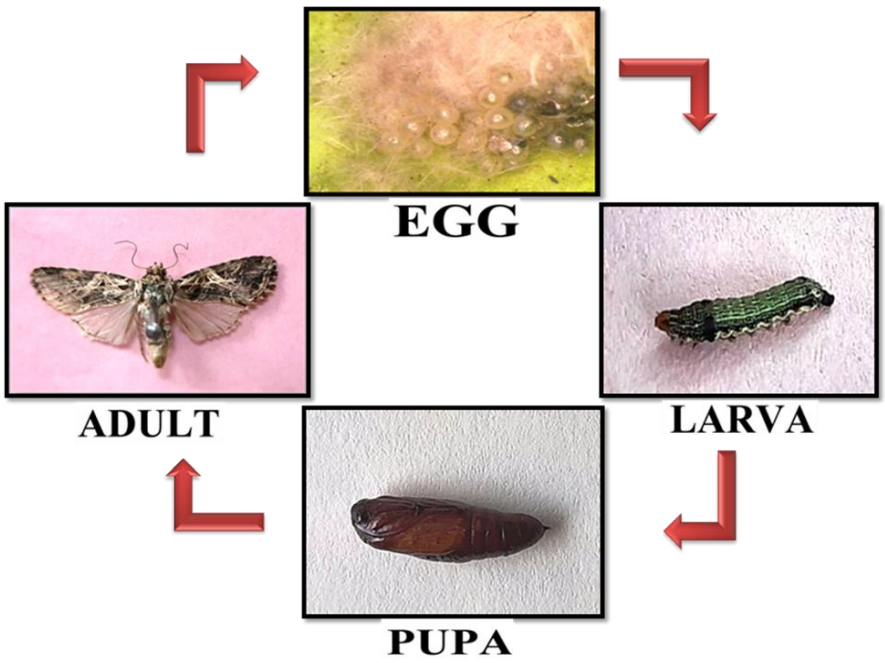


Plate 3.7. Life cycle of *Spodoptera litura*

Immediately after emergence, four to five pairs of adult moths were transferred to oviposition cages with butter papers for laying eggs. Cotton swabs soaked in 10 per cent honey solution were provided inside the cage to feed the adult moths. After oviposition, the eggs were collected periodically and placed on a moist filter paper in a petri plate. Overlapping generations were maintained for continuous supply of *H. armigera and S. litura* larvae.

3.4 STUDIES ON GROWTH AND DEVELOPMENT OF TEST INSECTS

3.4.1 Effect of *e*CO_2 and *e*Temp on Growth and Development of Test Insects

Upon hatching, the neonate larvae of *H. armigera* and *S. litura* were allowed to feed on tender cotton leaves (Bt and non-Bt) grown under *e*CO_2 and *e*Temp conditions *viz.*, 550 and 380 ± 25 ppm CO_2 and 28, 29, 31, 33 and 35 ± 1 °C in CTGCs up to three days. The light intensity of 550 µmol m^{-2} s^{-1} was maintained in the chambers during 14 hours photoperiod with 60 per cent relative humidity during day and 70 per cent at night. One larva was maintained in each petri dish individually (Plates: 3.8a, 3.8b), each individual larva forming one replication. Twenty five such replications were maintained at each set condition. A moist filter paper was kept at the bottom of the cell in rearing trays upon which larvae were released to maintain leaf turgidity On every alternative day the weights of larvae, their faecal matter, unfed leaves were recorded. And thereafter the faecal matter and unfed leaf were removed and replaced with new leaf after recording its weight.

Plate 3.8a: Cotton leaf collection

Plate 3.8b: Experimental set up

Plate 3.8. Cotton plants grown under controlled conditions

After cessation of feeding, pre-pupae were collected and transferred to glass jars. Total larval duration was calculated from the date of hatching to pupation. Pupal weight was recorded about 12 h after pupation. Pupal duration was calculated from the date of pupation to emergence of adults. To study the reproductive biology sexing was done at the pupal stage to differentiate male and female moths. The emerged pair of male and female moths was kept in a separate plastic container (22 x 25 x 30 cm dimensions) and fed with 10 per cent honey solution. The container was closed with cotton cloth to facilitate oviposition. Eggs laid on the cloth were collected everyday with the help of fine camel-hair brush and counted. All feeding trials were conducted as per procedure given by and observations like larval weight (mg), larval duration (days), weight of the leaf ingested (weight of fresh leaf –weight of left over leaf after ingestion expressed in mg), faecal matter weight (mg), pupal weight (mg), pupal duration (days) reproductive biology *viz.,* pre-oviposition (period from the emergence of the female moth to the laying of first egg), oviposition (period from laying of the first to the last egg) and post oviposition (period from laying of the last egg to the death of the moth) durations and fecundity (No. of eggs/ female) were recorded for both insects fed on Bt and Non-Bt cotton.

3.4.2 Determination of Feeding Indices of Test Insects

Energy and nutrients are quite essential to survive, grow and reproduce. The nutritional components (protein, carbohydrates, fats, vitamins and minerals) of ingested food may or may not be digested and absorbed properly. Several insect performance indices were determined by utilizing the data related to larval body weight, amount of food ingested and faecal matter excreted.

The effect of *e*CO_2 and *e*Temp on the feeding indices of *H. armigera* and *S. litura* were assessed at respective set conditions after feeding the larvae on non-Bt and Bt cotton leaves. The data on growth parameters *viz.,* larval weight, amount of food ingested and amount of faeces excreted were used to estimate insect performance or food conversion efficiency indices (Waldbauer, 1968) and the formulae for the estimation of the indices are mentioned here under:

3.4.2.1. Approximate digestibility (AD): It is the ratio of weight of leaf digested and weight of leaf ingested or the proportion of ingested food that is actually digested (expressed as %). From the nutrients absorbed, portions are expended in the processes of respiration and work. AD is an index that iterates the per cent of food effectively assimilated, assessed using formula,

$$\text{AD} = \frac{\text{Weight of food ingested} - \text{Weight of faeces}}{\text{Weight of food ingested}} \times 100$$

3.4.2.2. Relative consumption rate (RCR): It is the weight of leaf ingested per day as a fraction of larval body weight. It is expressed in mg g^{-1} d^{-1}.

$$\text{RCR} = \frac{\text{Weight of food ingested}}{\text{Average larval body weight per day}}$$

3.4.2.3. Efficiency of conversion of ingested food (ECI): It is nothing but the larval weight gain per unit weight of leaf ingested (expressed as %). The degree of food utilization depends on digestibility of food and the efficiency with which digested food is converted into biomass. ECI is an overall measure of an insect's ability to utilize the ingested food for growth and development.

$$\text{ECI} = \frac{\text{Weight gained by larvae during feeding period}}{\text{Weight of food ingested}} \times 100$$

3.4.2.4. Efficiency of conversion of digested food (ECD): It is the larval weight gain per unit weight of leaf digested (expressed as %). The proportion of digested food that is actually transformed into net insect biomass is denoted by ECD

$$\text{ECD} = \frac{\text{Weight gained by larvae during feeding period}}{\text{Weight of food ingested} - \text{Weight of faeces}} \times 100$$

3.4.2.5. Relative growth rate (RGR): It is defined as larval weight gained per day as a fraction of body weight. It is expressed in mg g^{-1} d^{-1}.

$$\text{RGR} = \frac{\text{Increase in larval body weight}}{\text{Average insect body weight per day}}$$

In brief, AD indicates how the digestibility of the food is, whereas, ECD and ECI indicate how efficient an herbivore is in converting that food into biomass. These indices provide a conceptual model for analysing the role of larval nutrition in the insect growth and development.

3.5 STUDIES ON BIOCHEMICAL CONSTITUENTS OF COTTON FOLIAGE AND TEST INSECTS

3.5.1 Effect of *e*CO_2 and *e*Temp. on Biochemical Constituents of Cotton Foliage

Tender leaves of peripheral branches of cotton grown under different test conditions *i.e.*, ambient (380 ± 25 ppm CO_2; 28 °C), *e*Temp (29, 31, 33 and 35 ± 1 °C), *e*CO_2 + *e*Temp (29, 31, 33 and 35 ± 1 °C with 550 ± 25 ppm CO_2) and *e*CO_2

(550 ± 25 ppm CO_2; 28 °C) were collected at 30, 45, 60, 75 and 90 DAS to estimate proteins, carbohydrates, carbon, nitrogen, C:N ratio, phenols and Bt endotoxin. The collected leaf samples were oven dried (YORCO hot air oven) at 80 °C for 72 hours and ground into fine powder with the help of blender. Estimation of nutritional constituents of cotton leaves was taken up as per the standard procedures described (Plates: 3.9 and 3.10).

3.5.1.1 Estimation of nitrogen: To determine the Nitrogen content in cotton foliage Kjeldahl method (Kjeldahl, 1883) was followed. The apparatus used in this experiment was K- ZELTECH (Model 8400) analyser. The organic nitrogen present in the sample gets converted into ammonium sulphate by H_2SO_4 during digestion. Boric acid collects the ammonia released during steam distillation. And this solution was titrated against standard hydrochloric acid.

Reagents required

1. Digestion catalyst mixture containing 100 parts of K_2SO_4 , 20 parts of $CuSO_4$
2. Hydrogen peroxide
3. NaOH solution – 40%
4. Boric acid – 4%
5. Concentrated sulphuric acid (Sp. Gr.1.84)
6. Bromocresol green
7. Methyl red indicator

Procedure: Five hundred milligram **(**0.5 g) of powdered plant sample was taken in Kjeldahl's digestion flask and 15 ml of conc. H_2SO_4 was added with a dispenser. The solution was digested in digestion chamber till colour changed to dark. To this, hydrogen peroxide is added till the colour changed to light or colourless. One gram catalyst was added to each sample in the digestion tube and then samples were digested at 200 °C.

Ten ml **(**10 ml) of the digested substance was transferred to micro-kjeldahl distillation apparatus. In a conical flask containing bromocresol green and methyl red indicator 10 ml of 4 per cent boric acid solution was added and the outlet of the condenser flask was dipped 10 ml of 40 per cent sodium peroxide was added to the distillation flask and 5 ml aliquot was distilled into the flask containing 10 ml of boric acid. After this boric acid was titrated against 0.1 N sulphuric acid until violet colour appears.

a. K- ZELTECH (Model 8400) Digestion unit

b. Distillation unit

c. Spectrophotometer (T60, LABINDIA)

d. CHNS analyzer (Elementar Analysensysteme GmbH)

e. Hot air oven (YORCO)

f. Centrifuge (REMI)

Plate 3.9. Instruments used for the estimation of biochemical constituents of cotton foliage and test insects

Plate 3.10. Estimation of biochemical constituents of cotton foliage

3.5.1.2 Estimation of carbon: The carbon content in the cotton foliage was estimated using Walkley and Black method (1934).

Reagents required

1. **Potassium dichromate ($K_2Cr_2O_7$) :** 49.07 g of $K_2Cr_2O_7$ was taken in a volumetric flask containing one litre of distilled water

2. **Ferrous Ammonium Sulphate (FAS):** 196 g of FAS was dissolved in distilled water and 20 ml of conc. H_2SO_4 was added and volume was made up to one litre in a volumetric flask.

3. 196 g of FAS was taken in another volumetric flask containing distilled water and 20 ml of H_2SO_4 was added and the volume was made upto one litre.

4. **Diphenyl amine indicator:** 0.5 g of Diphenyl amine indicator was dissolved in 20 ml of distilled water followed by the addition of 100 ml conc. H_2SO_4

5. Orthophosphoric acid 10 ml.

6. Pinch of Sodium fluoride

Procedure: Five hundred mg **(**0.5 g) of powdered leaf sample was taken in 500 ml conical flask and 10 ml potassium dichromate was added to it with a digital pipette. Then 20 ml of concentrated sulphuric acid was added and the contents were mixed gently for one minute. The solution was left undisturbed for 20 to 40 minutes. Later, 200 ml of distilled water and a pinch of sodium fluoride (NaF_4) were added followed by 10 ml of orthophosphoric acid. Finally 3-5 drops of diphenyl amine indicator was added and titrated against FAS solution till the colour changed to dark green.

3.5.1.3 Estimation of C: N ratio: Both the Carbon and nitrogen present in the leaf samples were determined (Jackson, 1973) by solid sample dry combustion method using Elementar Vario El Cube CHNS Analyzer (Elementar Analysensysteme GmbH, Germany). About 5 mg of finely grounded samples were weighed into tin weighing boats. And these boats were loaded into a sample carousel one at a time to transfer the samples into the combustion tube using a ball valve. These samples were combusted at a temperature of 950 °C and the gases released were passed through a reduction tube heated to 600 °C, which resulted in conversion of C to CO_2 and N to N_2. These gases, carried by helium carrier gas, were separated chromatographically and detected by a thermal conductivity detector (TCD).

3.5.1.4 Estimation of protein: The protein content was estimated by Lowry's method (Lowry *et al.,* 1951). Blue colour develops by the reduction of phosphotungistic components in the Folin- Ciocalteau reagent by the aminoacids tyrosine and tryptophan present in the protein.

Reagents required

1. Reagent A: 2 per cent sodium carbonate (Na_2CO_3) in 0.1 N sodium hydroxide
2. Reagent B: 0.5 per cent copper sulphate in 1 per cent potassium sodium tartarate
3. Reagent C: Alkaline copper solution. (Mix 50 ml of reagent A and 1ml of reagent B)
4. Reagent D: 1 part of Folin-Phenol (2N): 1 part water

Working standards: For estimation of protein, Bovine Serum Albumin (BSA) was used as a standard. For preparation of stock solution 50 mg of BSA was taken and dissolved in 50 ml of distilled water. Stock solution is diluted upto 5 times with distilled water in standard flask for preparing working standards. One ml of this final solution contains 200 µg proteins, and the quantity of protein in the sample is calculated accordingly.

Procedure: Extraction buffer was used to extract proteins from 0.5 g of leaf sample. Exactly 0.1 ml and 0.2 ml of extracts were taken into two test tubes and volume was made up to one ml in all the test tubes. A tube with water served as blank. After this 5 ml of reagent C (alkaline copper solution) was added in all test tubes and left undisturbed for 10 minutes. Then 0.5 ml of folin- ciocalteau reagent was added and kept in a dark place for 30 minutes. Later the intensity of the blue color developed was read at 660 nm in UV–Spectrophotometer (T60, LABINDIA). Proteins were calculated using Bovine Serum Albumin (BSA) standards (20-100 µg).

3.5.1.5 Estimation of carbohydrates: Carbohydrates are the important components of storage and structural materials in the plants (Yemm and Willis, 1954). They exist as free sugars and polysaccharides and estimated by anthrone method. The basic units of carbohydrates are the monosaccharide's which cannot be split by hydrolyzing into more sampler sugars. The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant

monosaccharides. Carbohydrates are hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 620 nm.

Reagents required

1. 2.5 N HCl
2. Anthrone reagent: 100 mg of anthrone reagent was dissolved in 100 ml of ice-cold 95 % H_2SO_4 (Prepared fresh before use).
3. Standard Glucose: Stock-Dissolved 100 mg in 100ml distilled water. Working standard-10 ml of stock diluted to 100 ml with distilled water

Procedure: Weighed 100 ml of the leaf sample into the boiling tube and boiled for three hours with 5 ml of 2.5 N HCl and cooled at room temperature. Neutralized with solid sodium carbonate until the effervescence ceases, volume was madeup to 100 ml and centrifuged. Aliquots 0.1 ml and 0.2 ml were taken and madeup to volume to one ml in the test tubes, to this 4 ml of anthrone reagent was added, allowed for heating for eight minutes in a boiling water bath. The change in colour from green to dark green was noted. After cooling the absorbance were read at 620 nm.

3.5.1.6 Estimation of polyphenols: Phenols were estimated by Folin- Ciocalteau reagent (FCR) given after Mallick and Singh (1980). Phenols are aromatic compounds with hydroxyl groups. Phenols react with phosphomolybdic acid present in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex.

Reagents required

1. Ethanol (80 per cent)
2. Folin- Ciocalteau reagent
3. 10 per cent Sodium carbonate
4. Standard (100 mg Catechol in 100 ml Water)

Working standard: Catechol and water are taken in 1:1 ratio. Series of volumes 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 ml were pipetted out into test tubes. 1ml of distilled water was added into each sample and diluted upto 10 times. A standard curve was drawn using the mentioned concentrations of catechol. From the standard curve, concentration of polyphenols in the leaf samples were derived and expressed as mg g^{-1} plant sample. The absorbance was measured at 650 nm against a reagent blank.

Procedure: Five hundred mg (0.5 g) of leaf sample was weighed and extracted in 80 per cent ethanol. The mixture was centrifuged for 20 min at 10,000 rpm and supernatant was saved. The residue is re-extracted with five times the volume of 80 per cent ethanol, and again centrifuged and supernatants were pooled. The left over residue was dissolved in 5 ml distilled water. Aliquots of different volumes (0.2 to 2 ml) were pipetted out into test tubes and volumes were made upto 3 ml each with water. Later 0.5 ml of folin-ciocalteau reagent was added. After 3 minutes, 2 ml of 20 per cent sodium carbonate solution was added and mixed thoroughly. The tubes were placed in boiling water for exactly one minute, and later tubes were cooled. Absorbance was measured at 650 nm against blank in spectrophotometer.

3.5.1.7 Estimation of Bt endotoxin: Enzyme linked Immunosorbent Assay (ELISA) was used for the estimation of Bt endotoxin (Chen *et al*., 2005a). ELISA plate is coated with capture antibody. To this tissue sample was added which bound to the antigen. Secondary antibody was added followed by enzyme linked antibody. This labelled antibody was used to detect amount of antigen present.

Reagents required

1. Cry1Ac/ Cry2Ab antibody coated ELISA plate.
2. Secondary antibody for Cry1Ac/ Cry 2Ab.
3. Tertiary antibody
4. Protein stocks
5. Quan T Extraction buffer
6. Powder A and B
7. Ovalbumin
8. 10 X buffer A
9. 5 X Substrate buffer
10. pNPP (p-Nitrophenyl Phosphate)

Buffer stocks preparation

1. 1 X Extraction Buffer (100 ml): 0.2 g powder A and 12 g powder B were added to the extraction buffer provided with the kit, freshly at the time of the sample extraction.

2. 1 X Wash Buffer: 100 ml of 10 X Buffer A was taken, diluted to 1 L by deionized water.

3. 1 X Diluent Buffer: 100 ml of 10 X buffer A was taken, diluted to 1L by adding deionized watter. To this 0.5 % Ovalbumin was added to 1X diluents buffer.

4. 1 X Substrate Buffer: 20 ml of deionized water was added to 5 ml of 5 X Substrate buffer provided

5. pNPP Substrate: 25 mg of pNPP was added to 25 ml of 1 X substrate buffer.

Working standards: For preparation of standards, 16 µg ml^{-1} of Cry1Ac/ Cry2Ab stock solution was taken and diluent buffer was added in the ratio of 1:100. And to this 100 µl sample buffer was added and the corresponding antigen @ 80 ng ml^{-1} was also added to make First Standard. For making Second Standard, 500 µl of First Standard was taken and to that 100 µl sample buffer and the corresponding antigen @ 40 ng ml^{-1} were added. For Third Standard, 500 µl of standard two was taken and to that 100 µl of sample buffer and the corresponding antigen @ 20 ng ml^{-1} were added. For Fourth Standard four, 500 µl of Third Standard three was taken and to that 100 µl sample buffer and antigen @ 10 ng ml^{-1} were added. For Fifth Standard five, 500 µl of Fourth standard was taken and to that 100 µl sample buffer and antigen @ 5 ng ml^{-1} were added. For Sixth Standard, 500 µl of Fifth standard was taken to which 100 µl of sample buffer and the corresponding antigen @ 2.5 ng ml^{-1} were added.

Procedure: Five mg (5mg) of leaf sample was taken in a 1.5 ml microfuge tube and 500 µl ice cold extraction buffer was added. Tissue was macerated at 3000 rpm for 30 seconds. After that sample was chilled for 10 minutes and again macerated for 30 seconds. It was spinned at 8000 rpm for 15 minutes and supernatant was pipetted out and diluted with 1X diluent by 80 times. ELISA plates were loaded with 50 µl sample followed by 50 µl secondary antibodies. They were incubated for 1.5 hr at 37 °C in darkness. Samples were discarded and plates were washed with wash buffer twice with an interval of five minutes. 250 µl of mixture of diluents AP conjugate per

well and diluents buffer @ 1:1000 ratio were added. It was incubated again for 45 minutes at 37 °C in darkness. The plate was washed twice with wash buffer with an interval of five minutes. One mg ml^{-1} of pNPP solution was added @ 250 µl per well and incubated for 30 minutes. The absorbance was read at 405 nm in ELISA reader.

3.5.2 Effect of *e*CO_2 and *e*Temp on Biochemical Constituents of Test Insects

3.5.2.1 Estimation of carbohydrates : The carbohydrate content in test insects was determined by Anthrone reagent method (Van Handel, 1985). Monosaccharides are the basic components of carbohydrates and they cannot be split into sugars by hydrolysis. Hence, to quantify carbohydrate content, the polysaccharides were hydrolysed into simple sugars by acid hydrolysis. Concentrated sulphuric acid was used to dehydrate carbohydrates to form 'furfural' which condenses with anthrone into a green colour complex. This green colour was measured at 620 nm using spectrophotometer (Plate: 3.11).

Reagents required

1. Hydrochloric acid (2.5 N) and sodium carbonate.

2. Anthrone reagent: 200 mg of anthrone was dissolved in 100 ml of ice cold 95 % H_2SO_4 (This reagent was made afresh every time).

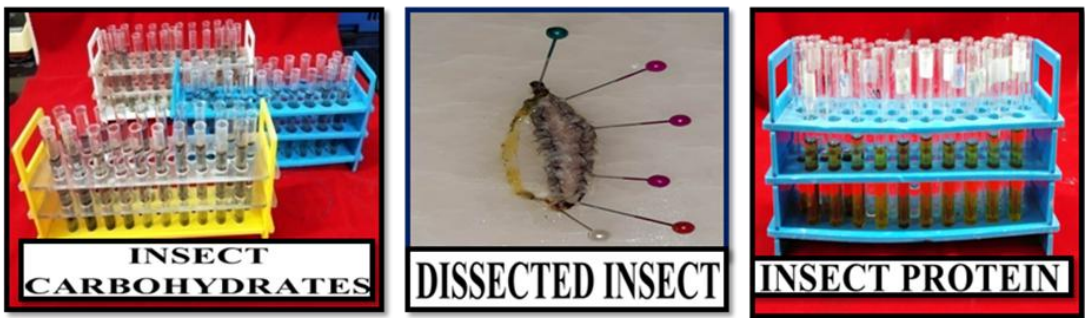


Plate 3.11: Estimation of biochemical constituents of insects

Working standards: Glucose was used as a standard for carbohydrate estimation. 100 mg of glucose was added in 100 ml of distilled water to obtain stock solution of 1000 µg of glucose/ ml. One ml of stock solution was taken and made upto 100 ml to get 10 µg/ ml. Thus different strengths of glucose solutions *viz.*, 100, 200, 300, 400 and 500 µg ml^{-1} were prepared. Glucose of different strengths was added individually to 5ml of anthrone taken in separate test tubes.

Procedure: One hundred mg **(**100 mg**)** of plant sample was taken into boiling tube and kept in boiling water bath for 3 hours for hydrolyzation with 5ml of 2.5 N HCL and cooled to room temperature. Solid sodium carbonate was added to neutralize the contents in the tube until the effervescence appears and then volume was made up to 100 ml. The mixture was subjected to centrifugation for 15 min at 5000 rpm and the supernatant was collected. Then 0.1 and 0.2 ml aliquots were extracted for analysis and the volume was made up to 1 ml with distilled water. After this 4 ml of anthrone reagent was added and the test tubes were kept in water bath for 8 min. The test tube were removed from bath and allowed to cool up to room temperature and the colour intensity was measured at 620 nm. The carbohydrate content was expressed in mg per gram fresh weight of larvae.

3.5.2.2 Estimation of protein: The total protein content in the test insects was determined by Bradford method (Bradford, 1976). In this estimation, Bovine Serum Albumin (BSA) was taken as standard. One gram of test larval sample was homogenized in 10 ml of 0.1 M sodium acetate buffer (pH 4.7) and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was saved for estimation of soluble protein. The reaction mixture consisted of 0.5 ml enzyme extract, 0.5 ml distilled water and 5 ml of diluted (5 times) dye solution. The absorbance was read at 595 nm in a spectrophotometer against reagent blank. The protein content was expressed as µg albumin equivalent of soluble protein per gram of fresh weight of larvae.

3.5.2.3 Estimation of Bt endotoxin in the larval midgut: Enzyme-linked Immunosorbent Assay (ELISA) was used for the estimation of Bt endotoxin. ELISA plate was coated with capture antibody. To this insect midgut tissue sample was added to bind to the antigen. Secondary antibody is added followed by enzyme linked antibody. This labelled antibody was used to detect amount of antigen present. The reagents required, preparation of buffer stocks, working standards and procedure of estimation of Bt endotoxin was similar as described earlier for plant sample.

3.5 STATISTICAL ANALYSIS

The data on growth parameters of both the test insects *H. armigera* and *S. litura* (*viz.,* weights and durations of larvae and pupae and reproductive biology) and insect feeding indices (AD, RCR, ECI, ECD and RGR) after exposure to different set conditions were analyzed using ANOVA in two Factorial CRD.

The biochemical constituents of cotton foliage (Bt and non-Bt) and that of test insect larvae under different treatment conditions were analyzed using two Factorial CRD, where levels of CO_2 and temperature were taken as two factors.

Chapter – IV

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

The present investigations on '**Interactive Effects of Elevated Carbon Dioxide and Temperature on *Helicoverpa armigera* Hub. and *Spodoptera litura* Fab. on Bt cotton**' of Department of Entomology, Agricultural College, Bapatla, Acharya N. G. Ranga Agricultural University (ANGRAU) were conducted at ICAR-Central Research Institute for Dryland Agriculture (ICAR-CRIDA), Hyderabad, Telangana during 2018-20. The results obtained from the investigations were presented and discussed with appropriate research literature hereunder

4.1 STUDIES ON THE EFFECTS OF *e*CO_2 AND *e*Temp ON GROWTH AND DEVELOPMENT OF HELICOVERPA ARMIGERA AND SPODOPTERA LITURA

Experiments were conducted to determine the effects of different combinations of elevated carbon dioxide (*e*CO_2) and elevated temperature (*e*Temp) *viz.*, two levels of CO_2 (380 or 550 $\pm$ 25 ppm) and five levels of temperature (28, 29, 31, 33 and 35 ± 1 °C) conditions on different parameters of growth and development of *Helicoverpa armigera* and *Spodoptera litura*. The results pertaining to these experiments are discussed in the light of available literature.

4.1.1 Food Ingestion

The perusal of results indicates significant variation in the amount of leaf ingested by the test insects (Tables 4.1- 4.4 and Fig. 4.1 - 4.2) throughout their larval period, across *e*CO_2 and *e*Temp conditions. The food ingested under *e*CO_2 was higher than that of *a*CO_2 at all temperatures. With increase in test temperatures (upto 35 °C), under *a*CO_2 (380 ± 25 ppm) and *e*CO_2 (550 ± 25 ppm) conditions, food ingested by larvae of test insects gradually decreased. Comparative performance of both the test insects was presented in Fig. 4.3.

4.1.1.1 Effect on food ingestion of *H. armigera* in non-Bt cotton

The data pertaining to food ingestion of *H. armigera* in non-Bt cotton for the first, second and third generation was presented in Table 4.1 and Fig. 4.1.

First generation: At *a*CO_2, food ingestion in the entire larval period ranged from 3282.40 to 2123.88 mg at 28 and 35 °C, respectively. Food ingestion increased significantly under *e*CO_2. At *e*CO_2, food ingestion ranged between 3851.92 and 2624.92 mg at temperatures 28 and 35 °C. It clearly indicates that with increase in temperature food ingestion decreased significantly under both *a*CO_2 and *e*CO_2. The interaction effect of *e*CO_2 and *e*Temp also showed significant influence on food ingestion.

Second generation: In non-Bt cotton, food ingestion varied significantly from 3441.60 to 2318.60 mg with highest food ingestion at 28 °C and lowest at 35 °C. At *e*CO_2, food ingestion ranged between 4084.90 to 2732.76 mg apparently decreasing with increase in temperature from 28 to 35 ºC. The results show that with increase in temperature food ingestion decreased significantly both in *a*CO_2 and *e*CO_2 conditions. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on food ingestion.

Third generation: At *a*CO_2, food ingestion ranged between 3556.84 and 2392.44 mg with highest food ingestion at 28 °C and lowest at 35 °C. Among *a*CO_2 and *e*CO_2, food ingestion was significantly lower in *a*CO_2. At *e*CO_2, decrease in food ingestion was recorded with increase in temperature from 28 to 35 °C with highest 4160.72 and lowest 2817.16 mg , respectively. The individual and interaction effect of *e*CO_2 and *e*Temp showed significant influence on food ingestion.

Mean of generations: The interactive effect of *e*CO_2 and *e*Temp in non-Bt cotton, also showed significant impact on mean food ingestion by *H. armigera*. The mean of three generations also indicated that with increase in temperature (28, 29, 31, 33 and 35 ºC) the food ingestion by larvae has decreased significantly (3426.95, 3174.29, 2943.20, 2613.31 and 2278.31 mg, respectively) corresponding to a decrease of 7.37, 14.12, 23.74 and 33.52 %. Similarly, under *e*CO_2 also the food ingestion decreased significantly (4032.51, 3643.55, 3407.35, 3109.35 and 2724.95 mg) with increase in temperatures which has corresponded to a decrease of 9.65, 15.50, 22.89 and 32.43 % at 28, 29, 31, 33 and 35 ºC, respectively. However, the decrease in food ingestion with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the food ingestion under *e*CO_2 was higher (17.67, 14.78, 15.77, 18.98 and 19.60 %, at the corresponding temperatures) compared to that of *a*CO_2 in non-Bt cotton.

Table 4.1. Effect of *e*CO_2 and *e*Temp on food ingestion of *H. armigera* in non-Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	3282.40	3851.92	**3567.16**	3010.14	3708.37	**3359.25**	3556.84	4160.72	**3858.78**	3426.95	4032.51	**3729.73**
29 ± 1°C	3089.48	3510.56	**3300.02**	2713.50	3519.09	**3116.29**	3251.36	3763.36	**3507.36**	3174.29	3643.55	**3408.92**
31 ± 1°C	2736.68	3275.00	**3005.84**	2398.71	3209.05	**2803.88**	3071.28	3502.36	**3286.82**	2943.20	3407.35	**3175.27**
33 ± 1°C	2441.32	2932.00	**2686.66**	2111.68	3214.94	**2663.31**	2748.48	3246.60	**2997.54**	2613.31	3109.35	**2861.33**
35 ± 1°C	2123.88	2624.92	**2374.40**	1828.35	3049.01	**2438.68**	2392.44	2817.16	**2604.80**	2278.31	2724.95	**2501.63**
Mean	**2734.75**	**3238.88**		**2412.47**	**3340.09**		**3004.08**	**3498.04**		**2887.21**	**3393.53**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	2029.885*	7.91	22.04	1450.138*	9.12	25.39	1811.591*	8.21	22.86	6048.62*	4.51	12.57
Temperature (°C)	1438.327*	12.51	34.85	1090.726*	14.41	40.15	1363.489*	12.98	36.15	4451.17*	7.13	19.87
Interaction (CO_2 + Temp(°C))	5.005*	17.69	49.29	10.244*	20.38	56.78	7.860*	18.35	51.12	19.85*	10.09	28.11
CV	2.96 **%**			3.22 %			2.82 %			1.61 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

The present findings suggest that mean food ingestion by *H. armigera* increased with increase in CO_2 (by 18 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 20 %. Food ingestion capacity seems toincrease with every generation and was highest in the third generation (3283, 3442 and 3557 mg; 3852, 4085 and 4161 mg; and 2625, 2733 and 2817 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively) implying a greater chance for adaptation with advancement of generations. The present findings are in line with Lincoln *et al*. (1984) who reported increase in foliage ingestion at *e*CO_2 by *Pseudoplusia includens* on soybean. Similarly Kinney *et al.* (1997) reported increased consumption and decreased conversion efficiencies in gypsy moth, *Limantria dispar* Linnaeus under *e*CO_2. Coviella and Trumble (1999) revealed that *e*CO_2 causes compensatory increase in food consumption and reduced digestive efficiency due to lower nitrogen availability in the crop foliage. Similar (Chen *et al.* (2004) reported significant difference in wheat food ingestion by *H. armigera* under *a*CO_2 (559.8 mg) and *e*CO_2 (817.5 mg).

4.1.1.2 Effect on food ingestion of *H. armigera* in Bt cotton

The data regarding food ingestion of *H. armigera* in Bt cotton for first, second and third generation was presented in Table 4.2 and Fig. 4.1.

First generation: The food ingestion was significantly influenced under both *e*CO_2 and *e*Temp. At *a*CO_2, food ingestion in the entire larval period ranged between 3010.14 to 1828.35 mg at 28 and 35 °C. At *e*CO_2, decrease in food ingestion was recorded from 3708.37 to 3049.01 mg with increase in temperature from 28 to 35 °C. The combined effect of *e*CO_2 and *e*Temp showed significant impact on food ingestion.

Second generation: At *a*CO_2, food ingestion ranged from 3215.12 to 1997.27 mg with highest food ingestion at 28 °C and lowest at 35 °C. At *e*CO_2, decrease in food ingestion was recorded with increase in temperature with highest at 28 °C (3887.11 mg) and lowest at 35 °C (3301.26 mg). The individual and interaction effect of *e*CO_2 and *e*Temp showed significant influence on food ingestion.

Table 4.2. Effect of *e*CO_2 and *e*Temp on food ingestion of *H. armigera* in Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	3010.14	3708.37	**3359.25**	3215.12	3887.11	**3551.11**	3305.62	4027.55	**3666.59**	3176.96	3874.34	**3525.66**
29 ± 1°C	2713.50	3519.09	**3116.29**	2891.81	3711.41	**3301.61**	3008.00	3815.46	**3411.73**	2871.10	3681.99	**3276.55**
31 ± 1°C	2398.71	3209.05	**2803.88**	2587.69	3506.20	**3046.94**	2740.64	3598.13	**3169.38**	2575.68	3437.79	**3006.74**
33 ± 1°C	2111.68	3214.94	**2663.31**	2309.47	3379.83	**2844.65**	2411.16	3453.64	**2932.40**	2277.44	3349.47	**2813.46**
35 ± 1°C	1828.35	3049.01	**2438.68**	1997.27	3301.26	**2649.26**	2078.63	3418.21	**2748.42**	1968.08	3256.16	**2612.13**
Mean	**2412.47**	**3340.09**		**2600.27**	**3557.16**		**2708.81**	**3662.60**		**2573.85**	**3519.95**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	5681.741*	8.70	24.24	6567.448*	8.35	23.26	5637.026*	8.98	25.03	1139.19*	4.83	13.47
Temperature (°C)	703.832*	13.76	38.33	736.006*	13.20	36.78	666.715*	14.20	39.57	2254.02*	7.64	21.30
Interaction (CO_2 + Temp(°C))	65.367*	19.46	54.21	84.222*	18.67	52.01	74.713*	20.09	55.96	235.21*	10.81	30.12
CV	3.38 **%**			3.03 %			3.15 %			1.77 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

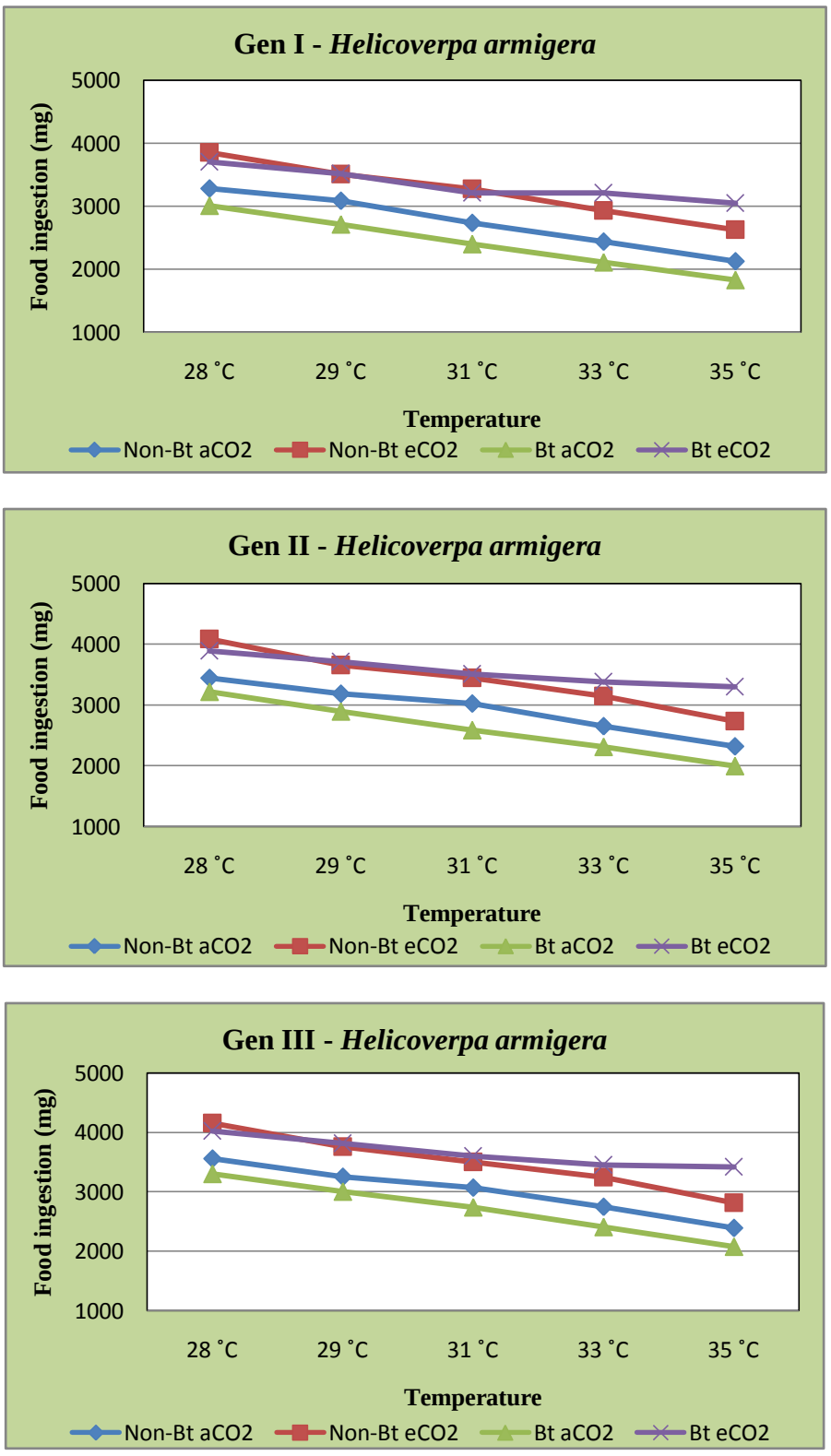


Fig. 4.1. Effect of eCO_2 and *e*Temp on food ingestion of *H. armigera* larvae on non-Bt and Bt cotton in first, second and third generation

Third generation: In Bt cotton, at *a*CO_2, decrease in food ingestion was recorded with increase in temperature with highest at 28 °C (3305.62 mg) and lowest at 35 °C (2078.63 mg). At *e*CO_2, food ingestion ranged from 4027.55 to 3418.21 mg with highest at 28 ºC and lowest at 35 ºC. The individual and interaction effect of *e*CO_2 and *e*Temp showed significant influence on food ingestion.

Mean of generations: The mean of three generations in Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28-35 °C) the food ingestion by larvae has decreased significantly (3176.96, 2871.10, 2575.68, 2277.44 and 1968.08 mg, respectively) corresponding to 9.63, 18.93, 28.31 and 38.05 %. Similarly, under *e*CO_2 also the food ingestion decreased significantly (3874.34, 3681.99, 3437.79, 3349.47 and 3256.16 mg) with increase in temperature (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 4.96, 11.27, 13.55 and 15.96 %. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on food ingestion on Bt cotton. And among *a*CO_2 and *e*CO_2, food ingestion was significantly lower in *a*CO_2. However, the decrease in food ingestion with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the food ingestion under *e*CO_2 was higher (21.95, 28.24, 33.47, 47.07 and 65.45 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These findings suggest that mean Bt cotton food ingestion by *H. armigera* increased with increase in CO_2 (by 22 %) and decreased with temperature and ultimately increased by a little extent with *e*CO_2 + *e*Temp by 2.4 %. This is in close proximity with Stiling and Cornelissen (2007) who estimated that elevated CO_2 can cause 17.0 % higher consumption in several herbivores. Likewise Chen *et al*. (2007) observed that *H. armigera* reared on Bt cotton exhibited higher consumption in elevated CO_2 (750 µl L^{-1}) over ambient CO_2. Further, Bt food ingestion capacity in the study seems to increase with every generation and was highest in the third generation (3010, 3215 and 3306 mg; 3708, 3887 and 4028 mg; and 3049, 3301 and 3418 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively) implying a greater chance for adaptation with advancement of generations.

4.1.1.3 Effect on food ingestion of *S. litura* in non-Bt cotton

The food ingestion data of *S. litura* in non-Bt cotton for all three generations was presented in Table 4.3 and Fig. 4.2.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has significant effect on food ingestion. At *a*CO_2, food ingestion in the entire larval period ranged from 3604.76 to 2382.56 mg with increase in temperatures from 28 to 35 °C. At *e*CO_2, decrease in food ingestion was recorded from 4097.76 to 2931.52 mg with highest at 28 °C and lowest at 35 °C. But the interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on food ingestion.

Second generation: At *a*CO_2, food ingestion ranged from 3806.72 to 2599.16 mg with highest at 28 °C and lowest at 35 °C. At *e*CO_2, decrease in food ingestion was recorded from 4286.56 mg (28 °C) to 3085.04 mg (35 °C). Under both *a*CO_2 and *e*CO_2, with increase in temperature there is a corresponding decrease in food ingestion by *S. litura*. The *e*CO_2 and *e*Temp showed significant effect individually but interaction effect showed non-significant influence on food ingestion.

Third generation: At *a*CO_2, decrease in food ingestion was recorded *viz.*, 3914.76, 3603.80, 3342.60, 2992.64 and 2653.36 mg, respectively at temperatures 28, 29, 31, 33 and 35 °C. At *e*CO_2, food ingestion ranged between 4416.68 mg to 3153.36 mg, respectively at temperatures 28 and 35 ºC. This clearly indicates the decrease in food ingestion with increase in temperature. The *e*CO_2 and *e*Temp showed significant effect individually but interaction effect showed non-significant influence on food ingestion.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the food ingestion by larvae significantly decreased (3775.41, 3506.12, 3238.63, 2878.55 and 2545.03 mg, respectively) with increase in temperature (28, 29, 31, 33 and 35 ºC) corresponding to a decrease of 7.13, 14.22, 23.76 and 32.59 %. Similarly, under *e*CO_2, the food ingestion significantly decreased (4267.00, 3921.33, 3634.95, 3313.15 and 3056.64 mg) with increase in temperatures (28, 29, 31, 33 and 35 ºC, respectively) which has corresponded to a decrease of 8.10, 14.81, 22.35 and 28.37 %. However, the interactive influence of *e*CO_2 and *e*Temp was non-significant.

Table 4.3. Effect of *e*CO_2 and *e*Temp on food ingestion of *S. litura* in non-Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	3604.76	4097.76	**3851.26**	3806.72	4286.56	**4046.64**	3914.76	4416.68	**4165.72**	3775.41	4267.00	**4021.21**
29 ± 1°C	3395.24	3816.76	**3606.00**	3519.32	3910.60	**3714.96**	3603.80	4036.64	**3820.22**	3506.12	3921.33	**3713.73**
31 ± 1°C	3110.92	3518.52	**3314.72**	3262.36	3642.72	**3452.54**	3342.60	3743.60	**3543.10**	3238.63	3634.95	**3436.79**
33 ± 1°C	2717.72	3199.80	**2958.76**	2925.28	3356.08	**3140.68**	2992.64	3383.56	**3188.10**	2878.55	3313.15	**3095.85**
35 ± 1°C	2382.56	2931.52	**2657.04**	2599.16	3085.04	**2842.10**	2653.36	3153.36	**2903.36**	2545.03	3056.64	**2800.83**
Mean	**3042.24**	**3512.87**		**3222.56**	**3656.20**		**3301.43**	**3746.76**		**3188.74**	**3638.61**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	309.649*	18.91	52.69	253.660*	19.25	53.63	239.949*	20.33	56.63	985.32*	10.13	28.23
Temperature (°C)	258.647*	29.90	83.30	240.349*	30.44	84.80	241.478*	32.14	89.55	911.66*	16.02	44.64
Interaction (CO_2 + Temp(°C))	0.92	42.29	NS	0.64	43.05	NS	0.68	45.46	NS	2.39	22.66	NS
CV	6.45**%**			6.26%			6.45%			3.32%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Further, the food ingestion in non-Bt cotton, under *e*CO_2 was higher (13.02, 11.84, 12.24, 15.10 and 20.10 %, at each test temperature, respectively) compared to that of *a*CO_2.

To sum up, *S. litura* mean food ingestion increased with increase in CO_2 (by 13 %) and decreased with increase in temperature and ultimately decreased with *e*CO_2 + *e*Temp by 19 %. Coley (1998) rightly corroborated that herbivory seems to increase by 2-4 fold with increasing CO_2. Similarly, Reddy *et al*. (2004) recorded that *S. litura* consumed almost twice the quantity of groundnut foliage under *e*CO_2 (4.24 g per larva at 550 ppm) than *a*CO_2 (2.4 g per larva at 350 ppm) and consequently larval duration increased. Srinivasa Rao *et al*. (2009) reported that with enhancement of CO_2, castor food ingestion in 4 day old *S. litura* larvae increased from 594 mg (350 ppm) to 820 (550 ppm) and 869 mg (700 ppm CO_2). On the same lines,

Shwetha *et al*. (2017) reported significantly higher leaf consumption (3758.07 mg) in *S. litura* on groundnut foliage grown under *e*CO_2 + *e*Temp compared to reference plot (3341.28 mg). Contrastingly, Sharma and Brar (2018) observed that food consumption of *S. litura* increased with *e*CO_2 (500 ppm) by 2.0 % and with 5 °C rise in temperature also by 43.23 %.In the study, it was observed that generation wise also, *S. litura* food ingestion increased (3605, 3807 and 3915 mg; 4098, 4287 and 4417 mg; and 2932, 3085 and 3153 mg, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp) implying a greater chance for adaptation with advancement of generations.

4.1.1.4 Effect on food ingestion of *S. litura* in Bt cotton

The food ingestion data of *S. litura* in Bt cotton for all three generations were presented in Table 4.4 and Fig. 4.2.

First generation: In Bt cotton, at *a*CO_2, food ingestion in the entire larval period ranged from 3110.88 to 2165.68 mg with highest food ingestion at 28 °C and least at 35 °C. At *e*CO_2, food ingestion was higher than *a*CO_2, but it decreased significantly with increase in temperature from 28 to 35 °C (3916.22 to 3091.05 mg, respectively). The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on food ingestion.

Table 4.4 Effect of *e*CO_2 and *e*Temp on food ingestion of *S. litura* in Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	3110.88	3916.22	**3513.55**	3265.56	4030.73	**3648.14**	3377.30	4074.82	**3726.06**	3251.25	4007.26	**3629.25**
29 ± 1°C	2897.00	3707.67	**3302.33**	3007.17	3788.33	**3397.75**	3045.92	3856.62	**3451.27**	2983.36	3784.21	**3383.78**
31 ± 1°C	2616.85	3485.13	**3050.99**	2713.79	3600.56	**3157.17**	2772.71	3664.00	**3218.36**	2701.12	3583.23	**3142.17**
33 ± 1°C	2388.67	3313.78	**2851.22**	2529.19	3382.37	**2955.78**	2673.53	3455.37	**3064.45**	2530.46	3383.84	**2957.15**
35 ± 1°C	2165.68	3091.05	**2628.36**	2291.05	3252.05	**2771.55**	2344.30	3188.70	**2766.50**	2267.01	3177.27	**2722.14**
Mean	**2635.81**	**3502.76**		**2761.34**	**3610.80**		**2842.75**	**3647.90**		**2746.63**	**3587.15**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	1773.319*	14.56	40.56	1276.873*	16.81	46.83	1193.423*	16.48	45.91	4193.69*	9.18	25.57
Temperature (°C)	233.094*	23.02	64.12	171.299*	26.58	74.04	197.436*	26.06	72.59	597.43*	14.51	40.43
Interaction (CO_2 + Temp(°C))	1.62	32.55	NS	2.27	37.59	NS	1.94	36.85	NS	4.59*	20.52	57.17
CV	5.30**%**			5.90%			5.68%			3.24%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

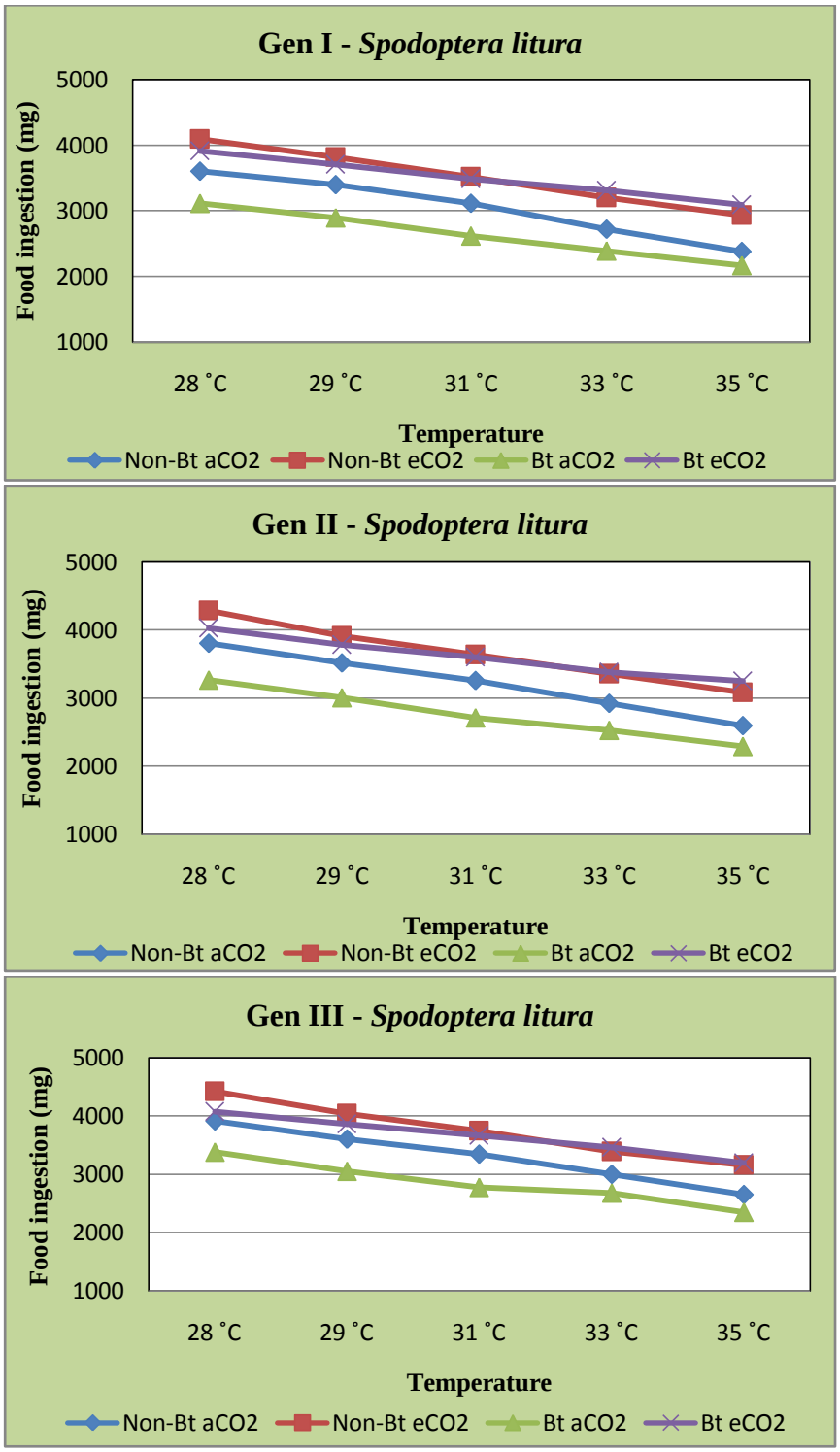


Fig. 4.2. Effect of *e*CO_2 and *e*Temp on food ingestion of *S. litura* larvae on non-Bt and Bt cotton in first, second and third generation

Second generation: At *a*CO_2, food ingestion ranged from 3265.56 to 2291.05 mg with highest and lowest at temperatures 28 and 35 °C, respectively. Similarly at *e*CO_2, food ingestion ranged in-between 4030 to 3252 mg with highest and lowest food ingestion at temperatures 28 and 35 °C, respectively. The *e*CO_2 and *e*Temp showed significant effect individually but interaction effect showed non-significant influence on food ingestion.

Third generation: At *a*CO_2, food ingestion ranged between 3377.30 to 2344.30 mg with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, food ingestion increased over *a*CO_2 where it reduced with increase in temperature with 4074.82 mg and 3188.70 mg as highest and lowest food ingestion at temperatures 28 and 35 °C, respectively. The *e*CO_2 and *e*Temp showed significant effect individually but interaction effect showed non-significant influence on food ingestion.

Mean of generations: The mean of three generations on Bt cotton also indicated that the food ingestion by larvae has decreased significantly (3251.25, 2983.36, 2701.12, 2530.46 and 2267.01 mg, respectively) with increase in temperature (28-35 ºC) corresponding to decrease of 8.24, 16.92, 22.17 and 30.27 %. Similarly, under *e*CO_2 also the food ingestion also decreased significantly (4007.26, 3784.21, 3583.23, 3383.84 and 3177.27 mg) with increase in temperatures (28, 29, 31, 33 and 35 ºC, respectively) which has corresponded to a decrease of 5.57, 10.58, 15.56 and 20.71 %. But the interaction effect of *e*CO_2 and *e*Temp showed significant influence on food ingestion. However, the decrease in food ingestion with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the food ingestion under *e*CO_2 was higher (23.25, 26.84, 32.66, 33.72 and 40.15 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The present findings suggest that mean Bt cotton foliage ingestion by *S. litura* increased with increase in CO_2 (by 23 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 2 %. Divya *et al.* (2018) reported that chickpea foliage consumption by *S. exigua* larvae under *e*CO_2 increased by 32.10 % in first generation, 31.58 % in second generation, 30.00 % in third generation and 25.21 % in fourth generation. The present study also shows that *S. litura* food ingestion on Bt cotton increased with every generation and was highest in the third generation (3111, 3266 and 3377 mg; 3916, 4031 and 4075 mg; and 3091, 3252 and

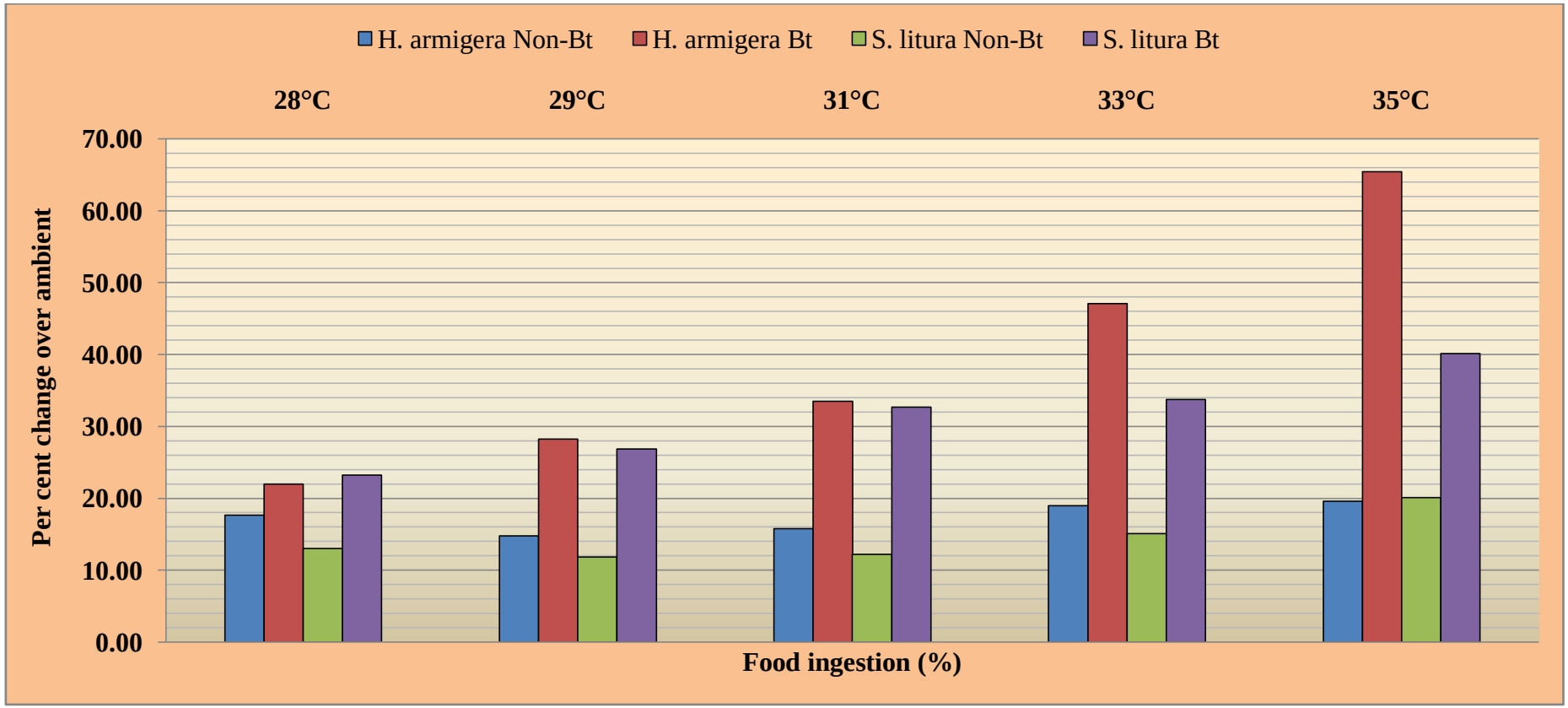


Fig. 4.3. Effect of *e*CO_2 and *e*Temp on mean food ingestion of *H. armigera* and *S. litura* on non-Bt and Bt cotton

3189 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). It is also notable that the consumption was relatively lesser than that in non-Bt cotton. This observation was made earlier in case of leaf consumption of *S. exigua* which was significantly lower in the first (F = 27.93, d.f. = 1, 6, P = 0.0019), second (F = 8.66, d.f. = 1, 6, P = 0.0259) and third (F = 9.23, d.f. = 1, 6, P = 0.0229) generations on Bt cotton compared with non-Bt cotton (Wu *et al*., 2009).

4.1.2 Larval Weight: The data on the effect of *e*CO_2 and *e*Temp conditions on the larval weights of *H. armigera* and *S. litura* was presented in the Tables 4.5 - 4.8 and Fig. 4.4 - 4.5. In either cotton hybrids, with increase in CO_2, weights increased and with temperature, weight of test insects gradually decreased. Comparative performance of both the test insects was presented in Fig. 4.6.

4.1.2.1 Effect on larval weight of *H. armigera* in non-Bt cotton

The data regarding larval weight of *H. armigera* in non-Bt cotton for first, second and third generation was presented in Table 4.5 and Fig. 4.4.

First generation: At *a*CO_2, larval weight decreased with increase in temperature from 28 °C (415.08 mg) to 35 °C (375.08 mg). At *e*CO_2 also, larval weight decreased with temperature and ranged between 497.08 to 457.24 mg at 28 and 35 °C, respectively. The individual effect of *e*CO_2 and *e*Temp has significant impact whereas interactive effect has non-significant influence on larval weight.

Second generation: The *e*CO_2 and *e*Temp has significant effect on food ingestion individually. In non-Bt cotton, at *a*CO_2, larval weight ranged from 453.52 to 420.92 mg, respectively at temperatures from 28 to 35 °C. At *e*CO_2, decrease in larval weight was recorded varying from 533.96 to 497.76 mg with highest and least at temperatures 28 and 35 ºC, respectively. It clearly indicates that there is a decrease in larval weight with increase in temperature in both *a*CO_2 and *e*CO_2 conditions. And the interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on larval weight.

Third generation: At *a*CO_2, the larval weight ranged from 472.32 to 423.80 mg at 28 and 35 °C, respectively. At *e*CO_2, larval weight ranged between 546.68 and 502.24 mg with highest at 28 °C and lowest at 35 °C. The individual effect of *e*CO_2 and *e*Temp has significant impact whereas interactive effect has non-significant influence on larval weight. And among *a*CO_2 and *e*CO_2, larval weight was significantly lower in *a*CO_2.

Table 4.5. Effect of *e*CO_2 and *e*Temp on larval weight of *H. armigera* in non-Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	415.08	497.08	**456.08**	453.52	533.96	**493.74**	472.32	546.68	**509.50**	446.97	525.91	**486.44**
29 ± 1°C	412.76	490.64	**451.70**	450.48	527.68	**489.08**	461.64	537.12	**499.38**	441.63	518.48	**480.05**
31 ± 1°C	402.52	478.88	**440.70**	442.00	520.68	**481.34**	448.80	528.92	**488.86**	431.11	509.49	**470.30**
33 ± 1°C	397.04	470.20	**433.62**	428.84	509.88	**469.36**	435.68	518.76	**477.22**	420.52	499.61	**460.07**
35 ± 1°C	375.08	457.24	**416.16**	420.92	497.76	**459.34**	423.80	502.24	**463.02**	406.60	485.75	**446.17**
Mean	**400.49**	**478.80**		**439.15**	**517.99**		**448.44**	**526.74**		**429.36**	**507.84**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	2219.218*	1.18	3.28	1672.676*	1.36	3.80	1441.789*	1.46	4.06	5764.89*	0.73	2.03
Temperature (°C)	72.690*	1.86	5.18	43.211*	2.16	6.00	62.65*	2.31	6.42	192.44*	1.15	3.22
Interaction (CO_2 + Temp(°C))	1.07	2.63	NS	0.19	3.05	NS	0.58	3.26	NS	0.17	1.63	NS
CV	2.99%			3.18%			3.34%			1.74%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant

*Significant @ 5 per cent level of significance

Mean of generations: The mean of three generations of *H. armigera* on non-Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the larval weight significantly decreased (446.97, 441.63, 431.11, 420.52 and 406.60 mg, respectively) corresponding to 1.20, 3.55, 5.92 and 9.03 %. Similarly, under *e*CO_2 also the larval weight significantly decreased (525.91, 518.48, 509.49, 499.61 and 485.75 mg) with increase in temperatures (28, 29, 31, 33 and 35 ºC, respectively) which has corresponded to a decrease of 1.41, 3.12, 5.00 and 7.64 %. And the interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on larval weight. Further, the larval weight under *e*CO_2 was higher (17.66, 17.40, 18.18, 18.81 and 19.47 %, at corresponding temperatures, respectively) compared to that of *a*CO_2.

The present findings suggest that mean larval weight of *H. armigera* increased with increase in CO_2 (by 18 %) and decreased with temperature; however it increased with *e*CO_2 + *e*Temp by meagre 9 % which appears to be due to the higher influence of CO_2. Similarly, Mallikarjuna *et al*. (2020) observed that *H. armigera* larval weights reduced under *e*CO_2 + *e*Temp (242 mg) by 33 % over reference ambient conditions (364 mg). Larval weight increased with every generation and was highest in the third generation (415, 454 and 472 mg; 497, 534 and 547 mg; and 457, 498 and 502 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). On the contrary, Akbar *et al*. (2016) recorded that larval weights of *H. armigera* were significantly lower under *e*CO_2 @ 750 ppm (340 mg) compared to *a*CO_2 @ 350 ppm (420 mg). Sharma *et al.* (2016) also reported that elevated CO_2 adversely affects the larval weight of *H. armigera*. Probably higher CO_2 levels above 550 ppm could alter the pattern of increase in weight gain of larvae, contradicting with the observations in the current study.

4.1.2.2 Effect on larval weight of *H. armigera* in Bt cotton

The data pertaining to larval weight of *H. armigera* in Bt cotton for first, second and third generation were presented in the Table 4.6 and Fig. 4.4.

First generation: In Bt cotton, individually *e*CO_2 and *e*Temp has shown significant effect. At *a*CO_2, larval weight varied from 346.71 to 323.75 mg at a temperature range of 28-35 °C. At *e*CO_2, decrease in larval weight was recorded ranging from 457.87 to 427.18 mg with increase in temperature (28 to 35 °C). The interaction effect

of *e*CO_2 and *e*Temp showed non-significant influence on larval weight. But with increase in temperatures *viz.*, 28, 29, 31, 33 and 35 °C, an increase in larval weight *viz.*, 32.06, 32.30, 28.21, 31.33 and 31.95 %, respectively was recorded at *e*CO_2 over *a*CO_2.

Second generation: At *a*CO_2, larval weight varied from 354.25 to 329.12 mg with highest and lowest larval weights at temperatures 28 and 35 ºC, respectively. At *e*CO_2, highest larval weight was recorded at 28 °C (476.88 mg) and lowest at 35 °C (444.13 mg).The *e*CO_2 and *e*Temp has shown significant impact individually but the interaction effect has shown non-significant influence on larval weight.

Third generation: At *a*CO_2, larval weight varied from 364.12 to 339.22 mg with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in larval weight was recorded with increase in temperature with highest larval weight at 28 ºC (477.11 mg) and lowest at 35 ºC (453.00 mg). The *e*CO_2 and *e*Temp has shown significant impact individually but the interaction effect has shown non-significant influence on larval weight.

Mean of generations: The mean of three generations on Bt cotton, also indicated that with increase in temperature (28, 29, 31, 33 and 35 °C) the larval weight has decreased significantly (355.03, 350.48, 345.43, 339.12 and 330.70 mg, respectively) corresponding to 1.28, 2.70, 4.48 and 6.85 %. Similarly, under *e*CO_2 also the larval weight decreased significantly (470.62, 460.97, 450.44, 448.23 and 441.44 mg) with increase in temperatures (28-35 °C) which has corresponded to a decrease of 2.05, 4.29, 4.76 and 6.20 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on larval weight. Further, the larval weight under *e*CO_2 was higher (32.56, 31.52, 30.40, 32.17 and 33.49 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These findings suggest that mean larval weight of *H. armigera* on Bt cotton increased with increase in CO_2 (by 33 %) and decreased with temperature; but however increased with *e*CO_2 + *e*Temp by 24 %. Whittaker (1999) showed that Bt cotton and *e*CO_2 slows the development of bollworms and consequently reduce larval weight gain. Larval weight increased with every generation and was highest in the third generation (347, 354 and 364 mg; 458, 477 and 477 mg; and 427, 444 and 453 mg in three successive generations at ambient condition, *e*CO_2 and

Table 4.6. Effect of *e*CO_2 and *e*Temp on larval weight of *H. armigera* in Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	346.71	457.87	**402.29**	354.25	476.88	**415.56**	364.12	477.11	**420.61**	355.03	470.62	**412.83**
29 ± 1°C	339.10	448.63	**393.86**	350.81	462.75	**406.78**	361.54	471.53	**416.54**	350.48	460.97	**405.73**
31 ± 1°C	336.28	431.13	**383.70**	343.38	457.00	**400.19**	356.64	463.20	**409.92**	345.43	450.44	**397.94**
33 ± 1°C	333.37	437.83	**385.60**	338.05	452.61	**395.33**	345.94	454.24	**400.09**	339.12	448.23	**393.68**
35 ± 1°C	323.75	427.18	**375.46**	329.12	444.13	**386.62**	339.22	453.00	**396.11**	330.70	441.44	**386.07**
Mean	**335.84**	**440.53**		**343.12**	**458.67**		**353.49**	**463.81**		**344.15**	**454.34**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	1319.159*	2.04	5.68	1606.882*	2.04	5.68	1137.398*	2.31	6.44	4436.60*	1.17	3.25
Temperature (°C)	10.107*	3.22	8.98	11.675*	3.22	8.98	8.177*	3.66	10.19	31.63*	1.85	5.15
Interaction (CO_2 + Temp(°C))	0.99	4.56	NS	0.41	4.56	NS	0.18	5.17	NS	1.05	2.61	NS
CV	5.87%			5.68%			6.33%			3.28%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

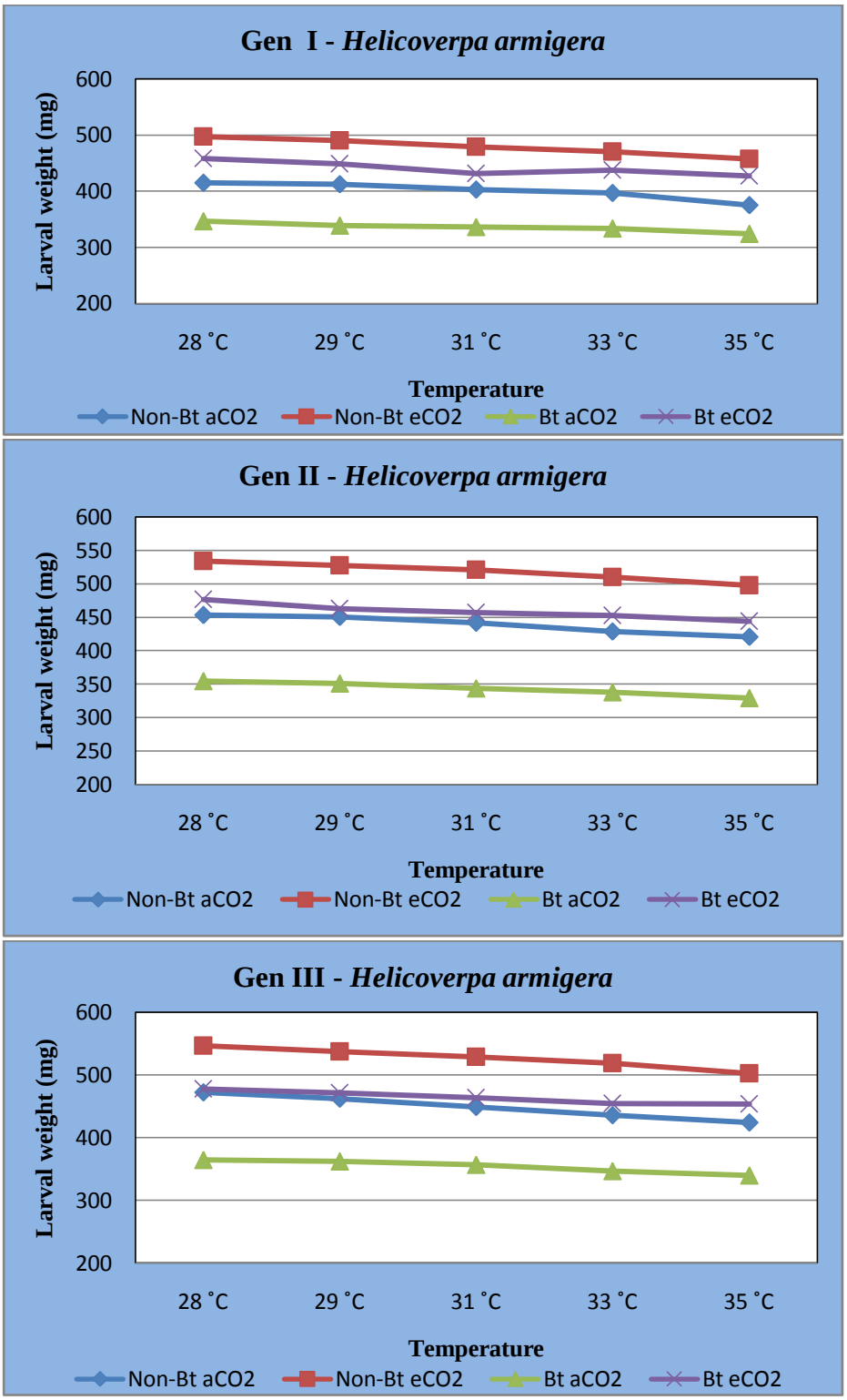


Fig. 4.4. Effect of *e*CO_2 and *e*Temp on larval weight of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

eCO$_2$ + eTemp, respectively).mg in three successive generations at ambient condition, eCO$_2$ and eCO$_2$ + eTemp, respectively). It was notable that larval weight gain across the generations in Bt cotton was lower than that on non-Bt cotton. In this regard, Chen *et al*. (2004) convincingly demonstrated that eCO$_2$ resulted in drastically reduced larval weight in bollworms on Bt cotton over those on non-Bt cotton. Ghazanafar *et al.* (2020) in tha same context, reported that when Bt-cotton leaf powder was mixed into the diet of *H. virescens*, larval weight (8 days after hatching) reduced by 10 % compared to non-Bt diet.

4.1.2.3 Effect on larval weight of *S. litura* in non-Bt cotton

The data pertaining to larval weight of *S. litura* in non-Bt cotton for first, second and third generation was presented in the Table 4.7 and Fig. 4.5.

First generation: In non-Bt cotton, at aCO$_2$, larval weight varied significantly from 538.80 to 396.12 mg with highest larval weight at temperature 28 ºC and lowest at 35 ºC. At eCO$_2$, larval weight increased significantly over ambient and ranged from 612.40 to 457.92 mg with highest and lowest at temperatures 28 and 35 ºC, respectively. The interaction effect of eCO$_2$ and eTemp showed non-significant influence on larval weight. And an increase in larval weight was recorded at eCO$_2$ with 13.66, 14.34, 15.58, 12.61 and 15.60 %, respectively at temperatures 28, 29, 31, 33 and 35 ºC over aCO$_2$.

Second generation: At aCO$_2$, larval weight ranged from 554.76 to 409.52 mg with highest and lowest, respectively at temperatures 28 and 35 ºC. Weight increased under eCO$_2$, and ranged between 632.52 and 481.40 mg with highest at 28 °C and lowest at 35 °C. The individual effect of eCO$_2$ and eTemp has shown significant impact whereas interaction effect showed non-significant influence on larval weight.

Third generation: At aCO$_2$, larval weight varied from 563.48 to 424.72 mg within a temperature range of 28 to 35 °C. At eCO$_2$, larval weight ranged between 643.52 to 492.64 mg with highest and lowest at temperatures 28 and 35 °C, respectively. The individual effect of eCO$_2$ and eTemp has shown significant impact whereas interaction effect showed non-significant influence on larval weight. But with increase in temperatures *viz.*, 28, 29, 31, 33 and 35 °C, an increase in larval weight by larvae *viz.*, 14.20, 14.72, 15.64, 15.07 and 15.99 %, respectively was recorded at eCO$_2$ over aCO$_2$.

Mean of generations: The mean of three generations on non-Bt cotton, also indicated that the larval weight significantly decreased (552.35, 517.35, 474.64, 440.47 and 410.12 mg, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to decrease of 6.34, 14.07, 20.26 and 25.75 %. Similarly, under *e*CO_2 also the larval weight decreased significantly (629.48, 592.95, 547.32, 502.91 and 477.32 mg) with increase in temperature (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 5.80, 13.05, 20.11 and 24.17 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on larval weight in non-Bt cotton. Further, the larval weight under *e*CO_2 was higher (13.96, 14.61, 15.31, 14.18 and 16.39 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The present findings suggest that mean larval weight of *S. litura* increased with increase in CO_2 (by 14 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 14 %. These results are in agreement with Srinivasa Rao *et al.* (2009) who reported that larval weight of 4 day old *S. litura* increased with increase in CO_2 from 117 mg (350 ppm) to 137 mg (700 ppm) commensurate with the castor leaf ingested. In the current study, larval weight increased with every generation and was highest in the third generation (539, 555 and 563 mg; 612, 633 and 644; and 458, 481 and 493 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). Srinivasa Rao *et al.* (2014) also recorded higher larval weights in groundnut, in all four generations of *S. litura* grown under *e*CO_2. Similarly, Shrikant and Bandeppa (2014) observed that *Spilosoma obliqua* larvae fed with castor foliage from *e*CO_2 conditions recorded more larval weight (786 mg) over those fed on foliage from *a*CO_2 conditions (731 mg). Khafagi *et al*. (2016) attributed enormous weight gain in 5^{th} and 6^{th} larval instars of *S. littoralis* due to apparent prolongation of the larval duration and concomitant prolonged feeding.

4.1.2.4 Effect on larval weight of *S. litura* in Bt cotton

The data regarding larval weight of *S. litura* in Bt cotton for all the three generations were presented in the Table 4.8 and Fig. 4.5.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant influence individually. At *a*CO_2, larval weight ranged from 389.88 to 310.53 mg with highest larval weight at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, larval weight was

Table 4.7. Effect of *e*CO_2 and *e*Temp on larval weight of *S. litura* in non-Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	538.80	612.40	**575.60**	554.76	632.52	**593.64**	563.48	643.52	**603.50**	552.35	629.48	**590.91**
29 ± 1°C	503.08	575.20	**539.14**	519.44	596.20	**557.82**	529.52	607.44	**568.48**	517.35	592.95	**555.15**
31 ± 1°C	461.20	533.04	**497.12**	475.40	545.40	**510.40**	487.32	563.52	**525.42**	474.64	547.32	**510.98**
33 ± 1°C	431.16	485.52	**458.34**	442.48	507.96	**475.22**	447.76	515.24	**481.50**	440.47	502.91	**471.69**
35 ± 1°C	396.12	457.92	**427.02**	409.52	481.40	**445.46**	424.72	492.64	**458.68**	410.12	477.32	**443.72**
Mean	**466.07**	**532.81**		**480.32**	**552.69**		**490.56**	**564.47**		**478.98**	**549.99**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	166.784*	3.65	10.18	206.875*	3.56	9.91	189.492*	3.80	10.58	632.019*	2.00	5.56
Temperature (°C)	107.194*	5.78	16.10	114.102*	5.63	15.67	99.286*	6.00	16.72	359.62*	3.16	8.80
Interaction (CO_2 + Temp(°C))	0.52	8.17	NS	0 .201	7.96	NS	0.24	8.49	NS	0.93	4.47	NS
CV	4.57%			4.77%			5.26%			4.34%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Table 4.8. Effect of *e*CO_2 and *e*Temp on larval weight of *S. litura* in Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	389.88	509.11	**449.49**	417.78	522.90	**470.34**	426.30	534.45	**480.38**	411.32	522.15	**466.74**
29 ± 1°C	369.00	492.42	**430.71**	381.00	503.42	**442.21**	391.92	507.15	**449.54**	380.64	501.00	**440.82**
31 ± 1°C	348.08	477.07	**412.57**	359.86	489.25	**424.55**	363.79	491.94	**427.86**	357.24	486.09	**421.66**
33 ± 1°C	333.53	461.50	**397.52**	344.75	465.26	**405.01**	348.29	472.32	**410.30**	342.19	466.36	**404.28**
35 ± 1°C	310.53	440.18	**375.35**	326.30	452.59	**389.45**	334.70	433.30	**384.00**	323.84	442.02	**382.93**
Mean	**350.20**	**476.05**		**365.93**	**486.68**		**372.99**	**487.83**		**363.04**	**483.52**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	874.447*	3.01	8.38	621.295*	3.43	9.54	683.634*	3.11	8.65	2039.82*	1.89	5.26
Temperature (°C)	36.464*	4.76	13.26	34.153*	5.42	15.09	56.274*	4.91	13.68	117.70*	2.98	8.31
Interaction (CO_2 + Temp(°C))	0.22	6.73	NS	0.75	7.66	NS	1.48	6.94	NS	1.27	4.22	NS
CV	3.14%			4.98%			4.07%			4.98%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

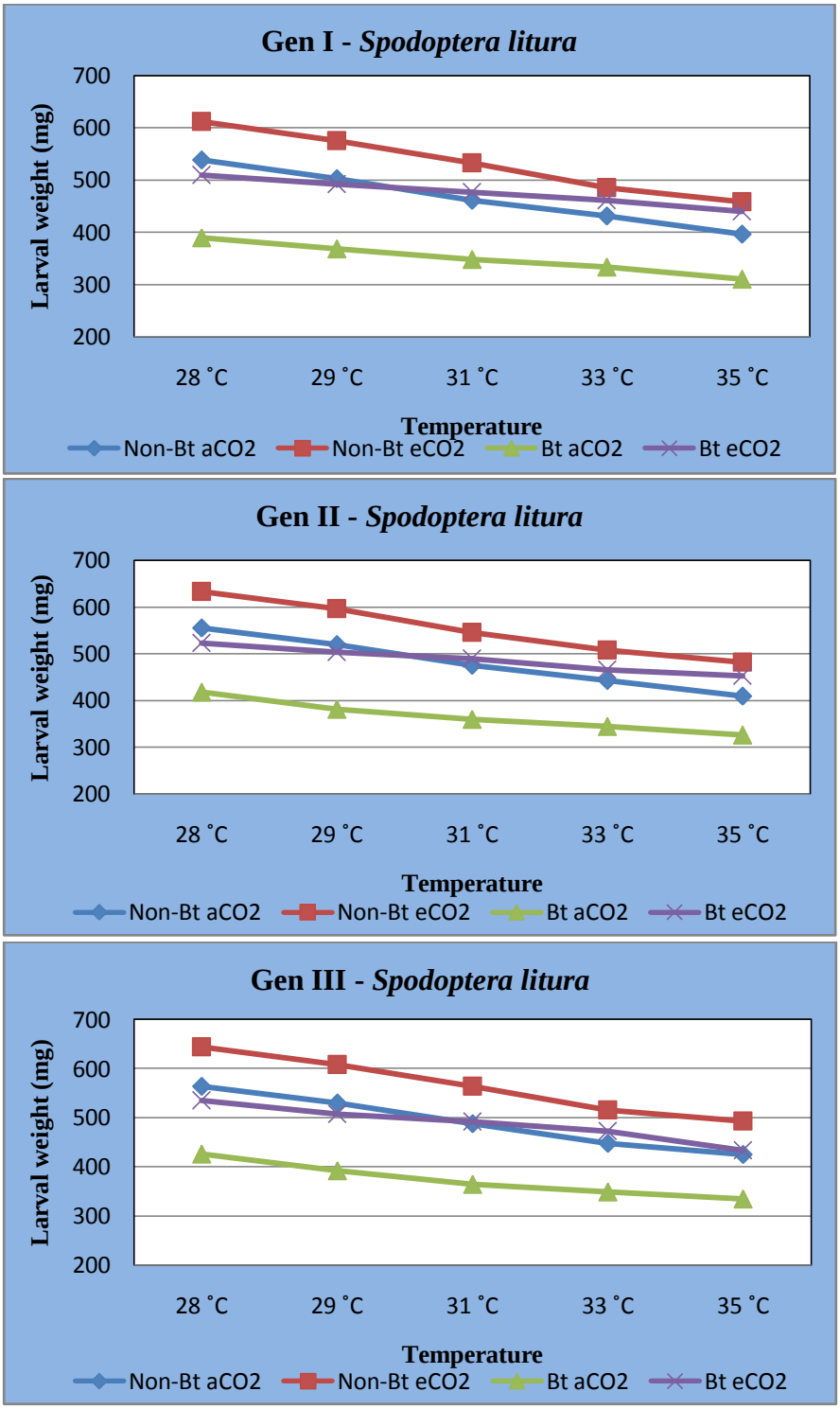


Fig. 4.5. Effect of *e*CO_2 and *e*Temp on larval weight of *S. litura* on non-Bt and Bt cotton in first, second and third generation

relatively higher and ranged between 509.11 to 440.18 mg (at 28 and 35 ºC, respectively). A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval weight.

Second generation: At *a*CO_2, larval weight varied from 417.78 to 326.30 mg within a temperature range of 28- 35 °C. At *e*CO_2, decrease in larval weight from 522.90 to 452.59 mg was recorded with increase in temperature from 28-35 ºC. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval weight whereas individually significant impact was recorded.

Third generation: At *a*CO_2, larval weight decreased in the range 426.30 - 334.70 mg with increase in temperature upto 35 ºC. At *e*CO_2, larval weight was higher but decreased with increase in temperature from 28-35 °C (534.45 - 433.30 mg). The *e*CO_2 and *e*Temp has shown significant impact on larval weight but interactive effect was non-significant.

Mean of generations: The mean of three generations on Bt cotton, also indicated that the larval weight by larvae has decreased significantly (411.32, 380.64, 357.24, 342.19 and 323.84 mg, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 7.46, 13.15, 16.81 and 21.27 %. Similarly, under *e*CO_2, the larval weight significantly decreased (522.15, 501.00, 486.09, 466.36 and 442.02 mg) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 4.05, 6.91, 10.69 and 15.35 %. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval weight. And larval weight was significantly lower in *a*CO_2 than *e*CO_2. Further, the larval weight under *e*CO_2 was higher (26.95, 31.62, 36.07, 36.29 and 36.49 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These findings suggest that mean larval weight of *S. litura* in Bt cotton increased with increase in CO_2 (by 27 %) and decreased with temperature and however increased with *e*CO_2 + *e*Temp by 8 %. Hence *S. litura* larvae may gain more weight on Bt cotton with extreme climate stress compared to that on non-Bt cotton.

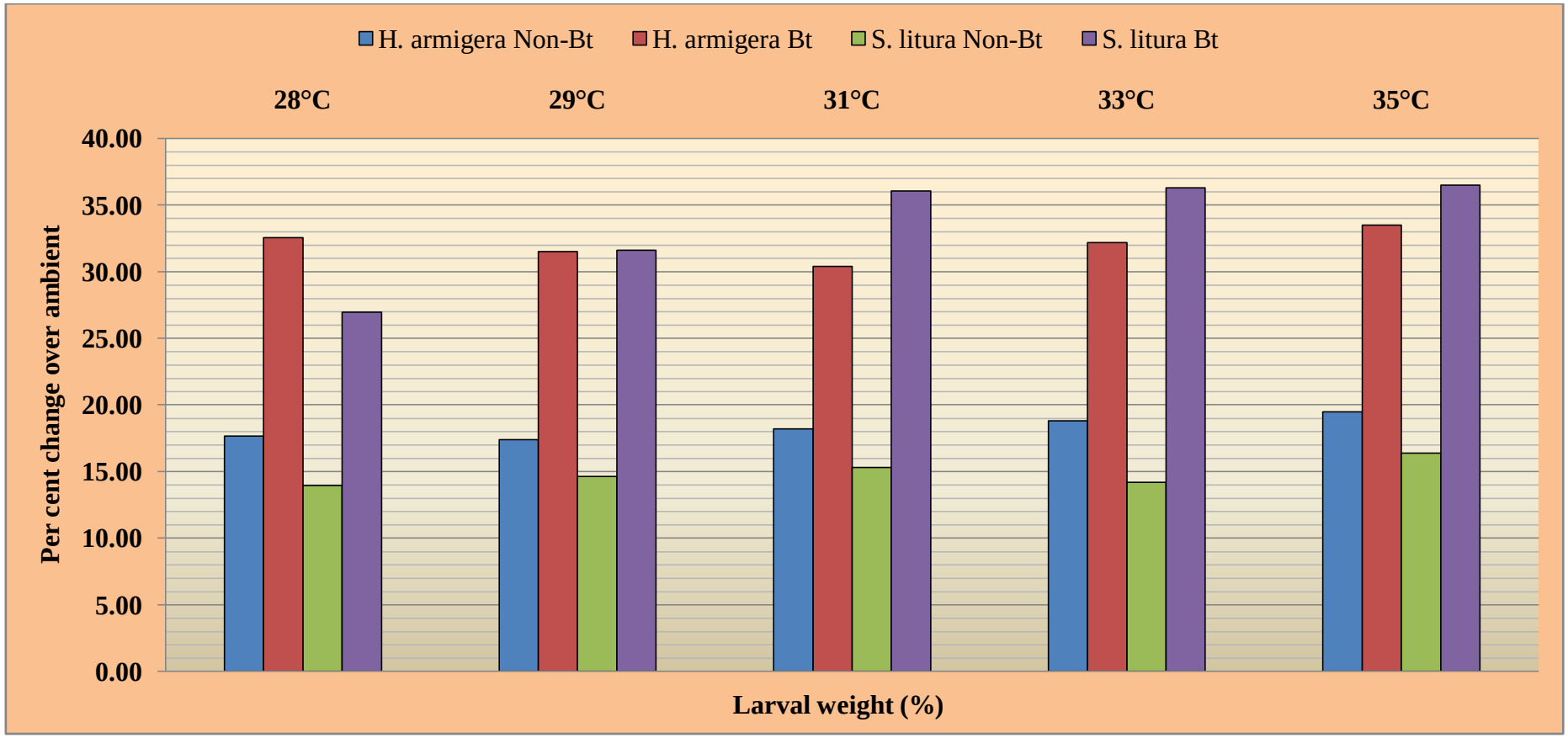


Fig. 4.6. Effect of *e*CO_2 and *e*Temp on mean larval weight of *H. armigera* and *S. litura* on non-Bt and Bt cotton

Larval weight in Bt cotton increased with every generation and was highest in the third generation (390, 418 and 426 mg; 509, 523 and 534 mg; and 440, 453 and 433 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). Similarly, Coviella *et al*. (2002) observed that larval weight of 10 day old *S. exigua* increased by 20 % with *e*CO_2 (900 µmol mol^{-1}) over ambient and by 40 % in Bt over non-Bt cotton. Rajesh *et al.* (2007) reported that application of *Bacillus thuringiensis* var. kurstaki 0.2 % in various treatment combinations with 5 per cent each of of neem oil, citronella oil, karanj oil, cottonseed oil and sesamum oil on *S. litura* resulted in significantly reduction in mean larval weight (30.0- 22.2 %), over untreated check. On the similar notes, Ghazanafar *et al.* (2020) observed that when pulverized leaf powder from Bt-cotton was mixed into the diet of 8 day old *S. littoralis*, larval weight was reduced by 30-40 % over normal diet without Bt powder.

4.1.3 Larval excretion: The perusal of the results (Tables 4.9 - 4.12 and Fig. 4.7 - 4.8) indicated significant variation in the amount of excrement by the test insects throughout their larval period, across *e*CO_2 and *e*Temp conditions test. With increase in CO_2, excretion increased. With increase in test temperatures (28, 29, 31, 33 and 35 °C), under both *a*CO_2 (380 $\pm$ 25 ppm) and *e*CO_2 (550 $\pm$ 25 ppm) conditions, larval excretion by test insects gradually decreased. Comparative performance of both the test insects was presented in Fig. 4.9.

4.1.3.1 Effect on larval excretion of *H. armigera* in non-Bt cotton

The data regarding larval excretion of *H. armigera* in non-Bt cotton for the first, second and third generation was presented in Table 4.9 and Fig. 4.7.

First generation: The *e*CO_2 and *e*Temp has shown significant effect on larval excretion. At *a*CO_2, larval excretion varied from 1054.12 to 771.28 mg with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in larval excretion was recorded in the range of 1322.04 to 887.84 mg with increase in temperature from 28-35 °C. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on larval excretion.

Second generation: At *a*CO_2, larval excretion varied from 1087.84 to 801.12 mg with highest at 28 ºC and lowest at 35 ºC temperature. At *e*CO_2, larval excretion ranged between 1397.56 to 922.20 mg with highest and lowest larval excretion at temperatures 28 and 35 °C, respectively. The individual effect of *e*CO_2 and *e*Temp and interaction showed significant influence on larval excretion.

Third generation: At *a*CO_2, larval excretion varied from 1135.44 to 837.88 mg with highest and lowest at temperatures 28 and 35 °C, respectively. At *e*CO_2, decrease in larval excretion was recorded in the range of 1406.72 to 950.72 mg with increase in temperature from 28 to 35 °C. The individual effect of *e*CO_2 and *e*Temp and interaction showed significant influence on larval excretion.

Mean of generations: The mean of three generations on non-Bt cotton, also indicated that with increase in temperature (28-35 °C) the larval excretion has decreased significantly (1092.47, 1055.07, 999.01, 880.17 and 803.43 mg, respectively) corresponding to 3.42, 8.55, 19.43 and 26.46 %. Similarly, under *e*CO_2 also the larval excretion decreased significantly (1375.44, 1254.28, 1135.63, 1055.32 and 920.25 mg) with increase in temperatures (28, 29, 31, 33 and 35 °C) which has corresponded to a decrease of 8.81, 17.44, 23.27 and 33.09 %. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on larval excretion. And larval excretion was significantly lower in *a*CO_2 than *e*CO_2. However, the decrease in larval excretion with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the larval excretion under *e*CO_2 was higher (25.90, 18.88, 13.67, 19.90 and 14.54 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It implies that mean larval excretion of *H. armigera* increased with increase in CO_2 (by 26 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 16 %. This is in conformity with Chen *et al.* (2004) who reported significantly 38 % increase in frass production of *H. armigera* from *a*CO_2 (139.1) to *e*CO_2 (191.7 mg) in wheat. In the study, larval excretion increased with every generation and was highest in the third generation (1054, 1088 and 1135 mg; 1322, 1398 and 1407 mg; and 888, 922 and 951 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). These results are also in general agreement with Wu *et al*. (2006) who reported that *H. armigera* larvae produced more frass when fed on wheat from *e*CO_2 compared with that from *a*CO_2 ($p < 0.05$) and was significantly higher in the third generation. Similarly, Chen *et al.* (2007) observed that *H. armigera* under *e*CO_2 (750 µl l^{-1}) exhibited decreased frass output by 9.9 % for three generations in non-Bt cotton. With exertion of salt stress (150 mmol L^{-1} NaCl) also, faeces production decreased by 23.06 % in Simian- 3 non-Bt cotton (Luo *et al*., 2018).

4.1.3.2 Effect on larval excretion of *H. armigera* in Bt cotton

The data regarding larval excretion of *H. armigera* in Bt cotton for all the three generations were presented in Table 4.10 and Fig. 4.7.

First generation: In Bt cotton, the *e*CO_2 and *e*Temp has shown significant effect on larval excretion. At *a*CO_2, larval excretion decreased in the range of 1014.42 to 608.65 mg with increase in temperature from 28 to 35 ºC. At *e*CO_2, decrease in larval excretion was recorded in the range of 1248.25 to 951.42 mg with increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on larval excretion.

Second generation: At *a*CO_2, larval excretion varied from 1062.75 to 657.42 mg with increase in temperature from 28 to 35 ºC in Bt cotton. At *e*CO_2, decreased larval excretion was recorded from 1301.44 to 1039.43 mg with increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and interaction showed significant influence on larval excretion.

Third generation: At *a*CO_2, decrease in larval excretion from 1110.75 to 688.95 mg with highest and lowest at temperatures 28 and 35 °C. At *e*CO_2, larval excretion ranged between 1359.88 and 1063.95 mg within a temperature range of 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and interaction showed significant influence on larval excretion.

Mean of generations: The mean of three generations in Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the larval excretion has decreased significantly (1062.64, 957.67, 857.07, 760.25 and 651.67 mg, respectively) corresponding to 9.88, 19.35, 28.46 and 38.67 %. Similarly, under *e*CO_2 also the larval excretion decreased significantly (1303.19, 1200.77, 1119.00, 1084.78 and 1018.27 mg) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 7.86, 14.13, 16.76 and 21.86 %. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on larval excretion of *H. armigera*. However, the decrease in larval excretion with increase in

Table 4.9. Effect of *e*CO_2 and *e*Temp on larval excretion of *H. armigera* in non-Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1054.12	1322.04	**1188.08**	1087.84	1397.56	**1242.70**	1135.44	1406.72	**1271.08**	1092.47	1375.44	**1233.95**
29 ± 1°C	1018.64	1192.44	**1105.54**	1074.24	1270.12	**1172.18**	1072.32	1300.28	**1186.30**	1055.07	1254.28	**1154.67**
31 ± 1°C	912.64	1076.64	**994.64**	1047.08	1144.84	**1095.96**	1037.32	1185.40	**1111.36**	999.01	1135.63	**1067.32**
33 ± 1°C	827.04	1004.72	**915.88**	894.24	1059.48	**976.86**	919.24	1101.76	**1010.50**	880.17	1055.32	**967.75**
35 ± 1°C	771.28	887.84	**829.56**	801.12	922.20	**861.66**	837.88	950.72	**894.30**	803.43	920.25	**861.84**
Mean	**916.74**	**1096.73**		**980.90**	**1158.84**		**1000.44**	**1188.97**		**966.02**	**1148.18**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	1525.789*	3.26	9.08	1189.242*	3.65	10.16	1564.389*	3.37	9.39	4483.85*	1.92	5.36
Temperature (°C)	776.529*	5.15	14.35	698.797*	5.77	16.07	766.027*	5.33	14.85	2351.70*	3.04	8.47
Interaction (CO_2 + Temp(°C))	28.386*	7.29	20.30	51.668*	8.16	22.73	34.776*	7.54	21.00	113.82*	4.30	11.98
CV	3.62 %			3.81 %			3.44 %			2.03 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Table 4.10. Effect of *e*CO_2 and *e*Temp on larval excretion of *H. armigera* in Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1014.42	1248.25	**1131.33**	1062.75	1301.44	**1182.09**	1110.75	1359.88	**1235.31**	1062.64	1303.19	**1182.92**
29 ± 1°C	903.10	1155.63	**1029.36**	964.00	1208.91	**1086.45**	1005.90	1237.76	**1121.83**	957.67	1200.77	**1079.22**
31 ± 1°C	807.35	1020.08	**913.71**	857.93	1144.00	**1000.96**	905.92	1192.93	**1049.43**	857.07	1119.00	**988.04**
33 ± 1°C	717.00	1015.33	**866.16**	773.70	1107.11	**940.40**	790.05	1131.89	**960.97**	760.25	1084.78	**922.52**
35 ± 1°C	608.65	951.42	**780.03**	657.42	1039.43	**848.43**	688.95	1063.95	**876.45**	651.67	1018.27	**834.97**
Mean	**810.10**	**1078.14**		**863.16**	**1160.18**		**900.32**	**1197.28**		**857.86**	**1145.20**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	3093.992*	3.41	9.49	3761.501*	3.42	9.54	3094.958*	3.78	10.52	1154.69*	1.92	5.36
Temperature (°C)	656.683*	5.39	15.01	566.894*	5.42	15.08	544.243*	5.97	16.63	1975.28*	3.04	8.47
Interaction (CO_2 + Temp(°C))	23.630*	7.62	21.23	31.516*	7.66	21.33	25.844*	8.44	23.51	84.13*	4.30	11.98
CV	4.04 %			3.78 %			4.02 %			2.15 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

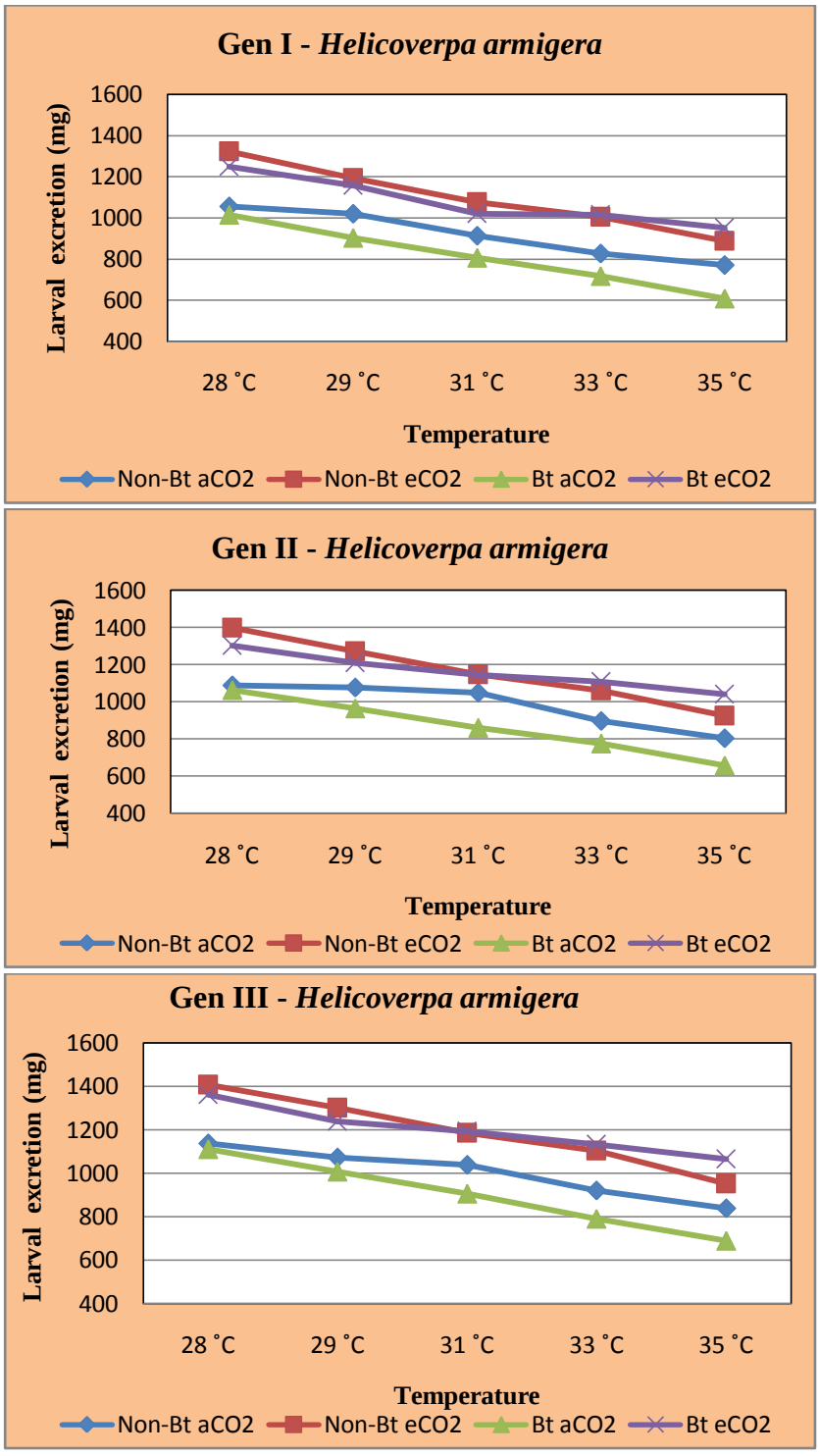


Fig. 4.7. Effect of *e*CO_2 and *e*Temp on larval excretion of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the larval excretion under *e*CO_2 was higher (22.64, 25.38, 30.56, 42.69 and 56.25 %, at corresponding temperatures, respectively) compared to that of *a*CO_2.

It implies that mean larval excretion of *H. armigera* in Bt cotton increased with increase in CO_2 (by 23 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 4 %. Larval excretion of *H. armigera* was relatively lower in Bt over non-Bt cotton upto 31 °C, after which it was the opposite outcome at 33 and 35 °C. Further excretion increased with every generation and was highest in the third generation (1014, 1062 and 1110 mg; 1248, 1301 and 1360 mg; and 951, 1039 and 1064 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). This is also in line with the findings of Chen *et al.* (2007) who recorded that bollworms reared on transgenic cotton had reduced frass output under *e*CO_2. Luo *et al*. (2018) reported that under NaCl stress, the amount of faeces produced by *H. armigera* on GK19 Bt cotton was significantly lower than that on Simian 3 non-transgenic cotton.

4.1.3.3 Effect on larval excretion of *S. litura* in non-Bt cotton

The data pertaining to larval excretion of *S. litura* in non-Bt cotton for all the three generations was presented in Table 4.11 and Fig. 4.8.

First generation: In non-Bt cotton, the *e*CO_2 and *e*Temp has shown significant effect on larval excretion. At *a*CO_2, larval excretion ranged between 1116.48 and 806.60 mg with highest and lowest larval excretion at temperatures 28 and 35 °C, respectively. At *e*CO_2, decrease in larval excretion was recorded in the range of 1354.96 to 944.48 mg with increase in temperature from 28 to 35 °C. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval excretion. And larval excretion was significantly lower in *a*CO_2 than *e*CO_2.

Second generation: At *a*CO_2, larval excretion ranged from 1209.12 to 865.64 mg within a temperature range of 28 to 35 °C. At *e*CO_2, decrease in larval excretion was recorded in the range of 1456.04 of 1011.88 mg with increase in temperature from 28 to 35 °C. The individual effect of *e*CO_2 and *e*Temp and interaction showed significant influence on larval excretion.

Third generation: At *a*CO_2, decrease in larval excretion of *S. litura* was recorded ranging from 1282.52 to 880.56 mg with increase in temperature from 28 to 35 °C. At *e*CO_2, larval excretion ranged from 1503.32 and 1053.40 mg with highest and lowest at temperatures 28 and 35 °C, respectively. The individual effect of *e*CO_2 and *e*Temp and interaction showed significant influence on larval excretion.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the larval excretion has decreased significantly (1202.71, 1147.79, 1069.72, 949.09 and 850.93 mg, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 4.57, 11.06, 21.09 and 29.25 %. Similarly, under *e*CO_2, the larval excretion decreased significantly (1438.11, 1295.77, 1199.97, 1099.93 and 1003.25 mg) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 9.90, 16.56, 23.52 and 30.24 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval excretion. And larval excretion was significantly lower in *a*CO_2 than *e*CO_2. However, the decrease in larval excretion with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the larval excretion under *e*CO_2 was higher (19.57, 12.89, 12.18, 15.89 and 17.90 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

From the findings it can be said that mean larval excretion of *S. litura* increased with increase in CO_2 (by 20 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 17 %. Shwetha *et al*. (2017) reported significantly higher faecal matter (893.72 mg per larva) in *S. litura* when fed with groundnut foliage from *e*CO_2 + *e*Temp compared to reference plot (884.38 mg per larva). Divya (2017) also reported higher faecal matter production due to extended larval duration in *S. exigua* under *e*CO_2. Larval excretion increased with every generation and was highest in the third generation (1116, 1209 and 1283 mg; 1355, 1456 and 1503 mg; and 944, 1011 and 1053 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively).

4.1.3.4 Effect on larval excretion of *S. litura* in Bt cotton

The data pertaining to larval excretion of *S. litura* in Bt cotton for all the three generations was presented in Table 4.12 and Fig. 4.8.

Table 4.11. Effect of *e*CO_2 and *e*Temp on larval excretion of *S. litura* in non-Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1116.48	1354.96	**1235.72**	1209.12	1456.04	**1332.58**	1282.52	1503.32	**1392.92**	1202.71	1438.11	**1320.41**
29 ± 1°C	1088.40	1243.92	**1166.16**	1149.96	1296.48	**1223.22**	1205.00	1346.92	**1275.96**	1147.79	1295.77	**1221.78**
31 ± 1°C	987.72	1147.56	**1067.64**	1093.08	1207.92	**1150.50**	1128.36	1244.44	**1186.40**	1069.72	1199.97	**1134.85**
33 ± 1°C	896.40	1056.32	**976.36**	960.96	1104.64	**1032.80**	989.92	1138.84	**1064.38**	949.09	1099.93	**1024.51**
35 ± 1°C	806.60	944.48	**875.54**	865.64	1011.88	**938.76**	880.56	1053.40	**966.98**	850.93	1003.25	**927.09**
Mean	**979.12**	**1149.44**		**1055.75**	**1215.39**		**1097.27**	**1257.38**		**1044.04**	**1207.40**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	281.471*	7.18	20.00	207.531*	7.84	21.83	205.214*	7.90	22.02	762.13*	4.18	11.66
Temperature (°C)	161.316*	11.35	31.62	156.374*	12.39	34.52	181.383*	12.50	34.81	553.51*	6.62	18.43
Interaction (CO_2 + Temp(°C))	2.976*	16.05	44.72	4.166*	17.52	48.81	2.498*	17.67	49.23	9.71*	9.36	26.07
CV	5.54 %			4.71 %			5.51 %			4.16 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Table 4.12. Effect of *e*CO_2 and *e*Temp on larval excretion of *S. litura* in Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1002.38	1287.22	**1144.80**	1073.78	1356.88	**1215.33**	1132.20	1386.36	**1259.28**	1069.45	1343.49	**1206.47**
29 ± 1°C	914.27	1205.42	**1059.84**	994.50	1247.42	**1120.96**	1038.83	1277.46	**1158.15**	982.53	1243.43	**1112.98**
31 ± 1°C	860.62	1153.00	**1006.81**	875.29	1178.06	**1026.67**	888.00	1193.44	**1040.72**	874.64	1174.83	**1024.73**
33 ± 1°C	748.07	1098.06	**923.06**	827.13	1122.21	**974.67**	850.12	1154.95	**1002.53**	808.44	1125.07	**966.75**
35 ± 1°C	682.63	1056.09	**869.36**	729.70	1077.77	**903.74**	736.35	1036.71	**886.53**	716.23	1056.86	**886.54**
Mean	**841.59**	**1159.95**		**900.07**	**1196.46**		**929.10**	**1209.78**		**890.25**	**1188.73**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	1509.918*	5.79	16.14	1287.171*	5.84	16.27	998.801*	6.28	17.50	3925.45*	3.37	9.39
Temperature (°C)	141.754*	9.16	25.52	175.729*	9.24	25.73	209.608*	9.93	27.66	547.99*	5.33	14.84
Interaction (CO_2 + Temp(°C))	4.897*	12.95	36.09	3.502*	13.06	36.39	2.570*	14.04	39.12	9.07*	7.53	20.98
CV	6.47 %			6.23 %			6.57 %			3.62 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

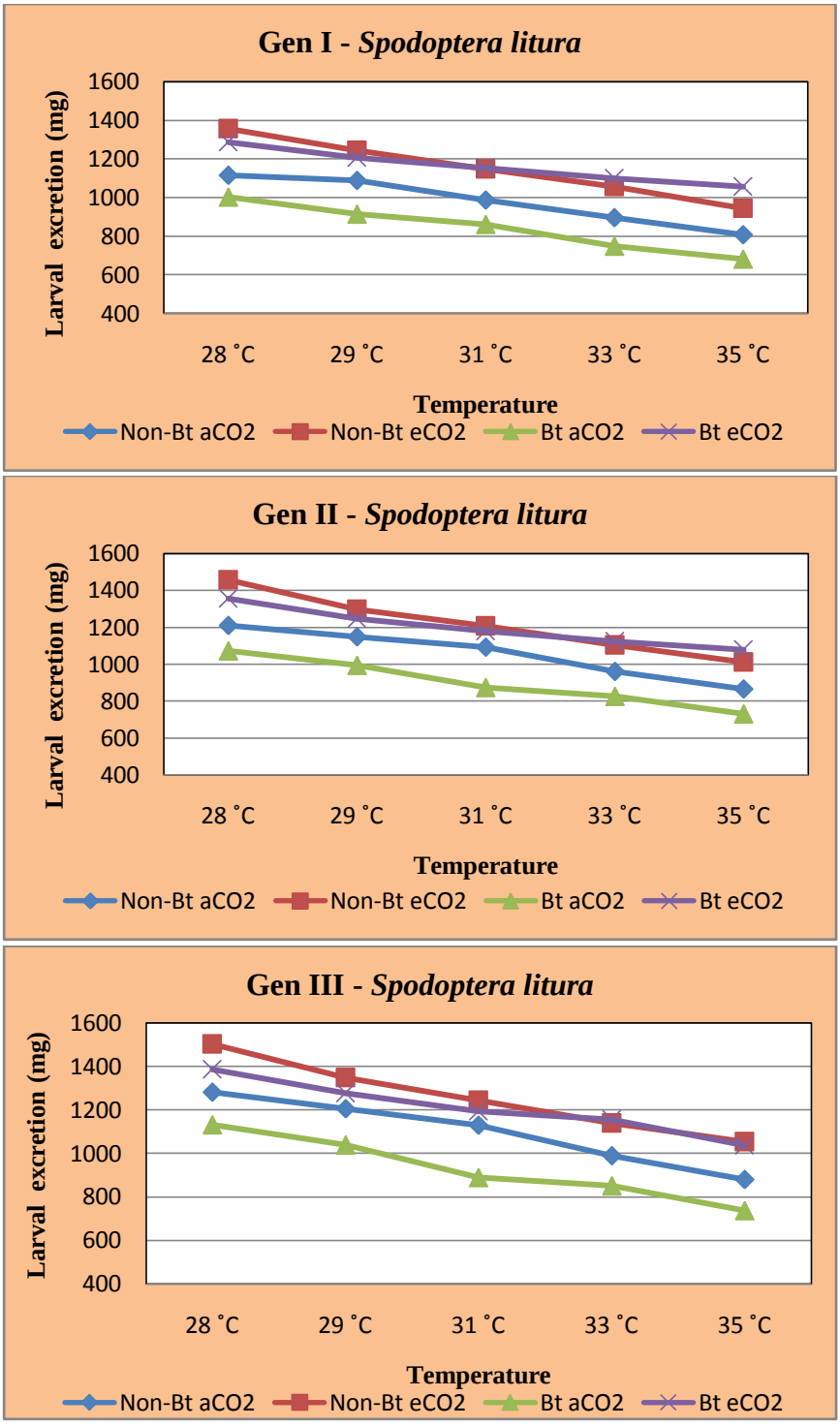


Fig. 4.8. Effect of *e*CO_2 and *e*Temp on larval excretion of *S. litura* on non-Bt and Bt cotton in first, second and third generation

First generation: The *e*CO_2 and *e*Temp has shown significant effect on larval excretion. In Bt cotton, at *a*CO_2, larval excretion ranged from 1002.38 to 682.63 mg within a temperature range of 28 to 35 ºC. Frass output increased with enhancement of CO_2, and decreased with temperature. At *e*CO_2, a decrease in larval excretion was observed in the range of 1287.22 to 1056.09 mg with highest and lowest at temperature 28 and 35 °C, respectively. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval excretion.

Second generation: At *a*CO_2, decrease in *S. litura* larval excretion was recorded in the range of 1073.78 to 729.70 mg with highest at 28 ºC and lowest at 35 ºC. At *e*CO_2, larval excretion ranged from 1356.88 and 1077.77 mg within a temperature range of 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and interaction showed significant influence on larval excretion.

Third generation: At *a*CO_2, larval excretion varied from 1132.20 to 736.35 mg with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, a decreased larval excretion was recorded in the range of 1386.36 to 1036.71 mg with increase in temperature from 28 to 35 °C. The individual effect of *e*CO_2 and *e*Temp and interaction showed significant influence on larval excretion.

Mean of generations: The mean of three generations in Bt cotton, also indicated that the larval excretion by larvae has decreased significantly (1069.45, 982.53, 874.64, 808.44 and 716.23 mg, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to decrease of 8.13, 18.22, 24.41 and 33.03 %. Similarly, under *e*CO_2 also the larval excretion decreased significantly (1343.49, 1243.43, 1174.83, 1125.07 and 1056.86 mg) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 7.45, 12.55, 16.26 and 21.33 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval excretion. And larval excretion was significantly lower in *a*CO_2 than *e*CO_2. However, the decrease in larval excretion with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the larval excretion under *e*CO_2 was higher (25.62, 26.55, 34.32, 39.17 and 47.56 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It implies that mean larval excretion of *S. litura* in Bt cotton increased with increase in CO_2 (by 26 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 1 %. Larval excretion increased with every generation and

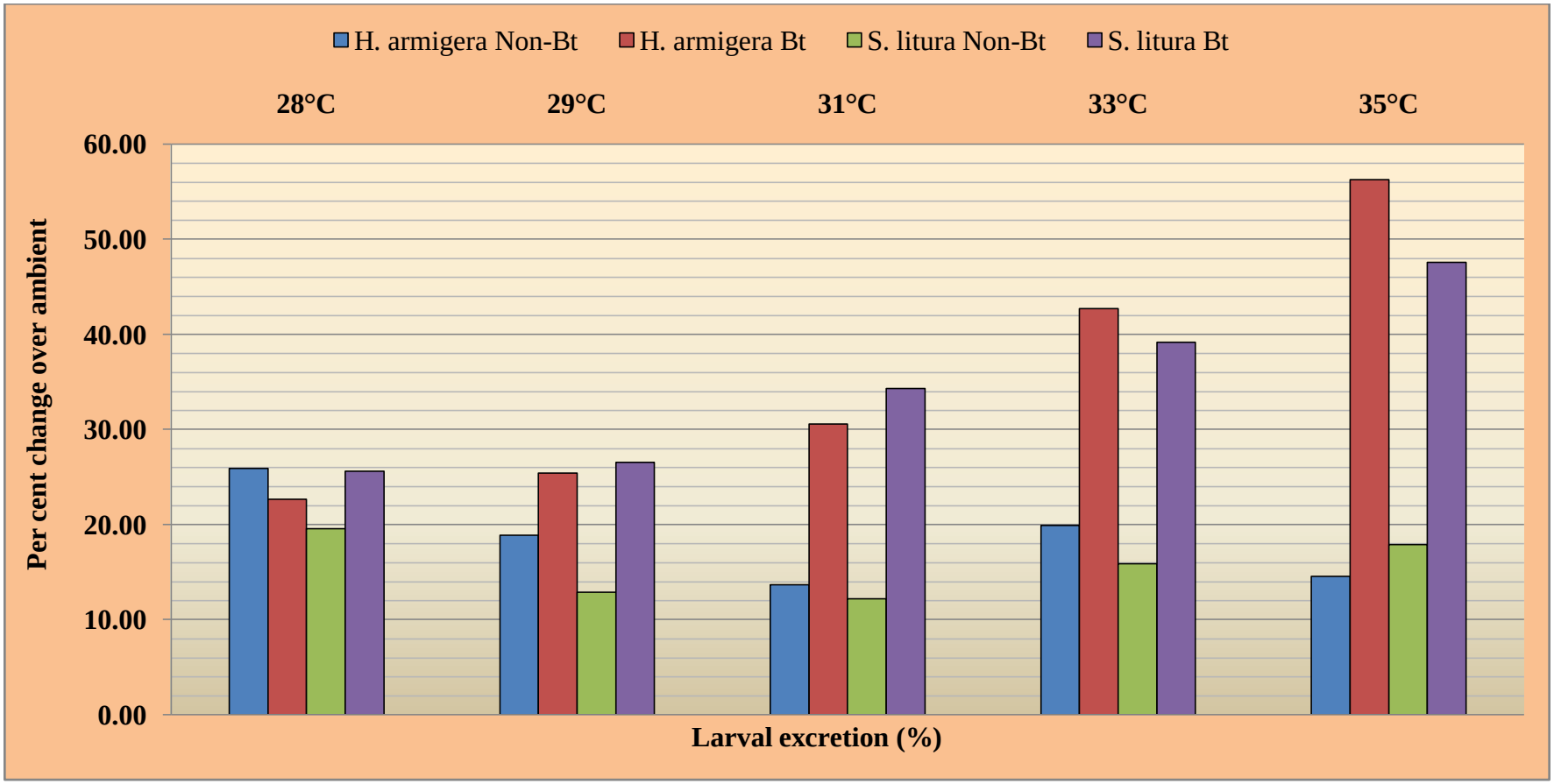


Fig. 4.9. Effect of *e*CO_2 and *e*Temp on mean larval excretion of *H. armigera* and *S. litura* on non-Bt and Bt cotton

was highest in the third generation (1002, 1074 and 1132 mg; 1287, 1357 and 1386 mg; and 1056, 1078 and 1037 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). The findings are in line with those of Wu *et al*. (2009) who recorded significantly lower larval frass in the first (F = 24.74, d.f. = 1, 6, P = 0.0025), second (F = 14.86, d.f. = 1, 6, P = 0.0084) and third (F = 20.82, d.f. = 1, 6, P = 0.0038) generations of *S. exigua* fed on Bt compared with non-Bt cotton.

4.1.4 Pupal weights: The effect of *e*CO_2 and *e*Temp conditions on the pupal weights of test insects under *e*CO_2 and *e*Temp conditions was presented in the Tables 4.13 - 4.16 and Fig. 4.10 - 4.11. With increase in CO_2, pupal weights decreased. With increase in test temperatures, under both CO_2 conditions, the pupal weight of test insects gradually decreased. Comparative performance of both the test insects was presented in Fig. 4.12.

4.1.4.1 Effect on pupal weight of *H. armigera* in non-Bt cotton

The data pertaining to pupal weight of *H. armigera* in non-Bt cotton for first, second and third generation was presented in Table 4.13 and Fig. 4.10.

First generation: The *e*CO_2 and *e*Temp has shown significant effect on pupal weight. At *a*CO_2, the pupal weights varied from 235.52 to 185.84 mg with highest and lowest at temperatures 28 and 35 °C, respectively. At *e*CO_2, decrease in pupal weight was recorded in the range of 207.80 to 181.04 mg with increase in temperature from 28 to 35 °C. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2.

Second generation: At *a*CO_2, pupal weight ranged between 238.92 and 188.08 mg corresponding to a temperature range of 28 to 35 ºC. At *e*CO_2, decrease in pupal weight was recorded in the range of 215.20 to 188.60 mg with increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has significant effect and interaction showed non-significant influence on pupal weight.

Third generation: At *a*CO_2, pupal weight varied from 240.76 to 204.20 mg corresponding to a temperature range of 28 to 35 ºC. At *e*CO_2, decrease in pupal weight was recorded in the range of 220.40 to 190.12 mg with increase in temperature from 28 to 35 °C. The individual effect of *e*CO_2 and *e*Temp has significant effect and interaction showed non-significant influence on pupal weight.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that at *a*CO_2 with increase in temperature (upto 35 ºC) the pupal weight has decreased significantly (238.40, 230.59, 214.88, 208.73 and 192.71 mg, respectively) corresponding to 3.28, 9.87, 12.44 and 19.17 %. Similarly, under *e*CO_2 also the pupal weight decreased significantly (214.47, 207.39, 199.84, 192.79 and 186.59 mg) with increase in temperature (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 3.30, 6.82, 10.11 and 13.00 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2. Further, the pupal weight under *e*CO_2 was lower (10.04, 10.06, 7.00, 7.64 and 3.18 %, at 28, 29, 31, 33 and 35 ºC, respectively) compared to that of *a*CO_2.

It implies that, mean pupal weight of *H. armigera* decreased with increase in both CO_2 (by 10 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 21 %. Similar observations were made by Chen *et al*. (2004) that *H. armigera* pupal weight decreased from *a*CO_2 (193 in female and 174 mg in male) to *e*CO_2 (186 in female and 166 mg in male) in wheat. Wu *et al*. (2006) also reported that CO_2 caused signficant decrease in pupal weight of *H. armigera* on spring wheat from 190-200 mg (*a*CO_2) to 170-180 mg (*e*CO_2). Ghazanafar *et al.* (2020) reported that, with increase in temperature, *H. virescens* mean pupal weight reduced by 44.44 % (270 mg at 25 °C and 150 mg at 35 °C). Mallikarjuna *et al*. (2020) observed that *H. armigera* pupal weights reduced from reference ambient conditions (231 mg) to *e*CO_2 +*e*Temp (169 mg) with 26.83 % reductions in weight. Paradoxically, Akbar (2016) reported that *H. armigera* pupal weights reduced from 350 to 330 mg with 5 °C rise in temperature and enhanced from 320 to 340 mg with doubling of ambient CO_2. From the data, pupal weight is noticed to increase with every generation and was highest in the third (236, 239 and 241 mg; 208, 215 and 220 mg; and 181, 189 and 190 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). Pupal weight, regarded as a fitness indicator seems to get affected with a probability for development of weaker adult moths that may not produce healthy offspring. But with increase in generation number, pupae seem to acquire more weight as part of adaptation to climate change.

Table 4.13. Effect of *e*CO_2 and *e*Temp on pupal weight of *H. armigera* in non-Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	235.52	207.80	**221.66**	238.92	215.20	**227.06**	240.76	220.40	**230.58**	238.40	214.47	**226.43**
29 ± 1°C	227.40	196.68	**212.04**	229.16	210.12	**219.64**	235.20	215.36	**225.28**	230.59	207.39	**218.99**
31 ± 1°C	213.80	190.44	**202.12**	215.20	206.76	**210.98**	215.64	202.32	**208.98**	214.88	199.84	**207.36**
33 ± 1°C	206.76	187.36	**197.06**	209.32	192.84	**201.08**	210.12	198.16	**204.14**	208.73	192.79	**200.76**
35 ± 1°C	185.84	181.04	**183.44**	188.08	188.60	**188.34**	204.20	190.12	**197.16**	192.71	186.59	**189.65**
Mean	**213.86**	**192.66**		**216.13**	**202.70**		**221.18**	**205.27**		**217.06**	**200.21**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	24.216*	3.05	8.49	8.487*	3.26	9.08	12.036*	3.24	9.04	41.08*	1.86	5.18
Temperature (°C)	9.140*	4.82	13.42	8.768*	5.16	14.36	7.655*	5.13	14.29	24.55*	2.94	8.19
Interaction (CO_2 + Temp(°C))	1.11	6.81	NS	0.86	7.29	NS	0.15	7.25	NS	1.51	4.16	NS
CV	5.73 %			4.98 %			5.35 %			6.56 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

4.1.4.2 Effect on pupal weight of *H. armigera* in Bt cotton

The data pertaining to pupal weight of *H. armigera* in Bt cotton for first, second and third generation was presented in Table 4.14 and Fig. 4.10.

First generation: The *e*CO_2 and *e*Temp has shown significant effect on pupal weight. At *a*CO_2, pupal weight ranged between 229.00 and 190.65 mg corresponding to temperature range of 28-35 ºC. At *e*CO_2, decrease in pupal weight was recorded in the range of 208.13 to 182.02 mg with increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2.

Second generation: At *a*CO_2, pupal weight varied from 235.75 to 200.05 mg corresponding to a temperature range of 28 to 35 ºC. At *e*CO_2, decreased pupal weight was recorded in the range of 214.11 to 189.26 mg with decrease in temperature from 28- 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interaction effect showed significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2.

Third generation: At *a*CO_2, pupal weight ranged between 238.88 and 202.82 mg with highest and lowest at temperature 28 and 35 ºC, respectively. At *e*CO_2, pupal weight decreased from 219.22 to 193.22 mg with corresponding increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant effect but the interaction showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2.

Mean of generations: The mean of three generations in Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the pupal weight decreased significantly (234.54, 226.23, 218.44, 212.51 and 197.84 mg, respectively) corresponding to 3.54, 6.86, 9.40 and 15.65 %. Similarly, under *e*CO_2 also the pupal weight decreased significantly (213.82, 208.57, 202.41, 194.48 and 188.17 mg) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 2.45, 5.34, 9.04 and 12.00 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2. However, the decrease in pupal weight with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the pupal weight under *e*CO_2 was lower (8.84, 7.81, 7.34, 8.48 and 4.89 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

Table 4.14. Effect of *e*CO_2 and *e*Temp on pupal weight of *H. armigera* in Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	229.00	208.13	**218.56**	235.75	214.11	**224.93**	238.88	219.22	**229.05**	234.54	213.82	**224.18**
29 ± 1°C	224.70	199.18	**211.94**	224.73	210.92	**217.82**	229.27	215.62	**222.44**	226.23	208.57	**217.40**
31 ± 1°C	219.57	194.71	**207.14**	221.69	205.47	**213.58**	214.07	207.05	**210.56**	218.44	202.41	**210.43**
33 ± 1°C	213.75	191.11	**202.43**	212.88	195.17	**204.02**	210.89	197.16	**204.02**	212.51	194.48	**203.49**
35 ± 1°C	190.65	182.02	**191.34**	200.05	189.26	**194.65**	202.82	193.22	**203.02**	197.84	188.17	**196.34**
Mean	**215.53**	**197.03**		**219.02**	**202.98**		**221.18**	**206.45**		**218.58**	**202.15**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	47.197*	1.90	5.31	29.493*	2.09	5.82	29.423*	1.92	5.35	97.53*	1.18	3.28
Temperature (°C)	11.629*	3.01	8.39	12.917*	3.30	9.20	14.357*	3.04	8.46	35.03*	1.86	5.18
Interaction (CO_2 + Temp(°C))	3.497*	4.26	11.86	0.38	4.67	NS	0.74	4.30	NS	1.23	2.63	NS
CV	4.93 %			5.28 %			4.96 %			6.25 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

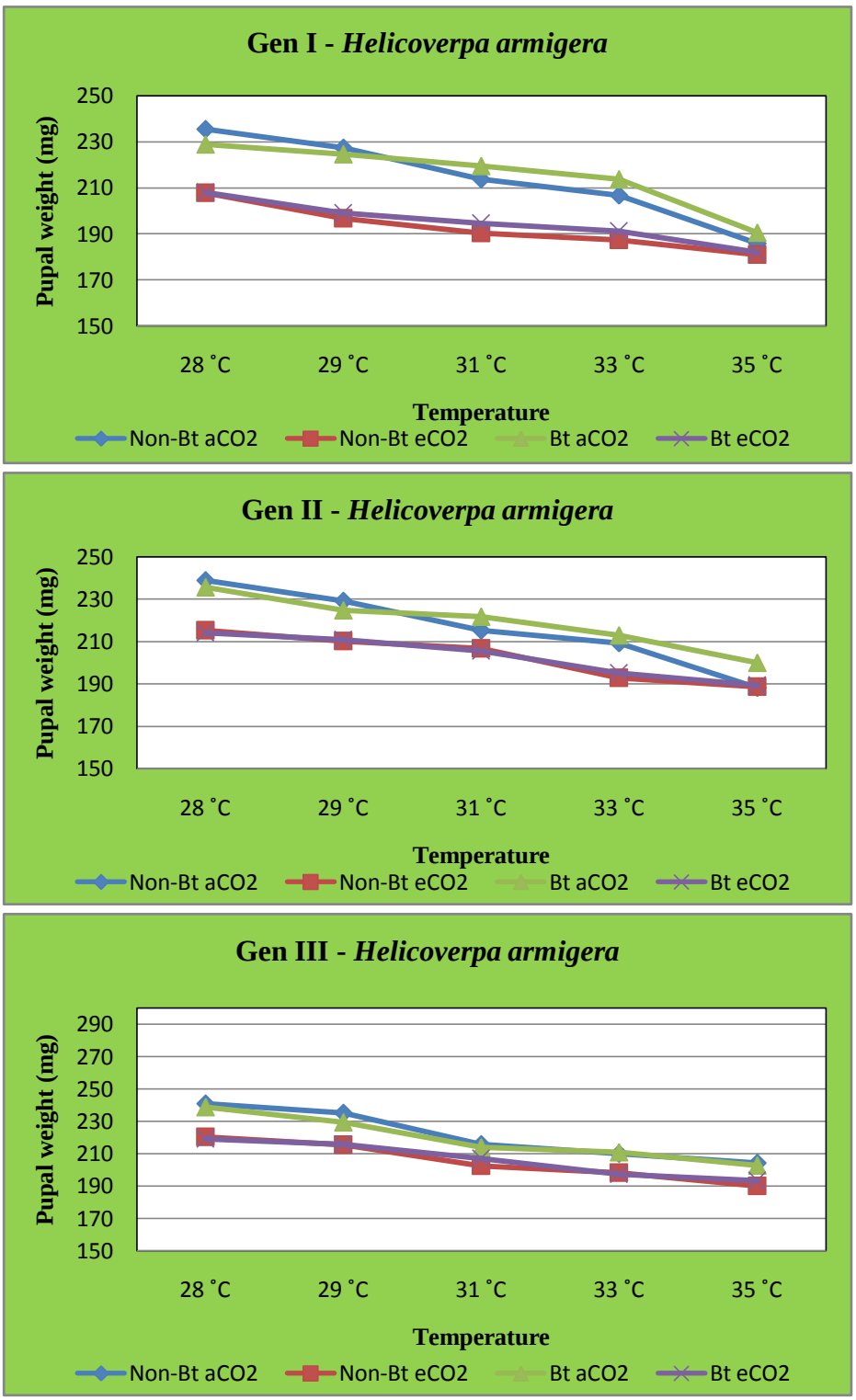


Fig. 4.10. Effect of *e*CO_2 and *e*Temp on pupal weight of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

When raised on transgenic cotton, lepidopterans had decreased pupal weight and fecundity (Stewart *et al*. 2001 and Carrie *et al*. 2003). The information generated revealed that, mean pupal weight of *H. armigera* on Bt cotton decreased with increase in both CO_2 (by 8 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 20 %. The pupal weight in interaction effect *e*CO_2 + eTemp was higher in Bt over non-Bt cotton. Liu *et al*. (2005) reported that Bt cotton may affect *H. armigera* pupal weight as it decreased when fed on a diet containing Cry1Ac toxin. Chen *et al*. (2007) reported that pupal weight reduced significantly in bollworms in Bt cotton over non-Bt cotton, regardless of the CO_2 level. Stiling and Cornelissen (2007) demonstrated that *e*CO_2 conditions can result in a decrease of almost 5.0 % in pupal weight from a meta analysis of several herbivore plant interactions. Ghazanafar *et al.* (2020) noted that when Bt leaf powder was mixed into diet, *H. virescens* pupal weight reduced significantly by 20–35 %. The study recorded decreased pupal weight with every generation and was highest in the third generation (229, 236 and 239 mg; 208, 214 and 219 mg; and 182, 189 and 193 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively).

4.1.4.3 Effect on pupal weight of *S. litura* in non-Bt cotton

The data pertaining to pupal weight of *S. litura* in non-Bt cotton for all three generations was presented in Table 4.15 and Fig. 4.11.

First generation: In non-Bt cotton, the *e*CO_2 and *e*Temp has shown significant effect on pupal weight. At *a*CO_2, pupal weight ranged between 246.88 and 200.12 mg with corresponding to a temperature range from 28 to 35ºC. At *e*CO_2, decrease in pupal weight was recorded in the range of 220.72 to 194.24 mg with increase in temperatures from 28 to 35 °C. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on pupal weight and was higher in *a*CO_2 than *e*CO_2.

Second generation: At *a*CO_2, pupal weight increased within a range of 247.48 to 209.28 mg with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, highest pupal weight was recorded with 227.00 at 28 °C and lowest at 35 °C with 198.68 mg. The individual effect of *e*CO_2 and *e*Temp has shown significant effect but the interaction showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2.

Table 4.15. Effect of *e*CO_2 and *e*Temp on pupal weight of *S. litura* in non-Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	246.88	220.72	**233.80**	247.48	227.00	**237.24**	251.92	232.96	**242.44**	248.76	226.89	**237.83**
29 ± 1°C	233.24	211.56	**222.40**	238.32	225.36	**231.84**	240.16	228.24	**234.20**	237.24	221.72	**229.48**
31 ± 1°C	228.68	206.92	**217.80**	230.08	215.28	**222.68**	228.04	217.72	**222.88**	228.93	213.31	**221.12**
33 ± 1°C	220.68	202.64	**211.66**	222.20	203.72	**212.96**	225.08	210.44	**217.76**	222.65	205.60	**214.13**
35 ± 1°C	200.12	194.24	**197.18**	209.28	198.68	**203.98**	211.20	202.56	**206.88**	206.87	198.49	**202.68**
Mean	**225.92**	**207.21**		**229.47**	**214.00**		**231.28**	**218.38**		**228.89**	**213.20**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	14.556*	3.47	9.66	8.484*	3.75	10.46	8.562*	3.12	8.68	27.40*	2.12	5.90
Temperature (°C)	6.095*	5.48	15.27	5.220*	5.94	16.54	7.969*	4.93	13.73	16.43*	3.35	9.33
Interaction (CO_2 + Temp(°C))	0.50	7.75	NS	0.12	8.39	NS	0.17	6.97	NS	0.52	4.74	NS
CV	6.17 %			5.93 %			4.53 %			5.72 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

Third generation: At *a*CO_2, decrease in pupal weight was recorded with increase in temperature with highest pupal weight at 28 °C (251.92 mg) and lowest at 35 °C (211.20 mg), respectively. At *e*CO_2, pupal weight ranged from 232.96 to 202.56 mg corresponding to temperature range from 28 to 35 °C. The individual effect of *e*CO_2 and *e*Temp has shown significant effect but the interaction showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2.

Mean of generations: The mean of three generations of *S. litura* in non-Bt cotton, also indicated that the pupal weight has decreased significantly (248.76, 237.24, 228.93, 222.65 and 206.87 mg, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 4.63, 7.97, 10.49 and 16.84 %. Similarly, under *e*CO_2, the pupal weight decreased significantly (226.89, 221.72, 213.31, 205.60 and 198.49 mg) with increase in temperatures (28, 29, 31, 33 and 35 °C) which has corresponded to a decrease of 2.28, 5.99, 9.38 and 12.52 %. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on pupal weight and was lower in *a*CO_2 than *e*CO_2. Further, the pupal weight under *e*CO_2 was lower (8.79, 6.54, 6.83, 7.66 and 4.05 %, at corresponding temperatures, respectively) compared to that of *a*CO_2.

These results infer that, mean pupal weight of *S. litura* decreased with increase in both CO_2 (by 9 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 20 %. Similar decrease in pupal weight by 59 % was reported by Ghazanafar *et al*. (2020) in *S. littoralis* in cotton (365 mg at 25 °C and 149 mg at 35 °C). Ismail (2021) recorded decrease in pupal weight of *S.littoralis* on castor from 293.6 mg (25 °C) to 182.5 mg (35 °C).Pupal weight has not changed by a greater degree along the generations and was highest in the third generation (247, 247 and 252 mg; 221, 227 and 233 mg; and 194, 199 and 203 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). The findings are also in conformity with Wu *et al*. (2009) who recorded pupal weight that lied in the range 77-79 mg which did not vary significantly among three generations of *S. exigua* on non-transgenic cotton (F = 0.29, p = 0.7570) and transgenic Bt cotton (F = 2.44, p = 0.1423).

4.1.4.4 Effect on pupal weight of *S. litura* in Bt cotton

The data pertaining to pupal weight of *S. litura* in Bt cotton for all three generations was presented in Table 4.16 and Fig. 4.11.

First generation: In Bt cotton, the *e*CO_2 and *e*Temp has shown significant effect on pupal weight. At *a*CO_2, pupal weight decreased from 235.50 to 196.95 mg with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, a decrease in pupal weight from 215.22 to 190.68 mg was recorded with highest and lowest pupal weight at 28 and 35 ºC, respectively. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on pupal weight and was higher in *a*CO_2 than *e*CO_2.

Second generation: At *a*CO_2, pupal weight ranged from 239.67 to 205.35 mg with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, pupal weight decreased from 221.00 to 194.50 mg with increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant effect but the interaction showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2.

Third generation: At *a*CO_2, pupal weight varied from 244.30 to 216.45 mg with highest and lowest at temperature 28 and 35 ºC, respectively. At *e*CO_2, highest pupal weights were recorded at 28 ºC with 225.36 mg and lowest at 35 º C with 198.57 mg. The individual effect of *e*CO_2 and *e*Temp has shown significant effect but the interaction showed non-significant influence on pupal weight and was significantly lower in *e*CO_2 than *a*CO_2.

Mean of generations: The mean of three generations in Bt cotton, also indicated that the pupal weight has decreased significantly (239.82, 232.53, 224.01, 219.22 and 206.25 mg, respectively) with increase in temperature (29, 31, 33 and 35 °C) corresponding to a decrease of 3.04, 6.59, 8.59 and 14.00 %. Similarly, under *e*CO_2 also the pupal weight decreased significantly (220.53, 215.93, 208.27, 200.57 and 194.58 mg) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 2.08, 5.56, 9.05 and 11.76 %. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on pupal weight and was higher in *a*CO_2 than *e*CO_2. However, the decrease in pupal weight with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the pupal weight under *e*CO_2 was lower (8.05, 7.14, 7.03, 8.50 and 5.66 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to *a*CO_2.

Table 4.16. Effect of *e*CO_2 and *e*Temp on pupal weight of *S. litura* in Bt cotton

Temperature	First generation (mg)			Second generation (mg)			Third generation (mg)			Mean (mg)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	235.50	215.22	**225.36**	239.67	221.00	**230.33**	244.30	225.36	**234.83**	239.82	220.53	**230.18**
29 ± 1°C	229.27	209.25	**219.26**	232.50	217.92	**225.21**	235.83	220.62	**228.22**	232.53	215.93	**224.23**
31 ± 1°C	226.54	202.73	**214.64**	225.29	209.44	**217.36**	220.21	212.63	**216.42**	224.01	208.27	**216.14**
33 ± 1°C	221.47	195.83	**208.65**	218.88	200.47	**209.67**	217.30	205.42	**211.39**	219.22	200.57	**209.90**
35 ± 1°C	196.95	190.68	**193.81**	205.35	194.50	**199.93**	216.45	198.57	**207.51**	206.25	194.58	**200.42**
Mean	**221.94**	**202.74**		**224.33**	**208.66**		**226.83**	**212.51**		**224.37**	**207.97**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	35.440*	2.28	6.35	24.944*	2.22	6.18	21.934*	2.16	6.02	72.95*	1.36	3.78
Temperature (°C)	11.142*	3.61	10.05	11.972*	3.51	9.77	11.354*	3.42	9.52	29.78*	2.15	5.98
Interaction (CO_2 + Temp(°C))	1.11	5.10	NS	0.21	4.96	NS	0.46	4.83	NS	0.49	3.04	NS
CV	5.01 %			5.46 %			5.04 %			6.02 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

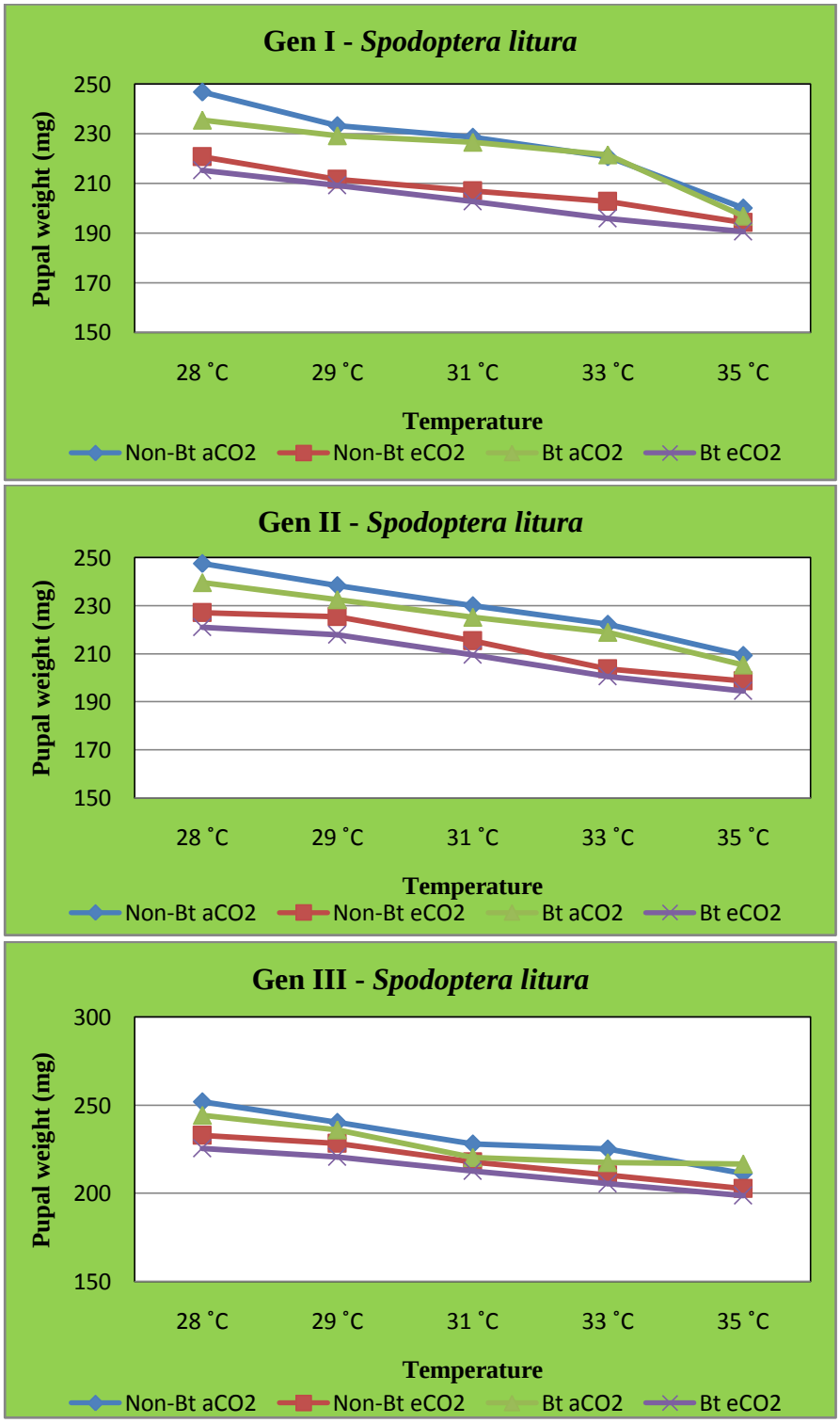


Fig. 4.11. Effect of *e*CO_2 and *e*Temp on pupal weight of *S. litura* on non-Bt and Bt cotton in first, second and third generation

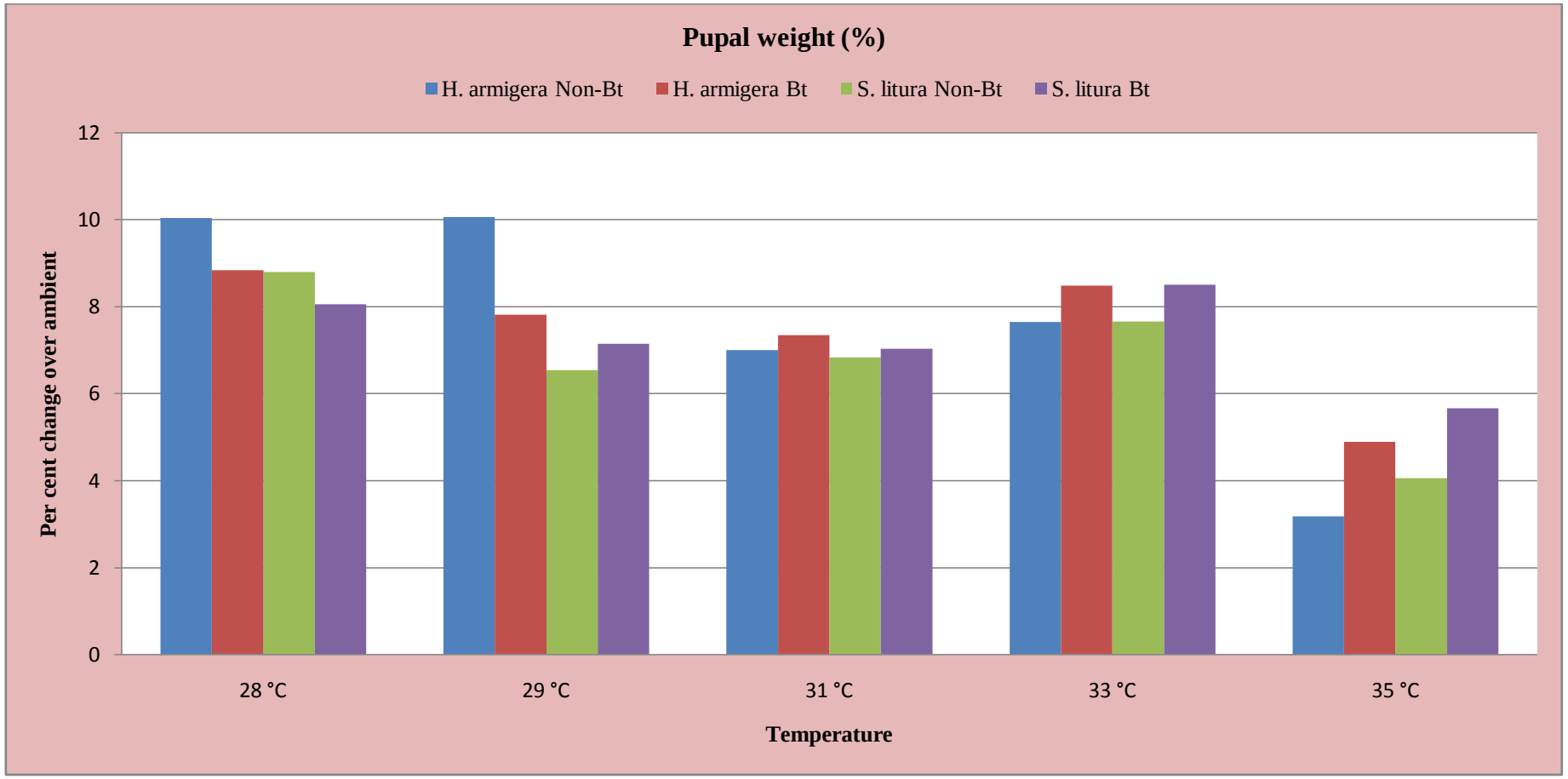


Fig. 4.12. Effect of *e*CO_2 and *e*Temp on mean pupal weight of *H. armigera* and *S. litura* on non-Bt and Bt cotton

It can be inferred from the data that mean pupal weight of *S. litura* decreased with increase in both CO_2 (by 8 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 19 %. It was also observed that pupal weight was less in Bt over non-Bt cotton. The present findings are in line with Ghazanafar *et al*. (2020) who observed that when pulverized leaf powder from Bt-cotton was mixed into the diet of *S. littoralis*, pupal weight at 25 °C was 16 % lower than that on non-Bt treatment. From the data obtained with our experiments, pupal weight of *S. litura* increased with every generation and was highest in the third generation, but was not substantially high (236, 240 and 244 mg; 215, 221 and 225 mg; and 191, 195 and 199 mg in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp, respectively). There is no substantial variation in the pupal weights on Bt and non-Bt cotton. Similar observation was made by Wu *et al*., (2009) who recorded lower pupal weight in the first (69 mg), second (69 mg) and third (71 mg) generations of *S. exigua* on Bt cotton, but were similar to non-Bt cotton. Rajesh *et al.* (2007) reported that application of *Bacillus thuringiensis* var. kurstaki 0.2 % in various treatment combinations with 5 per cent each of of neem oil, citronella oil, karanj oil, cottonseed oil and sesamum oil on *S. litura* resulted in significantly reduction in pupal weight reduction (31.0-15.1 %) over untreated check.

4.1.5 Larval Duration: The data on the effect of *e*CO_2 and *e*Temp conditions on the larval duration of *H. armigera* and *S. litura* is presented in the Tables 4.17 - 4.20 and Fig 4.13 - 4.14. With increase in test temperatures (28, 29, 31, 33 and 35 °C), under both *a*CO_2 (380 $\pm$ 25 ppm) and *e*CO_2 (550 $\pm$ 25 ppm) conditions, larval duration of test insects gradually decreased. Comparative performance of both the test insects was presented in Fig. 4.15.

4.1.5.1 Effect on larval duration of *H. armigera* in non-Bt cotton

The data pertaining to larval duration of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.17 and Fig. 4.13.

First generation: The *e*CO_2 and *e*Temp has shown significant impact on larval duration. At *a*CO_2, larval duration ranged from 14.60 to 11.44 days with highest larval duration at temperature 28 and lowest at 35 ºC. At *e*CO_2, decrease in larval duration was recorded in the range of 16.52 to 13.48 days with increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed non-significant effect on larval duration. And larval duration was higher in *e*CO_2 than *a*CO_2.

Second generation: In non-Bt cotton, larval duration varied from 14.72 to 11.68 days with highest and lowest larval duration at temperatures 28 and 35 ºC, respectively. At *e*CO_2, highest larval duration was recorded at 28 ºC with 16.68 days and lowest at 35 ºC with 13.68 days, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant effect but the interaction showed non-significant influence on larval duration and was significantly lower in *e*CO_2 than *a*CO_2. And larval duration was higher in *e*CO_2 than *a*CO_2.

Third generation: At *a*CO_2, decrease in larval duration from 14.68 to 11.80 days was recorded corresponding to increase in temperature from 28 to 35 ºC. At *e*CO_2, larval duration ranged between 16.80 to 13.76 days with highest larval duration at temperature 28 ºC and lowest at 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant effect but the interaction showed non-significant influence on larval duration and was significantly lower in *e*CO_2 than *a*CO_2. And larval duration was higher in *e*CO_2 than *a*CO_2.

Mean of generations: The mean of three generations of *H. armigera* in non-Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the larval duration has decreased significantly (14.67, 14.32, 13.48, 12.61 and 11.64 days, respectively) corresponding to 2.36, 8.09, 14.00 and 20.64 %. Similarly, under *e*CO_2 also the larval duration decreased significantly (16.67, 16.32, 15.39, 14.53 and 13.64 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 2.08, 7.68, 12.80 and 18.16 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant effect on larval duration. And larval duration was higher in *e*CO_2 than *a*CO_2. However, the decrease in larval duration with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the larval duration under *e*CO_2 was higher (13.64, 13.97, 14.14, 15.22 and 17.18 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

Table 4.17. Effect of *e*CO_2 and *e*Temp on larval duration of *H. armigera* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	14.60	16.52	**15.56**	14.72	16.68	**15.70**	14.68	16.80	**15.74**	14.67	16.67	**15.67**
29 ± 1°C	14.20	16.16	**15.18**	14.32	16.36	**15.34**	14.44	16.44	**15.44**	14.32	16.32	**15.32**
31 ± 1°C	13.28	15.24	**14.26**	13.56	15.40	**14.48**	13.60	15.52	**14.56**	13.48	15.39	**14.43**
33 ± 1°C	12.48	14.36	**13.42**	12.64	14.52	**13.58**	12.72	14.72	**13.72**	12.61	14.53	**13.57**
35 ± 1°C	11.44	13.48	**12.46**	11.68	13.68	**12.68**	11.80	13.76	**12.78**	11.64	13.64	**12.64**
Mean	**13.20**	**15.15**		**13.38**	**15.32**		**13.44**	**15.44**		**13.34**	**15.30**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	1066.316*	0.04	0.12	999.419*	0.04	0.12	1139.81*	0.04	0.12	2737.51*	0.03	0.07
Temperature (°C)	360.824*	0.07	0.19	327.986*	0.07	0.19	340.696*	0.07	0.19	879.68*	0.04	0.12
Interaction (CO_2 + Temp(°C))	0.20	0.10	NS	0.36	0.10	NS	0.32	0.09	NS	0.32	0.06	NS
CV	3.33 %			3.39 %			3.24 %			2.07 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

These results infer that, mean larval duration of *H. armigera* increased with increase in both CO_2 (by 14 %) and decreased with increase in temperature and ultimately decreased with *e*CO_2 + *e*Temp by 7 %. The results are on par with the observations of Wu *et al*. (2006) reported the *H. armigera* larval duration in three generations on spring wheat is in the range of 11.3-11.7 days in *a*CO_2 (370 ppm) and 12-12.4 in *e*CO_2 (750 ppm). Observations on the pupal weight across the three generations is as follows: 15, 15 and 15 days; 17, 17 and 17 days; and 13, 14 and 14 days, respectively in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp). Abdul *et al*. (2014) also reported that larval duration of *H. armigera* increased by 10.60-12.45 % in successive four generations under *e*CO_2 compared with *a*CO_2. Ghazanafar *et al*. (2020) reported that at constant temperature of 35 ºC, larval duration significantly reduced by 34 % for *Heliothis virescens* in cotton. Viswajyothi *et al*. (2020) reported that larval duration of *Sesamia inferens* in maize increased by 5.1 % at *e*CO_2 (450 ppm) and reduced by 19.0 % at *e*Temp (32 ºC).

4.1.5.2 Effect on larval duration of *H. armigera* in Bt cotton

The data pertaining to larval duration of *H. armigera* in Bt cotton for all three generations was presented in Table 4.18 and Fig. 4.13.

First generation: The *e*CO_2 and *e*Temp has shown significant influence on larval duration. At *a*CO_2, larval duration varied from 13.42 to 11.15 days with highest and lowest larval duration at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in larval duration was recorded varying from 14.50 to 13.04 days corresponding to increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp has significant effect on larval duration. And larval duration was higher in *e*CO_2 than *a*CO_2.

Second generation: In Bt cotton, at *a*CO_2, larval duration varied from 13.65 to 11.23 days with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in larval duration was recorded from 14.77 to 13.21 days with increase in temperature from 28 to 35 ºC. And larval duration was higher in *e*CO_2 than *a*CO_2. The individual effect of *e*CO_2 and *e*Temp and the interaction has significant effect on larval duration.

Table 4.18. Effect of *e*CO_2 and *e*Temp on larval duration of *H. armigera* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	13.42	14.50	**13.96**	13.65	14.77	**14.21**	13.62	14.88	**14.25**	13.56	14.72	**14.15**
29 ± 1°C	13.20	14.18	**13.69**	13.27	14.41	**13.84**	13.54	14.53	**14.04**	13.34	14.37	**13.86**
31 ± 1°C	12.35	13.85	**13.10**	12.61	14.13	**13.37**	12.78	14.20	**13.49**	12.58	14.06	**13.32**
33 ± 1°C	12.25	13.55	**12.90**	12.35	13.72	**13.03**	12.44	13.84	**13.14**	12.35	13.70	**13.03**
35 ± 1°C	11.15	13.04	**12.09**	11.23	13.21	**12.22**	11.40	13.34	**12.37**	11.26	13.20	**12.23**
Mean	**12.47**	**13.82**		**12.62**	**14.05**		**12.76**	**14.16**		**12.62**	**14.01**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	990.685*	0.03	0.09	950.967*	0.03	0.09	893.234*	0.03	0.09	3208.17*	0.02	0.05
Temperature (°C)	230.492*	0.05	0.13	219.119*	0.05	0.14	204.171*	0.05	0.15	738.24*	0.03	0.08
Interaction (CO_2 + Temp(°C))	14.573*	0.07	0.19	11.345*	0.07	0.20	10.791*	0.07	0.21	40.29*	0.04	0.11
CV	2.58 %			2.74 %			2.75 %			1.46%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Third generation: At *a*CO_2, larval duration decreased from 14.68 to 11.80 days corresponding to decrease in temperature from 28 to 35 ºC. At *e*CO_2, decrease in larval duration was recorded from 16.80 to 13.76 days corresponding increase in temperature from 28 to 35 ºC. And larval duration was higher in *e*CO_2 than *a*CO_2. The individual effect of *e*CO_2 and *e*Temp and the interaction has significant effect on larval duration.

Mean of generations: The mean of three generations in Bt cotton, also indicated that with increase in temperature (28, 29, 31, 33 and 35 °C) the larval duration has decreased significantly (13.56, 13.34, 12.58, 12.35 and 11.26 days, respectively) corresponding to 1.67, 7.25, 8.97 and 16.98 %. Similarly, under *e*CO_2 also the larval duration decreased significantly (14.72, 14.37, 14.06, 13.70 and 13.20 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 2.33, 4.46, 6.89 and 10.33%. The interaction effect of *e*CO_2 and *e*Temp has significant effect on larval duration. And larval duration was higher in *e*CO_2 than *a*CO_2. However, the decrease in larval duration with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the larval duration under *e*CO_2 was higher (8.50, 7.77, 11.76, 10.99 and 17.20 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The results are in close proximity to Chen *et al.* (2007) who observed that *H. armigera* reared on transgenic cotton under elevated CO_2 (750 µl L^{-1}) exhibited higher consumption and longer larval duration. Similarly Yin *et al.* (2009) reported that CO_2 enrichment (750 µl/ L) significantly delayed the larval stage of *H. armigera* in spring wheat, wherein the first generation had longer developmental durations in all instars except the first (DF = (1, 137), F= 2.845, P= 0.094) and sixth instar (DF = (1, 78), F= 0.831, P= 0.365). However, in the second generation, only the developmental duration of sixth instar increased by 31.5 %. Ghazanafar *et al.* (2020) reported that larval duration of *H. armigera* prolonged by 2–3 days when Bt cotton leaf powder was mixed with semi-synthetic diet.

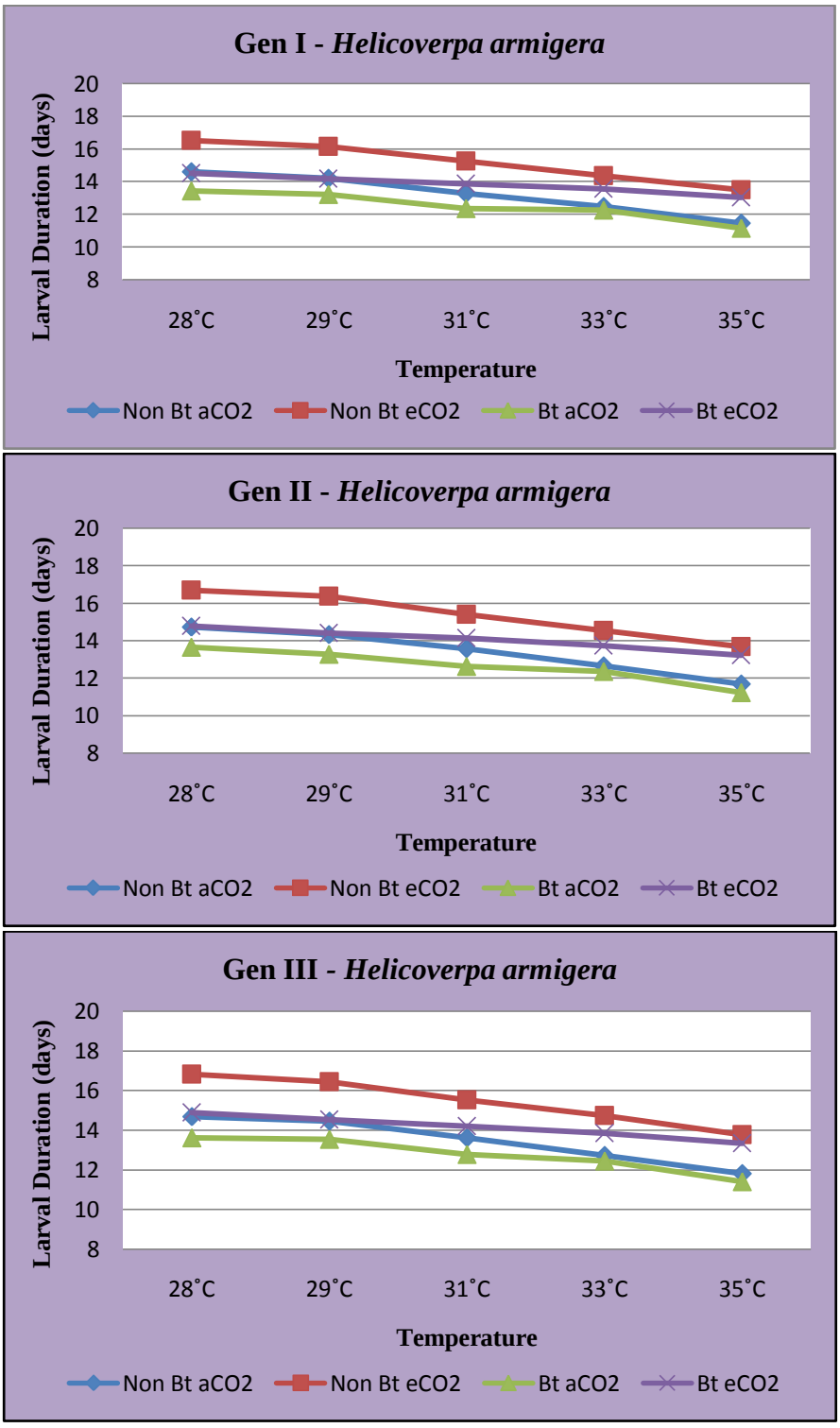


Fig. 4.13. Effect of *e*CO_2 and *e*Temp on larval duration of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

4.1.5.3 Effect on larval duration of *S. litura* in non-Bt cotton

The data pertaining to larval duration of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.19 and Fig. 4.14.

First generation: In non-Bt cotton, the *e*CO_2 and *e*Temp has shown significant effect on larval duration. It ranged from 15.32 to 12.32 days corresponding to increase in temperatures from 28 to 35 ºC. At *e*CO_2, a decrease in larval duration from 17.24 to 14.36 days was recorded against increase in temperature from 28 to 35 ºC. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on larval duration. And among *a*CO_2 and *e*CO_2, larval duration was higher at *e*CO_2.

Second generation: At *a*CO_2, decrease in larval duration ranging from 15.52 to 12.52 days was recorded with highest and lowest larval duration at temperatures 28 and 35 ºC, respectively. At *e*CO_2, larval duration ranged between 17.48 to 14.48 days with highest larval duration at temperature 28 ºC and lowest at 35 ºC. And among *a*CO_2 and *e*CO_2, larval duration was higher at *e*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant impact whereas the interactive effect has non-significant impact on larval duration.

Third generation: At *a*CO_2, larval duration varied from 15.64 to 12.72 days with highest and lowest larval durations at temperatures 28 and 35 ºC, respectively. At *e*CO_2, a decrease in larval duration was recorded from 17.64 to 14.64 days with corresponding increase in temperature from 28 to 35 ºC. And among *a*CO_2 and *e*CO_2, larval duration was higher at *e*CO_2.The individual effect of *e*CO_2 and *e*Temp has shown significant impact whereas the interactive effect has non-significant impact on larval duration.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the larval duration has decreased significantly (15.49, 14.59, 14.15, 13.56 and 12.52 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to decrease of 5.85, 8.69, 12.48 and 19.19 %. Similarly, under *e*CO_2 also the larval duration decreased significantly (17.45, 16.61, 16.12, 15.44 and 14.49 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 4.81, 7.64, 11.54 and 16.96 %. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on larval duration. And among

aCO_2 and *e*CO_2, larval duration was higher at *e*CO_2. However, the decrease in larval duration with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the larval duration under *e*CO_2 was higher (12.65, 13.89, 13.95, 13.86 and 15.76 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The observations are in accordance with Ranga Rao *et al*. (1989) who reported that *S. litura* larval duration decreased from 19.9 days (25 °C) to 12.4 days (35 °C) on groundnut. The larvae of *S. litura* were reported to have longer larval duration by two days grown under *e*CO_2 (Reddy *et al*., 2004). Srinivasa Rao *et al.* (2009) observed that larval duration prolonged from 16 days to 18 days with *e*CO_2 (700 ppm) in castor. Wu *et al*., (2009) recorded significantly longer larval duration in first (12.02), second (12.13) and third (12.2 days) generations of *S. exigua* on Bt cotton compared with non-Bt cotton. Srinivasa Rao *et al.* (2014b) reported that larval duration of *S. litura* on peanut foliage increased by 6-8 % across the four generations under *e*CO_2 compared with *a*CO_2 and was 10 % higher in the last generation compared to the first.

Tejinder and Sharma (2017) examined that *S. litura* larval duration was higher at 25:11 ºC (23.4 ± 0.8 days) than at 25:14 ºC (21.2 ± 0.9 days). Shwetha *et al*. (2017) reported that *S. litura* larval duration prolonged by two days in groundnut under *e*CO_2 over ambient. Divya (2017) recorded that larval period of *S. exigua* was significantly longer in second generation (15.75 days) followed by third (15.43 days), fourth (15.35days) and first (13.70 days) generations. Ismail (2021) recorded increase in larval duration of *S.littoralis* with temperature from 11.32 (25 °C) to 18.44 days (35 °C). Ghazanafar *et al.* (2020) reported that at constant temperature of 35 ºC, *S. littoralis* larval duration was 14 days at 25 °C and 13 days at 35 °C. Plessis *et al*. (2020) also recorded that *Spodoptera frugiperda* larval duration on sweet corn decreases from 14.86 days (26 °C) to 11.38 (30 °C) and 10.45 days (32 °C).

Table 4.19. Effect of *e*CO_2 and *e*Temp on larval duration of *S. litura* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	15.32	17.24	**16.28**	15.52	17.48	**16.50**	15.64	17.64	**16.64**	15.49	17.45	**16.47**
29 ± 1°C	14.40	16.44	**15.42**	14.64	16.68	**15.66**	14.72	16.72	**15.72**	14.59	16.61	**15.60**
31 ± 1°C	13.84	15.80	**14.82**	14.28	16.24	**15.26**	14.32	16.32	**15.32**	14.15	16.12	**15.13**
33 ± 1°C	13.40	15.20	**14.30**	13.60	15.52	**14.56**	13.68	15.60	**14.64**	13.56	15.44	**14.50**
35 ± 1°C	12.32	14.36	**13.34**	12.52	14.48	**13.50**	12.72	14.64	**13.68**	12.52	14.49	**13.51**
Mean	**13.85**	**15.80**		**14.11**	**16.08**		**14.21**	**16.18**		**14.06**	**16.02**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	856.633*	0.05	0.13	1001.644*	0.04	0.12	1061.684*	0.04	0.12	3309.75*	0.02	0.07
Temperature (°C)	222.647*	0.08	0.21	266.417*	0.07	0.19	273.16	0.07	0.19	862.55*	0.04	0.11
Interaction (CO_2 + Temp(°C))	0.45	0.11	NS	0.10	0.10	NS	0.11	0.10	NS	0.48	0.05	NS
CV	3.55 %			3.26 %			3.14 %			1.79 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

4.1.5.4 Effect on larval duration of *S. litura* in Bt cotton

The data pertaining to larval duration of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.20 and Fig. 4.14.

First generation: In Bt cotton, the *e*CO_2 and *e*Temp has shown significant effect on larval duration. At *a*CO_2, larval duration ranged between 13.88 to 11.79 days with highest and lowest larval duration at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in larval duration was recorded from 16.22 to 13.41 days with corresponding increase in temperature from 28 to 35 ºC. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval duration. And among *a*CO_2 and *e*CO_2, larval duration was higher at *e*CO_2.

Second generation: In Bt cotton, at *a*CO_2, larval duration decreased from 14.22 to 11.95 days with increase in corresponding temperatures from 28 to 35 ºC. At *e*CO_2, decrease in larval duration was recorded from 16.44 to 13.59 days with highest larval duration at temperature 28 ºC and lowest at 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant impact on larval duration.

Third generation: At *a*CO_2, decrease in larval duration was recorded from 14.30 to 12.20 days with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, larval duration ranged between 16.55 to 13.78 days with highest at temperature 28 ºC and lowest at 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant impact on larval duration.

Mean of generations: The mean of three generations in Bt cotton, also indicated that the larval duration by larvae has decreased significantly (14.13, 13.54, 12.97, 12.49 and 11.98 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 4.22, 8.25, 11.60 and 15.24 %. Similarly, under *e*CO_2, the larval duration decreased significantly (16.40, 15.67, 15.14, 14.39 and 13.59 days) with increase in temperatures (28-35 °C, respectively) which has corresponded to a decrease of 4.45, 7.68, 12.27 and 17.13 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on larval duration. And among *a*CO_2 and *e*CO_2, larval duration was higher at *e*CO_2. Further, the larval duration under *e*CO_2 was higher (16.06, 15.78, 16.79, 15.18 and 13.47 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to *a*CO_2.

Table 4.20. Effect of *e*CO_2 and *e*Temp on larval duration of *S. litura* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	13.88	16.22	**15.05**	14.22	16.44	**15.33**	14.30	16.55	**15.42**	14.13	16.40	**15.27**
29 ± 1°C	13.36	15.50	**14.43**	13.58	15.75	**14.67**	13.67	15.77	**14.72**	13.54	15.67	**14.61**
31 ± 1°C	12.69	14.80	**13.75**	13.00	15.25	**14.13**	13.21	15.38	**14.29**	12.97	15.14	**14.06**
33 ± 1°C	12.33	14.22	**13.28**	12.50	14.42	**13.46**	12.65	14.53	**13.59**	12.49	14.39	**13.44**
35 ± 1°C	11.79	13.41	**12.60**	11.95	13.59	**12.77**	12.20	13.78	**12.99**	11.98	13.59	**12.79**
Mean	**12.81**	**14.83**		**13.05**	**15.09**		**13.20**	**15.20**		**13.02**	**15.04**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	2035.65*	0.03	0.09	1392.25*	0.04	0.11	1885.13*	0.03	0.09	6308.60*	0.02	0.05
Temperature (°C)	366.53*	0.05	0.14	268.61*	0.06	0.17	342.21*	0.05	0.14	1163.66*	0.03	0.79
Interaction (CO_2 + Temp(°C))	7.62*	0.07	0.20	4.46*	0.09	0.24	6.76*	0.07	0.20	21.66*	0.04	0.11
CV	2.56 %			3.07 %			2.56 %			1.43 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

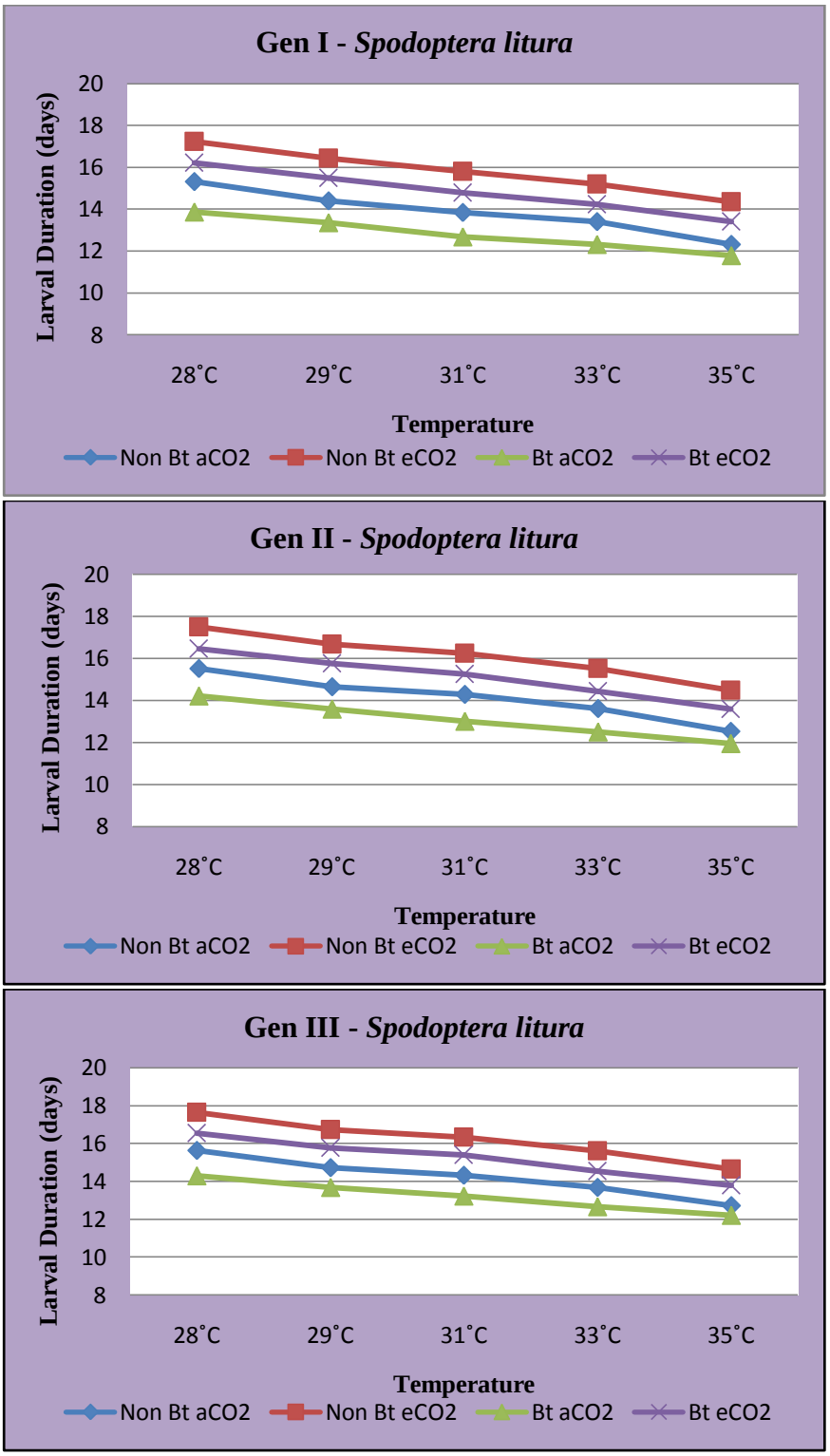


Fig. 4.14. Effect of eCO_2 and *e*Temp on larval duration of *S. litura* on non-Bt and Bt cotton in first, second and third generation

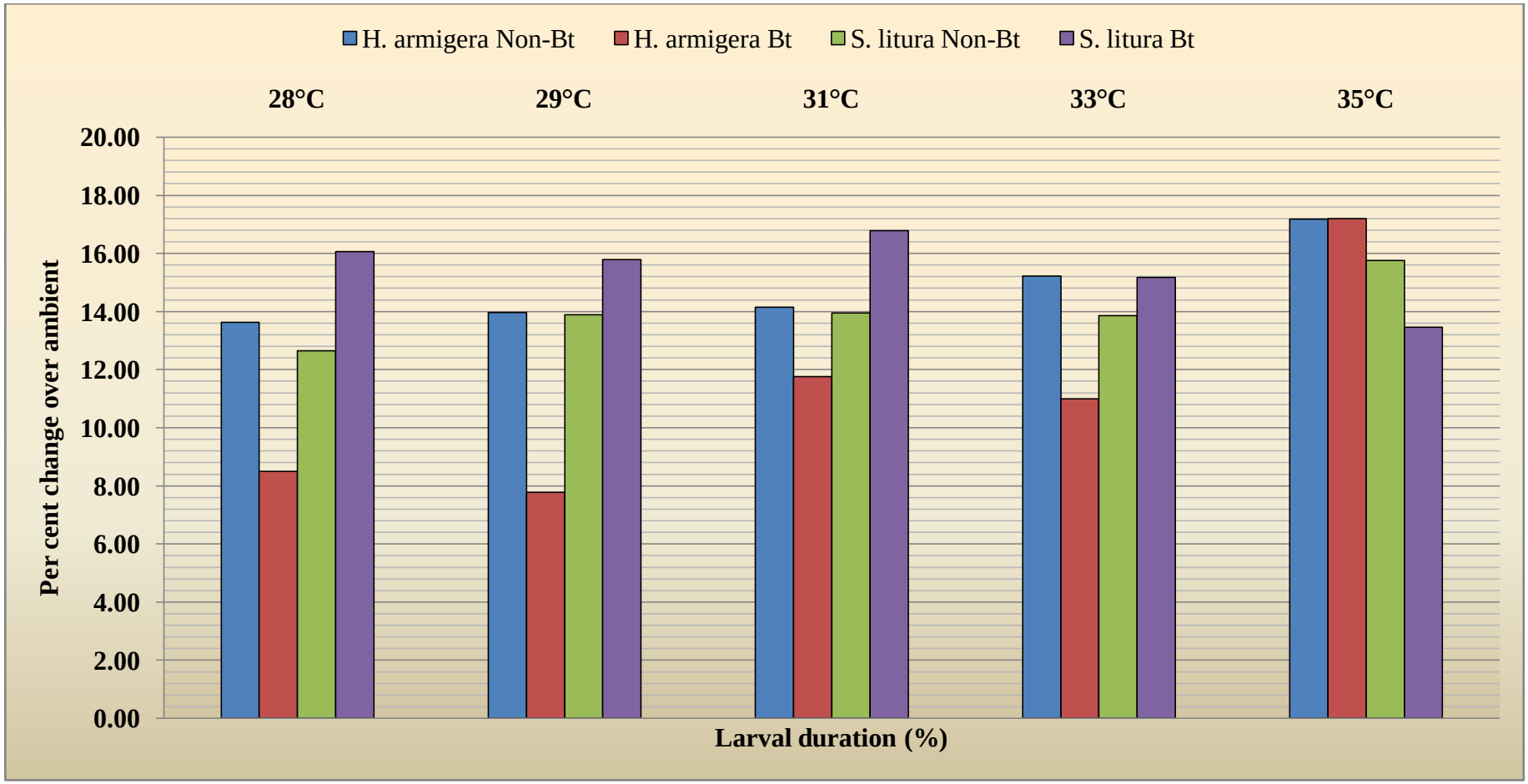


Fig. 4.15. Effect of *e*CO_2 and *e*Temp on mean larval duration of *H. armigera* and *S. litura* on non-Bt and Bt cotton

Rajesh *et al.* (2007) reported that application of *Bacillus thuringiensis* var. kurstaki 0.2 % in various treatment combinations with 5 per cent each of of neem oil, citronella oil, karanj oil, cottonseed oil and sesamum oil on *S. litura* resulted in significant extension of larval period (11.0 - 9.3 days) over untreated check. Wu *et al*., (2009) recorded significantly longer larval duration in first (12.02), second (12.13) and third (12.2 days) generations of *S. exigua* on Bt cotton compared to non-Bt (11, 11.22 and 11.24 days respectively). Srinivasa Rao *et al*., (2015) reported that with increase in temperatures, the generation time of *Spodoptera litura* in cotton would decrease by 18–22 % and subsequently increase number of generations. Report of El-Sayed *et al*., (2018) also supports the report that development rate of *S. litura* immature stages in cotton increased linearly as a function of temperature until approximately 34-36 °C, after which it became nonlinear.

4.1.6 Pre-pupal duration: The perusal of the results provided in the Tables 4.21 - 4.24 and Fig. 4.16 - 4.17 indicated significant variation in the pre-pupal duration of the test insects, across *e*CO_2 and *e*Temp conditions. With increase in test temperatures (28, 29, 31, 33 and 35 °C), under and CO_2 levels, pre-pupal duration of *H. armigera* decreased. Comparative performance of both the test insects was presented in Fig. 4.18.

4.1.6.1 Effect on pre-pupal duration of *H. armigera* in non-Bt cotton

The data pertaining to pre-pupal duration of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.21 and Fig. 4.16.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on pre-pupal duration. At *a*CO_2, pre-pupal duration varied from 1.76 to 1.40 days with highest and lowest pre-pupal duration at temperature 28 and 35 ºC, respectively. At *e*CO_2, decrease in pre-pupal duration was recorded from 2.04 to 1.56 days with corresponding increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp has non-significant effect on pre-pupal duration. And pre-pupal duration was higher at *e*CO_2 than *a*CO_2.

Second generation: At *a*CO_2, pre-pupal duration varied from 1.84 to 1.40 days with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in pre-pupal duration was recorded ranging from 2.12 to 1.64 days with corresponding

increase in temperature from 28 to 35 ºC. And pre-pupal duration was higher at *e*CO_2 than *a*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant impact whereas the interactive effect has non-significant impact on pre-pupal duration.

Third generation: At *a*CO_2, highest pre-pupal duration was recorded at 28 ºC with 2.12 days and lowest at 35 ºC with 1.52 days. At *e*CO_2, decrease in pre-pupal duration was recorded with increase in temperature with highest at 28 ºC (2.24 days) and lowest at 35 ºC (1.76 days). And pre-pupal duration was higher at *e*CO_2 than *a*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant impact whereas the interactive effect has non-significant impact on pre-pupal duration.

Mean of generations: The mean of three generations also in non-Bt cotton, indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the pre-pupal duration has decreased significantly (1.91, 1.73, 1.67, 1.60 and 1.44 days, respectively) corresponding to 9.09, 12.59, 16.08 and 24.48 %. Similarly, under *e*CO_2 also the pre-pupal duration of *H. armigera* decreased significantly (2.13, 2.01, 1.85, 1.81 and 1.65 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 5.63, 13.13, 15.00 and 22.50 %. The interaction effect of *e*CO_2 and *e*Temp has non-significant effect on pre-pupal duration. And pre-pupal duration was higher at *e*CO_2 than *a*CO_2. Further, the pre-pupal duration under *e*CO_2 was higher (11.89, 16.15, 11.20, 13.33 and 14.81 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred from the data that mean pre-pupal duration of *H. armigera* increased with increase in CO_2 (by 12 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 14 %. With advancement of generations, pre-pupal period did not vary greatly in days count (~2 days in all conditions), but definitely there is a variation if accounted in hours in all the three major test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively. Literatures on the effects of the factors, in study, on pre-pupal duration were meagre to compare.

4.1.6.2 Effect on pre-pupal duration of *H. armigera* in Bt cotton

The data pertaining to pre-pupal duration of *H. armigera* in Bt cotton for all three generations was presented in Table 4.22 and Fig. 4.16.

Table 4.21. Effect of *e*CO_2 and *e*Temp on pre-pupal duration of *H. armigera* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1.76	2.04	**1.90**	1.84	2.12	**1.98**	2.12	2.24	**2.18**	1.91	2.13	**2.02**
29 ± 1°C	1.72	1.84	**1.78**	1.68	2.04	**1.86**	1.80	2.16	**1.98**	1.73	2.01	**1.87**
31 ± 1°C	1.60	1.72	**1.66**	1.68	1.88	**1.78**	1.72	1.96	**1.84**	1.67	1.85	**1.76**
33 ± 1°C	1.48	1.68	**1.58**	1.64	1.84	**1.74**	1.68	1.92	**1.80**	1.60	1.81	**1.71**
35 ± 1°C	1.40	1.56	**1.48**	1.40	1.64	**1.52**	1.52	1.76	**1.64**	1.44	1.65	**1.55**
Mean	**1.59**	**1.76**		**1.64**	**1.90**		**1.76**	**2.00**		**1.66**	**1.89**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	4.047*	0.06	0.17	8.376*	0.06	0.17	6.822*	0.07	0.18	20.48*	0.04	0.10
Temperature (°C)	2.843*	0.10	0.27	2.953*	0.10	0.17	3.915*	0.10	0.29	10.32*	0.06	0.15
Interaction (CO_2 + Temp(°C))	0.12	0.14	NS	0.12	0.14	NS	0.17	0.15	NS	0.09	0.08	NS
CV	5.17 %			5.37 %			5.74 %			5.97 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Table 4.22 Effect of *e*CO_2 and *e*Temp on pre-pupal duration of *H. armigera* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1.71	1.92	**1.81**	2.00	2.18	**2.09**	2.13	2.33	**2.23**	1.95	2.14	**1.99**
29 ± 1°C	1.70	1.77	**1.73**	1.73	1.83	**1.78**	1.73	1.92	**1.83**	1.72	1.84	**1.77**
31 ± 1°C	1.57	1.68	**1.63**	1.69	1.74	**1.72**	1.79	1.81	**1.80**	1.68	1.74	**1.72**
33 ± 1°C	1.50	1.56	**1.53**	1.52	1.68	**1.60**	1.67	1.79	**1.73**	1.56	1.68	**1.63**
35 ± 1°C	1.40	1.61	**1.50**	1.46	1.62	**1.54**	1.55	1.74	**1.64**	1.47	1.66	**1.58**
Mean	**1.57**	**1.70**		**1.68**	**1.81**		**1.77**	**1.91**		**1.68**	**1.79**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	4.775*	0.04	0.12	4.175*	0.05	0.13	4.486*	0.05	0.14	8.58*	0.03	0.08
Temperature (°C)	4.087*	0.07	0.18	9.114*	0.07	0.20	8.333*	0.08	0.22	14.27*	0.04	0.12
Interaction (CO_2 + Temp(°C))	0.30	0.09	NS	0.13	0.10	NS	0.25	0.11	NS	0.42	0.06	NS
CV	5.31 %			5.79 %			5.03 %			5.28 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

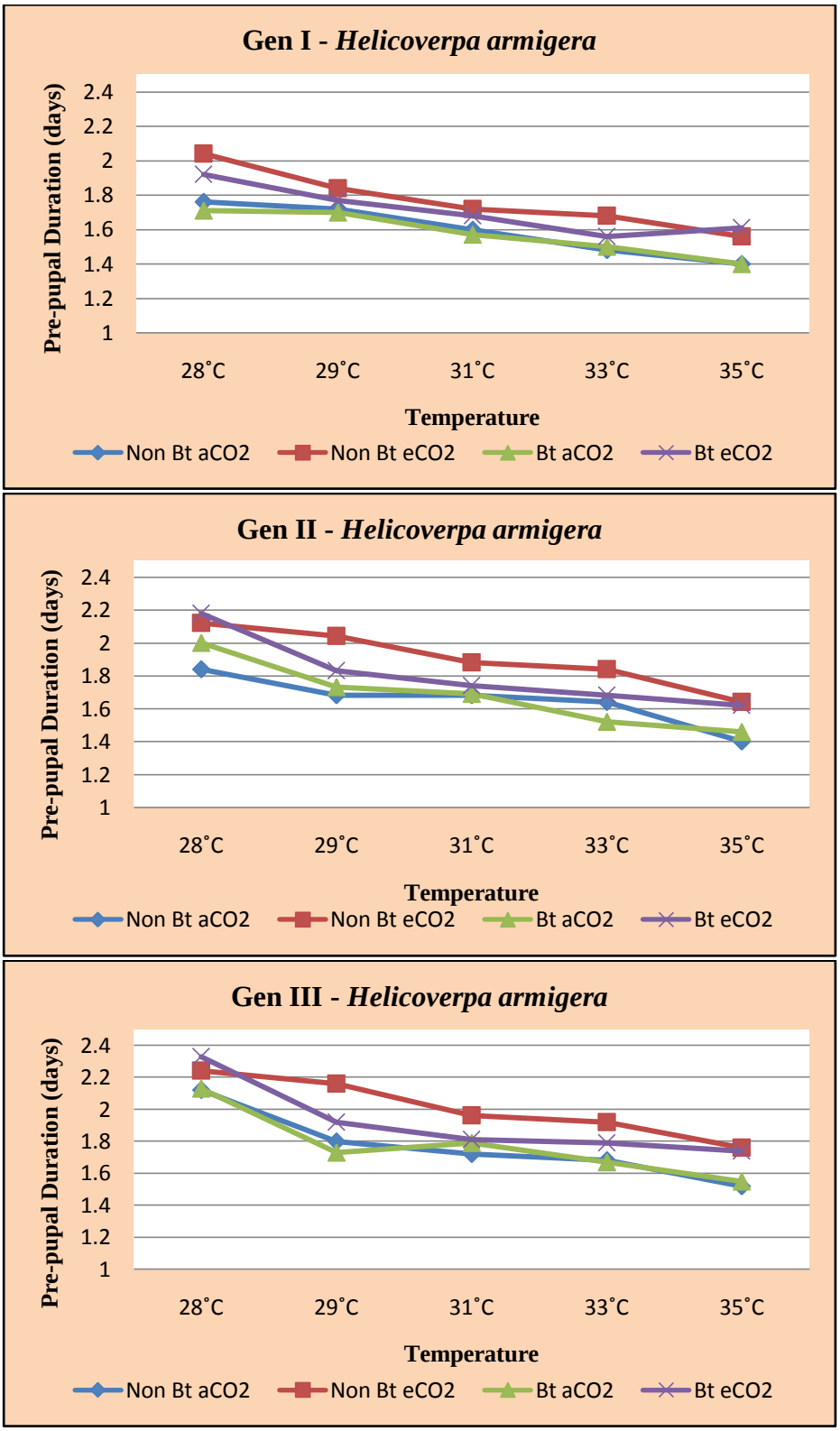


Fig. 4.16. Effect of *e*CO_2 and *e*Temp on pre-pupal duration of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on pre-pupal duration. At *a*CO_2, pre-pupal duration ranged between 1.71 and 1.40 days with highest pre-pupal duration at 28 ^{o}C and lowest at 35 ºC. At *e*CO_2, decrease in pre-pupal duration was recorded with increase in temperature from 28 to 35 ºC with highest and lowest as 1.92 and 1.61 days, respectively. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pre-pupal duration and was significantly lower in *a*CO_2 than *e*CO_2.

Second generation: At *a*CO_2, pre-pupal duration varied from 2.00 to 1.46 days with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decreased pre-pupal duration was recorded from 2.18 to 1.62 days with increase in corresponding temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-pupal duration.

Third generation: At *a*CO_2, decrease in pre-pupal duration ranging from 2.13 to 1.55 days was recorded corresponding to increase in temperature from 28 to 35 ºC. At *e*CO_2, pre-pupal duration decreased from 2.33 to 1.74 days with increase in corresponding temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant influence on pre-pupal duration.

Mean of generations: The mean of three generations in Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the pre-pupal duration of *H. armigera* has decreased significantly (1.95, 1.72, 1.68, 1.56 and 1.47 days, respectively) corresponding to 11.64, 13.53, 19.69 and 24.49 %. Similarly, under *e*CO_2 also the pre-pupal duration decreased significantly (2.14, 1.84, 1.74, 1.68 and 1.66 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 14.15, 18.66, 21.77 and 22.71 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pre-pupal duration and was significantly lower in *a*CO_2 than *e*CO_2. Further, the pre-pupal duration under *e*CO_2 was higher (10.10, 6.98, 3.56, 7.25 and 12.70 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred from the data that, mean pre-pupal duration of *H. armigera* raised on Bt cotton increased with increase in CO_2 (by 10 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 15 %. With

advancement of generations, pre-pupal period did not vary greatly in days count (~2 days in all conditions), but definitely there could be a variation if accounted in hours in all the three major test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively. da Silva *et al*. (2017) reported that *S. frugiperda* pre-pupal duration was highest in cotton (1.97) followed by soybean, maize (1.89 each) and semi-synthetic diet (1.87). Literature on the effects of these factors, on pre-pupal duration was meagre to compare.

4.1.6.3 Effect on pre-pupal duration of *S. litura* in non-Bt cotton

The data pertaining to pre-pupal duration of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.23 and Fig. 4.17.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on pre-pupal duration. At *a*CO_2, pre-pupal duration decreased from 1.78 to 1.47 days with increase in temperature from 28 to 35 ºC. At *e*CO_2, decrease in pre-pupal duration was recorded from 1.90 to 1.62 days with corresponding increase in temperature from 28 to 35 ºC. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on pre-pupal duration. And among *a*CO_2 and *e*CO_2, pre-pupal duration was higher at *e*CO_2.

Second generation: At *a*CO_2, highest pre-pupal duration was recorded at 28 ºC with 1.83 days and lowest at 35 ºC with 1.32 days. At *e*CO_2, decrease in pre-pupal duration was recorded from 2.21 to 1.56 days with increase in corresponding temperature from 28 to 35 ºC. And among *a*CO_2 and *e*CO_2, pre-pupal duration was higher at *e*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-pupal duration.

Third generation: At *a*CO_2, decrease in pre-pupal duration was recorded from 2.20 to 1.52 days with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, pre-pupal duration ranged from 2.41 to 1.64 days with highest and lowest at temperatures 28 and 35 ºC, respectively. And among *a*CO_2 and *e*CO_2, pre-pupal duration was higher at *e*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-pupal duration.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the pre-pupal duration has decreased significantly (1.90, 1.73, 1.67, 1.53 and 1.40 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 8.77, 12.28, 19.30 and 26.32 %. Similarly, under *e*CO_2, the pre-pupal duration decreased significantly (2.17, 1.93, 1.87, 1.73 and 1.60 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 10.77, 13.85, 20.00 and 26.15 %. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on pre-pupal duration. And among *a*CO_2 and *e*CO_2, pre-pupal duration was higher at *e*CO_2. Further, the pre-pupal duration under *e*CO_2 was higher (14.04, 11.54, 12.00, 13.04 and 14.29 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred from the data that mean pre-pupal duration of *S. litura* increased with increase in CO_2 (by 14 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 16 %. With advancement of generations, pre-pupal period did not vary greatly in days count (~2 days in all conditions), but definitely there is a variation if accounted in hours in all the three major test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp, respectively. Literature on this aspect on cotton crop is scanty.

4.1.6.4 Effect on pre-pupal duration of *S. litura* in Bt cotton

The data pertaining to pre-pupal duration of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.24 and Fig. 4.17.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on pre-pupal duration. It decreased from 1.63 to 1.38 days with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, highest pre-pupal duration of 1.78 days was recorded at temperature 28 ºC and lowest at temperature 35 ºC. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on pre-pupal duration. And among *a*CO_2 and *e*CO_2, pre-pupal duration was higher at *e*CO_2.

Second generation: Decrease in pre-pupal duration ranging from 1.71 to 1.36 days was recorded with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, pre-pupal duration ranged between 1.85 and 1.52 days with highest and lowest pre-pupal duration at temperatures 28 and 35 ºC, respectively. And among *a*CO_2 and

Table 4.23. Effect of *e*CO_2 and *e*Temp on pre-pupal duration of *S. litura* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1.73	1.95	**1.80**	1.81	2.22	**2.00**	2.24	2.47	**2.30**	1.90	2.17	**2.04**
29 ± 1°C	1.68	1.86	**1.70**	1.76	1.93	**1.80**	1.96	2.16	**2.00**	1.73	1.93	**1.85**
31 ± 1°C	1.63	1.87	**1.70**	1.65	1.85	**1.70**	1.85	2.08	**1.90**	1.67	1.87	**1.75**
33 ± 1°C	1.52	1.66	**1.60**	1.57	1.81	**1.60**	1.67	1.84	**1.70**	1.53	1.73	**1.64**
35 ± 1°C	1.44	1.62	**1.50**	1.35	1.67	**1.40**	1.53	1.68	**1.60**	1.40	1.60	**1.49**
Mean	**1.55**	**1.73**		**1.57**	**1.84**		**1.80**	**2.00**		**1.64**	**1.85**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	5.688*	0.06	0.15	5.161*	0.08	0.23	3.961*	0.07	0.19	16.55*	0.04	0.10
Temperature (°C)	2.618*	0.09	0.24	2.578*	0.13	0.36	6.781*	0.11	0.30	12.72*	0.06	0.16
Interaction (CO_2 + Temp(°C))	0.02	0.12	NS	0.12	0.18	NS	0.02	0.15	NS	0.02	0.08	NS
CV	5.10 %			4.85 %			5.06 %			3.66 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Table 4.24. Effect of *e*CO_2 and *e*Temp on pre-pupal duration of *S. litura* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1.63	1.78	**1.70**	1.71	1.85	**1.78**	1.80	1.99	**1.89**	1.71	1.87	**1.79**
29 ± 1°C	1.55	1.67	**1.61**	1.58	1.67	**1.63**	1.67	1.77	**1.72**	1.60	1.70	**1.65**
31 ± 1°C	1.50	1.60	**1.55**	1.51	1.58	**1.54**	1.62	1.81	**1.72**	1.54	1.66	**1.60**
33 ± 1°C	1.47	1.56	**1.51**	1.44	1.56	**1.50**	1.53	1.74	**1.63**	1.48	1.62	**1.55**
35 ± 1°C	1.38	1.51	**1.44**	1.36	1.52	**1.44**	1.48	1.65	**1.57**	1.41	1.56	**1.48**
Mean	**1.50**	**1.62**		**1.52**	**1.63**		**1.61**	**1.79**		**1.54**	**1.68**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	4.852*	0.04	0.11	3.965*	0.04	0.11	7.224*	0.05	0.13	15.52*	0.02	0.07
Temperature (°C)	2.619*	0.06	0.17	4.261*	0.06	0.12	2.951*	0.07	0.20	9.29*	0.04	0.11
Interaction (CO_2 + Temp(°C))	0.04	0.09	NS	0.08	0.09	NS	0.08	0.10	NS	0.10	0.05	NS
CV	5.52 %			5.76 %			5.71 %			4.75 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

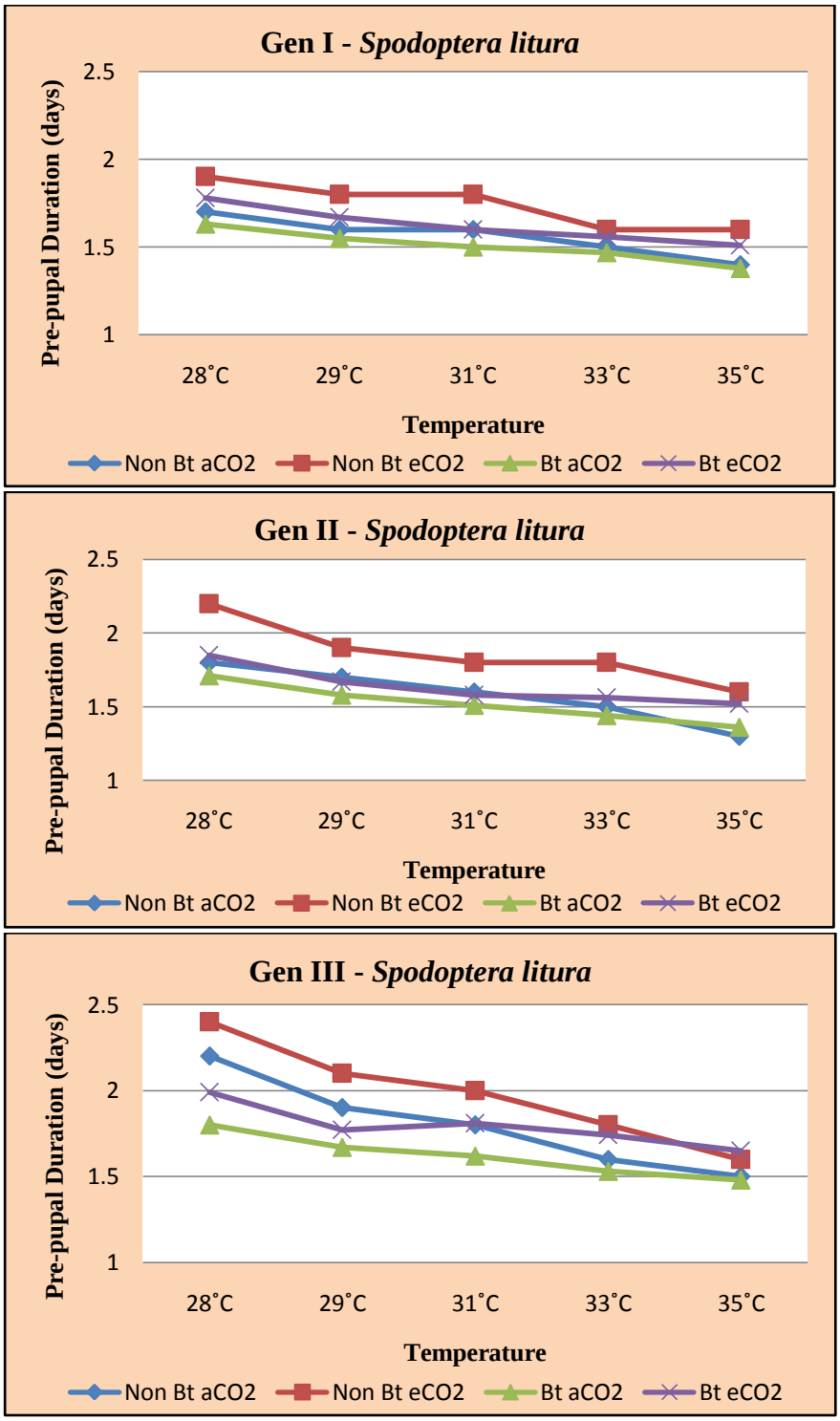


Fig. 4.17. Effect of *e*CO_2 and *e*Temp on pre-pupal duration of *S. litura* on non-Bt and Bt cotton in first, second and third generation

eCO_2, pre-pupal duration was higher at *e*CO_2.The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-pupal duration.

Third generation: Pre-pupal duration varied from 1.80 to 1.48 days with highest pre-pupal duration at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, decrease in pre-pupal duration was recorded from 1.99 to 1.65 days with increase in temperature from 28 to 35 ºC. And among *a*CO_2 and *e*CO_2, pre-pupal duration was higher at *e*CO_2.The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-pupal duration.

Mean of generations: The mean of three generations in Bt cotton, also indicated that the pre-pupal duration has decreased significantly (1.71, 1.60, 1.54, 1.48 and 1.41 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to decrease 6.61, 9.92, 13.62 and 17.90 %. Similarly, under *e*CO_2 also the pre-pupal duration decreased significantly (1.87, 1.70, 1.66, 1.62 and 1.56 days) with increase in temperatures (28-35 ºC) which has corresponded to a decrease of 9.07, 11.21, 13.52 and 16.73 %. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on pre-pupal duration. And among *a*CO_2 and *e*CO_2, pre-pupal duration was higher at *e*CO_2. Further, the pre-pupal duration under *e*CO_2 was higher (9.34, 6.46, 7.78, 9.46 and 10.90 %, at 28, 29, 31, 33 and 35 °C) compared to that of *a*CO_2.

It can be inferred from the data that, mean pre-pupal duration of *S. litura* in Bt cotton increased with increase in CO_2 (by 9 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 9 %. With advancement of generations, pre-pupal period did not vary greatly in days count (~2 days in all conditions), but definitely there is a variation if accounted in hours in all the three major test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp, respectively. Literature on this aspect on cotton crop is scanty.

4.1.7 Pupal duration: The data pertaining to the effect of *e*CO_2 and *e*Temp conditions on the pupal duration of *H. armigera* and *S. litura* was presented in the Tables 4.25 - 4.28. and Fig. 4.19 - 4.20. They indicated significant variation in the pupal duration of the test insects, across *e*CO_2 and *e*Temp conditions. The pupal duration under *e*CO_2 was higher than that of *a*CO_2. With increase in test temperatures

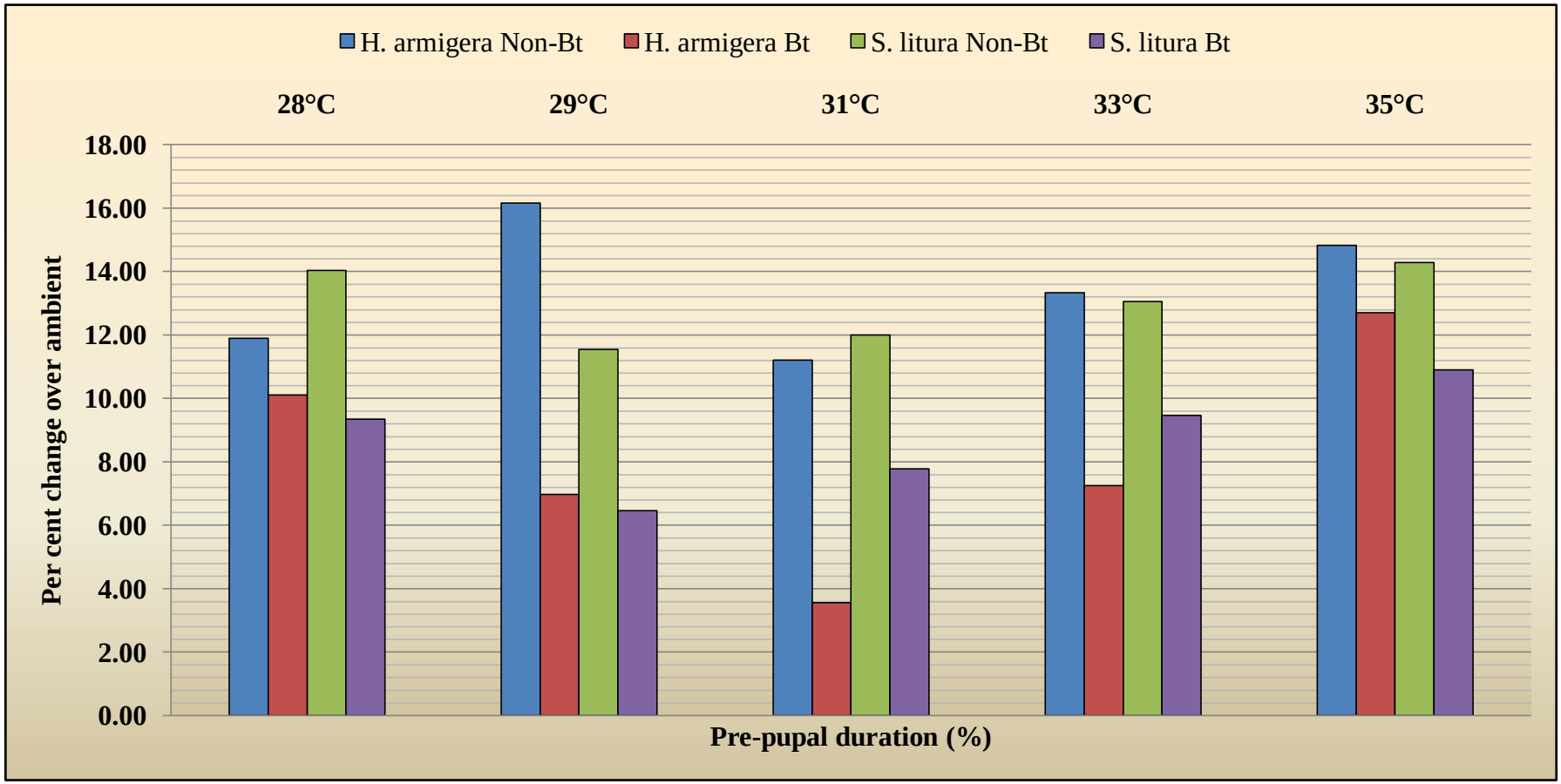


Fig. 4.18. Effect of *e*CO_2 and *e*Temp on mean pre-pupal duration of *H. armigera* and *S. litura* on non-Bt and Bt cotton

(28, 29, 31, 33 and 35 °C), under both *a*CO_2 (380±25 ppm) and *e*CO_2 (550± 25 ppm) conditions, pupal duration of test insects decreased. Comparative performance of both the test insects was presented in Fig. 4.21.

4.1.7.1 Effect on pupal duration of *H. armigera* in non-Bt cotton

The data pertaining to pupal duration of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.25 and Fig. 4.19.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on pupal duration. At *a*CO_2, pupal duration varied from 10.04 to 7.92 days with highest and lowest pupal duration at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in pupal duration was recorded from 10.64 to 8.64 days with corresponding increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp has shown non-significant impact on pupal duration. And among *a*CO_2 and *e*CO_2, pupal duration was significantly lower in *a*CO_2.

Second generation: At *a*CO_2, pupal duration varied from 10.48 to 8.20 days with highest pupal duration at temperature 28 and lowest at 35 ºC. At *e*CO_2, decrease in pupal duration was recorded with increase in temperature with highest pupal duration at 28 ºC (11.00 days) and lowest at 35 ºC (8.88 days). And among *a*CO_2 and *e*CO_2, pupal duration was significantly lower in *a*CO_2.The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pupal duration.

Third generation: At *a*CO_2, highest pupal duration was recorded at 28 and 35ºC with a pupal duration of 10.56 and 8.44 days, respectively. At *e*CO_2, decrease in pupal duration was recorded from 11.28 to 9.04 days with corresponding increase in temperature from 28 to 35 ºC. And among *a*CO_2 and *e*CO_2, pupal duration was significantly lower in *a*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pupal duration.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the pupal duration has decreased significantly (10.36, 9.68, 8.96, 8.72 and 8.19 days,

Table 4.25. Effect of *e*CO_2 and *e*Temp on pupal duration of *H. armigera* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	10.04	10.64	**10.34**	10.48	11.00	**10.74**	10.56	11.28	**10.92**	10.36	10.97	**10.67**
29 ± 1°C	9.24	10.04	**9.64**	9.56	10.36	**9.96**	10.24	10.84	**10.54**	9.68	10.41	**10.05**
31 ± 1°C	8.68	9.68	**9.18**	8.88	10.04	**9.46**	9.32	10.44	**9.88**	8.96	10.05	**9.51**
33 ± 1°C	8.44	9.24	**8.84**	8.84	9.60	**9.22**	8.88	9.76	**9.32**	8.72	9.53	**9.13**
35 ± 1°C	7.92	8.64	**8.28**	8.20	8.88	**8.54**	8.44	9.04	**8.74**	8.19	8.85	**8.52**
Mean	**8.86**	**9.64**		**9.19**	**9.97**		**9.48**	**10.27**		**9.18**	**9.96**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	37.873*	0.09	0.25	36.333*	0.09	0.26	35.051*	0.09	0.26	94.62*	0.06	0.16
Temperature (°C)	30.241*	0.14	0.40	32.103*	0.15	0.41	35.703*	0.15	0.41	84.14*	0.09	0.25
Interaction (CO_2 + Temp(°C))	0.26	0.20	NS	0.66	0.21	NS	0.55	0.21	NS	1.09	0.13	NS
CV	4.54 %			3.12 %			4.08 %			6.66 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

respectively) corresponding to 6.56, 13.51, 15.83 and 20.98 %. Similarly, under *e*CO_2 also the pupal duration decreased significantly (10.97, 10.41, 10.05, 9.53 and 8.85 days) with increase in temperatures (28- 35 ºC) which has corresponded to a decrease of 5.10, 8.38, 13.12 and 19.32 %. The interaction effect of *e*CO_2 and *e*Temp has shown non-significant impact on pupal duration. And among *a*CO_2 and *e*CO_2, pupal duration was significantly lower in *a*CO_2. However, the decrease in pupal duration with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the pupal duration under *e*CO_2 was higher (5.92, 7.58, 12.20, 9.33 and 8.14 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The data obtained suggests that, mean pupal duration of *H. armigera* increased with increase in CO_2 (by 6 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 15 %. Similarly, Akbar *et al*. (2016) recorded that *H. armigera* pupal period was significantly higher under *e*CO_2 (11.33 days) compared to *a*CO_2 conditions (10.67 days) and lower at 35 °C (8.33 days) compared to 25 °C (10.33 days). With advancement of every generation, pupal period increased to some extent (10.04, 10.48 and 10.56 days; 10.64, 11 and 11.28 days; and 8.64, 8.88 and 9.04 days), in the major test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp, respectively. It can be iterated from the increase in the third value in the third set of values, that with extreme climate stress also (*e*CO_2 +*e*Temp), *H. armigera* is building fitness by extending pupal duration and is definitely an indication for adaptation.

4.1.7.2 Effect on pupal duration of *H. armigera* in Bt cotton

The data pertaining to pupal duration of *H. armigera* in Bt cotton for all three generations was presented in Table 4.26 and Fig. 4.19.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on pupal duration. At *a*CO_2, pupal duration decreased from 9.86 to 7.85 days with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, decrease in pupal duration was recorded ranging from 10.38 to 8.60 days with highest and lowest pupal duration at temperatures 28 and 35 ºC, respectively. The interaction effect of *e*CO_2 and *e*Temp has shown non-significant impact on pupal duration.

Table 4.26. Effect of *e*CO_2 and *e*Temp on pupal duration of *H. armigera* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	9.86	10.38	**10.12**	10.25	10.44	**10.35**	10.38	10.56	**10.47**	10.16	10.46	**10.31**
29 ± 1°C	9.10	9.73	**9.41**	9.36	10.08	**9.72**	9.82	10.23	**10.02**	9.43	10.01	**9.72**
31 ± 1°C	8.71	9.50	**9.11**	9.15	9.87	**9.51**	9.21	10.00	**9.61**	9.02	9.79	**9.41**
33 ± 1°C	8.38	9.06	**8.72**	8.76	9.33	**9.05**	9.00	9.42	**9.21**	8.71	9.27	**8.99**
35 ± 1°C	7.85	8.60	**8.23**	8.19	8.52	**8.36**	8.41	8.70	**8.55**	8.15	8.61	**8.38**
Mean	**8.77**	**9.45**		**9.14**	**9.65**		**9.36**	**9.78**		**9.09**	**9.62**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	24.923*	0.10	0.27	17.207*	0.09	0.24	13.519*	0.08	0.22	52.04*	0.05	0.15
Temperature (°C)	22.451*	0.15	0.42	30.006*	0.14	0.38	33.758*	0.13	0.35	78.48*	0.08	0.23
Interaction (CO_2 + Temp(°C))	0.13	0.21	NS	0.74	0.19	NS	0.81	0.18	NS	1.07	0.12	NS
CV	5.41 %			5.83 %			3.74 %			6.23 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

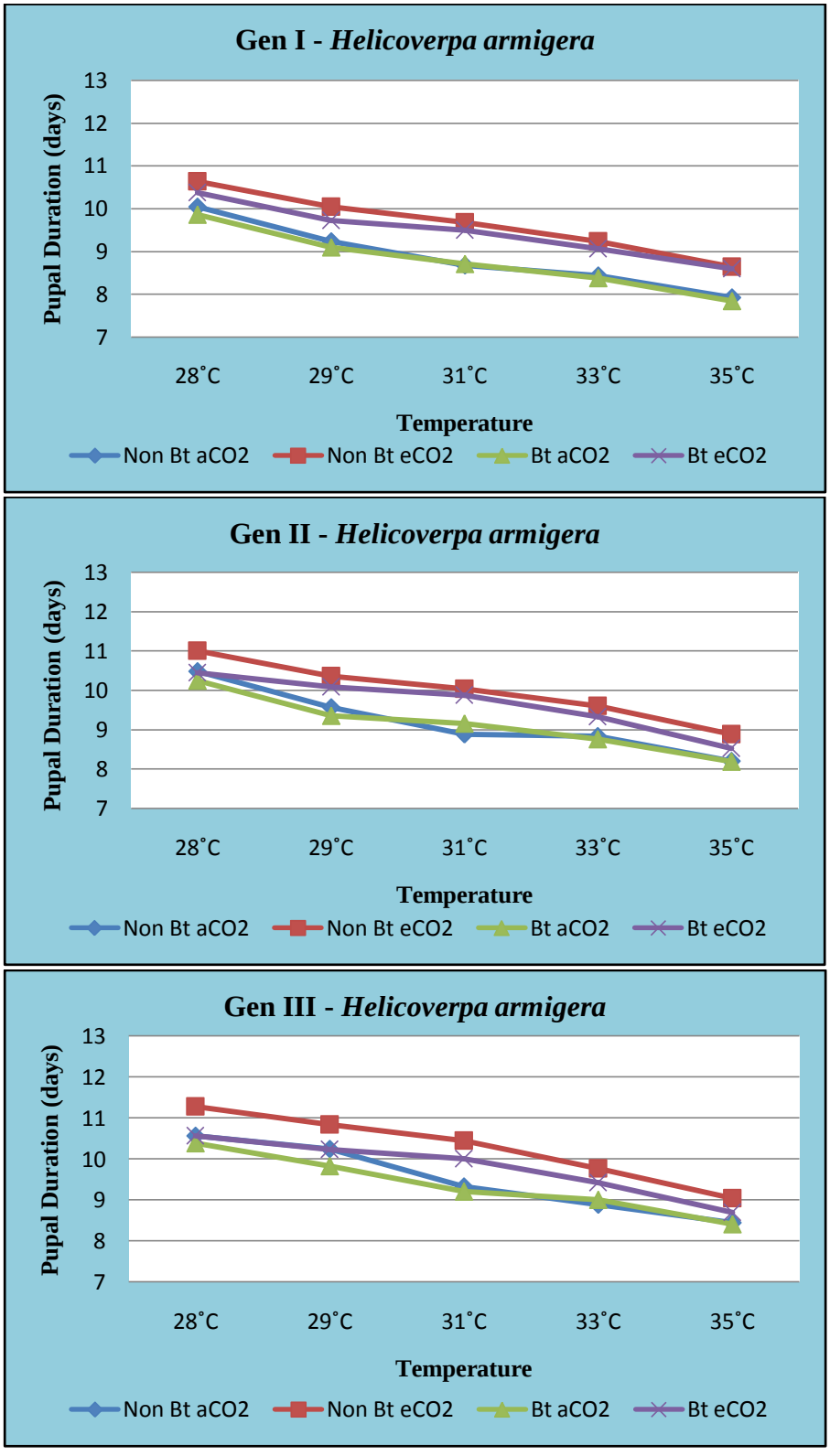


Fig. 4.19. Effect of *e*CO_2 and *e*Temp on pupal duration of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

Second generation: In Bt cotton, at *a*CO_2, pupal duration varied from 10.25 to 8.19 days with highest and lowest pupal duration at temperature at 28 and 35 ºC, respectively. At *e*CO_2, decrease in pupal duration was recorded with increase in temperature with highest pupal duration of 10.44 days at 28 ºC and lowest at 35 ºC with 8.52 days. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pupal duration.

Third generation: At *a*CO_2, highest pupal duration was recorded at 28 ºC with 10.8 days and lowest at 35 ºC with 8.41 days. At *e*CO_2, pupal duration ranged between 10.56 to 8.70 days with highest and lowest pupal duration at temperatures 28 ºC and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pupal duration.

Mean of generations: The mean of three generations in Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the pupal duration by larvae has decreased significantly (10.16, 9.43, 9.02, 8.71 and 8.15 days, respectively) corresponding to 7.25, 11.22, 14.27 and 19.81 %. Similarly, under *e*CO_2 also the pupal duration decreased significantly (10.46, 10.01, 9.79, 9.27 and 8.61 days) with increase in temperatures (28- 35 ºC, respectively) which has corresponded to a decrease of 4.27, 6.41, 11.38 and 17.72 %. The interaction effect of *e*CO_2 and *e*Temp has shown non-significant impact on pupal duration. And among *a*CO_2 and *e*CO_2, pupal duration was significantly lower in *a*CO_2. However, the decrease in pupal duration with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the pupal duration under *e*CO_2 was higher (2.92, 6.22, 8.50, 6.39 and 5.60 %, at 28, 29, 31, 33 and 35 °C) compared to that of *a*CO_2.

The data obtained suggests that, mean pupal duration of *H. armigera* in Bt cotton, increased with increase in CO_2 (by 3 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 15 %. With advancement of every generation, pupal duration increased to some extent (9.86, 10.25 and10.38 days; 10.38, 10.44 and 10.56 days; and 8.6, 8.52 and 8.7 days), in the major test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp, respectively. It can be iterated from the

increase in the third value in the third set of values, that with extreme climate stress also (eCO$_2$ +eTemp), *H. armigera* is improving fitness by extending pupal duration and is definitely an indication for adaptation.

4.1.7.3 Effect on pupal duration of *S. litura* in non-Bt cotton

The data pertaining to pupal duration of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.27 and Fig. 4.20.

First generation: In non-Bt cotton, eCO$_2$ and eTemp has shown significant impact on pupal duration. At aCO$_2$, pupal duration ranged between 10.08 to 7.88 days with highest and lowest pupal duration at temperatures 28 and 35 ºC. At eCO$_2$, decrease in pupal duration was recorded ranging between 10.60 to 8.68 days with increase in temperature from 28 to 35 ºC. The interaction effect of eCO$_2$ and eTemp showed non-significant influence on pupal duration. And among aCO$_2$ and eCO$_2$, pupal duration was lower in aCO$_2$.

Second generation: At aCO$_2$, pupal duration decreased from 10.52 to 8.36 days with highest and lowest pupal duration at temperatures 28 and 35 ºC, respectively. At eCO$_2$, decrease in pupal duration was recorded with increase in temperature with highest pupal duration at 28 ºC with 11.04 days and lowest at 35 ºC with 8.92 days. And among aCO$_2$ and eCO$_2$, pupal duration was lower in aCO$_2$.The individual effect of eCO$_2$ and eTemp has shown significant influence whereas the interactive effect has non-significant influence on pupal duration.

Third generation: At aCO$_2$, decrease in pupal duration was recorded with increase in temperature from 28 ºC (10.70 days) to 35 ºC (8.60 days). At eCO$_2$, pupal duration ranged between 11.40 and 9.20 days with highest and lowest pupal duration at temperatures 28 ºC and 35 ºC, respectively. The individual effect of eCO$_2$ and eTemp has shown significant influence whereas the interactive effect has non-significant influence on pupal duration. And among aCO$_2$ and eCO$_2$, pupal duration was lower in aCO$_2$.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the pupal duration has decreased (10.43, 9.77, 9.12, 8.64 and 8.28 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 6.39, 12.59, 17.19 and 20.64 % respectively. Similarly, under eCO$_2$, the

Table 4.27. Effect of *e*CO_2 and *e*Temp on pupal duration of *S. litura* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	10.08	10.60	**10.34**	10.52	11.04	**10.78**	10.70	11.40	**11.10**	10.43	11.01	**10.73**
29 ± 1°C	9.20	10.08	**9.64**	9.60	10.40	**10.00**	10.50	10.80	**10.70**	9.77	10.43	**10.10**
31 ± 1°C	8.72	9.64	**9.18**	9.24	9.96	**9.60**	9.40	10.30	**9.80**	9.12	9.97	**9.54**
33 ± 1°C	8.44	9.20	**8.82**	8.68	9.56	**9.12**	8.80	9.70	**9.30**	8.64	9.49	**9.07**
35 ± 1°C	7.88	8.68	**8.28**	8.36	8.92	**8.64**	8.60	9.20	**8.90**	8.28	8.93	**8.59**
Mean	**8.86**	**9.64**		**9.28**	**9.97**		**9.59**	**10.29**		**9.24**	**9.97**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	44.122*	0.08	0.23	34.947*	0.08	0.23	35.415*	0.08	0.23	107.15*	0.05	0.14
Temperature (°C)	36.209*	0.13	0.36	38.964*	0.13	0.37	49.018*	0.13	0.37	115.22*	0.08	0.22
Interaction (CO_2 + Temp(°C))	0.36	0.19	NS	0.34	0.19	NS	0.81	0.19	NS	0.60	0.11	NS
CV	3.98 %			4.67 %			3.41 %			5.77 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

pupal duration decreased (2.17, 1.93, 1.87, 1.73 and 1.60 days) with increase in temperatures (28-35 °C) which has corresponded to a decrease of 5.33, 9.50, 13.86 and 18.89 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pupal duration. And among *a*CO_2 and *e*CO_2, pupal duration was lower in *a*CO_2. However, the decrease in pupal duration with increase in temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the pupal duration under *e*CO_2 was higher (5.56, 6.76, 9.28, 9.80 and 7.89 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The data obtained suggests that, mean pupal duration of *S. litura* increased with increase in CO_2 (by 6 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 14 %. Analogously, Shwetha *et al*. (2017) reported that pupal duration was prolonged by two days under *e*CO_2 over ambient. Nanditha *et al*. (2017) reported that *S. litura* pupal duration (15.33 to 6.00 days) on jute decreased with increase in temperature from 18 to 33 ºC. Tejinder and Sharma (2017) examined that *S. litura* pupal duration was higher with lower night temperatures i.e. 25:11 ºC (9.3 ± 0.3 days) than at 25:14 ºC (8.7 ± 0.4 days). Dai *et al.*, (2017) reported *Spodoptera exigua* had reduced pupal duration (from 7.68 to 3.12 days) with increase in temperature from 20 to 38 ºC. Likewise, pupal period (6.80 days) of *S. litura* on sunflower foliage decreased (4.80 days) with increase in temperature from 20 to 30 ºC (Rama Devi and Jha, 2017).

Divya *et al.* (2018) reported that mean developmental time of pupa (16.46 to 7.93 days) decreased at 35 ºC + *e*CO_2 (16.80 to 6.66 days). Plessis *et al*. (2020) recorded that *S. frugiperda* pupal duration on sweet corn decreased from 11.43 to 9 and 7.82 days at 25, 30 and 32 °C, respectively. Ismail (2021) revealed that pupalduration of *S.littoralis* increased along the temperature from 12 (25 °C) to 23 days (35 °C). From the findings of the study, it was observed that with advancement of every generation, pupal period increased to some extent (10.08, 10.52 and 10.7 days; 10.6, 11.04 and11.4 days; and 8.68, 8.92 and 9.2 days), in the major test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp, respectively. It can be iterated from the increase in the third value in the third set of values, that with extreme climate stress also (*e*CO_2 +*e*Temp), *S. litura* is building fitness by extending pupal duration and is definitely an indication for adaptation.

4.1.7.4 Effect on pupal duration of *S. litura* in Bt cotton

The data pertaining to pupal duration of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.28 and Fig. 4.20.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on pupal duration. It ranged from 9.88 to 7.84 days with highest pupal duration at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, decrease in pupal duration with increase in temperature from 28 to 35ºC was recorded with highest and lowest pupal duration as 10.22 and 8.27 days, respectively. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pupal duration.

Second generation: A decrease in pupal duration ranging from 10.22 to 8.15 days was recorded with increase in temperature from 28 to 35 ºC. At *e*CO_2, pupal duration ranged between 10.33 to 8.59 days with highest and lowest pupal duration at temperatures 28 and 35 ºC, respectively.The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pupal duration.

Third generation: Pupal duration varied from 10.30 to 8.55 days with highest pupal duration at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, a decrease in pupal duration was recorded with increase in temperature with highest and lowest pupal duration at 28 ºC and 35 ºC with 10.55 and 8.83 days, respectively.The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pupal duration.

Mean of generations: The mean of three generations in Bt cotton, also indicated that the pupal duration has decreased significantly (10.13, 9.45, 9.04, 8.62 and 8.18 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to decrease 6.78, 10.79, 14.97 and 19.28 %. Similarly, under *e*CO_2 also the pupal duration decreased significantly (10.37, 10.10, 9.78, 9.26 and 8.56 days) with increase in temperatures (28- 35 ºC, respectively) which has corresponded to a decrease of 2.54, 5.66, 10.64 and 17.40 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pupal duration. And among *a*CO_2 and *e*CO_2, pupal duration was lower in *a*CO_2. However, the decrease in pupal duration with increase in

Table 4.28. Effect of *e*CO_2 and *e*Temp on pupal duration of *S. litura* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	9.88	10.22	**10.05**	10.22	10.33	**10.28**	10.30	10.55	**10.42**	10.13	10.37	**10.25**
29 ± 1°C	9.18	9.83	**9.51**	9.33	10.17	**9.75**	9.83	10.31	**10.07**	9.45	10.10	**9.78**
31 ± 1°C	8.69	9.53	**9.11**	9.14	9.75	**9.45**	9.29	10.06	**9.67**	9.04	9.78	**9.41**
33 ± 1°C	8.47	9.00	**8.73**	8.56	9.26	**8.91**	8.82	9.53	**9.17**	8.62	9.26	**8.94**
35 ± 1°C	7.84	8.27	**8.06**	8.15	8.59	**8.37**	8.55	8.83	**8.69**	8.18	8.56	**8.37**
Mean	**8.81**	**9.37**		**9.08**	**9.62**		**9.35**	**9.85**		**9.08**	**9.61**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	22.801*	0.08	0.23	19.595*	0.09	0.24	18.902*	0.08	0.22	56.31*	0.05	0.14
Temperature (°C)	33.171*	0.13	0.37	29.456*	0.14	0.38	29.549*	0.13	0.36	84.49*	0.08	0.22
Interaction (CO_2 + Temp(°C))	0.54	0.19	NS	1.05	0.19	NS	0.90	0.18	NS	1.81	0.11	NS
CV	4.31 %			5.29 %			4.37 %			5.99 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

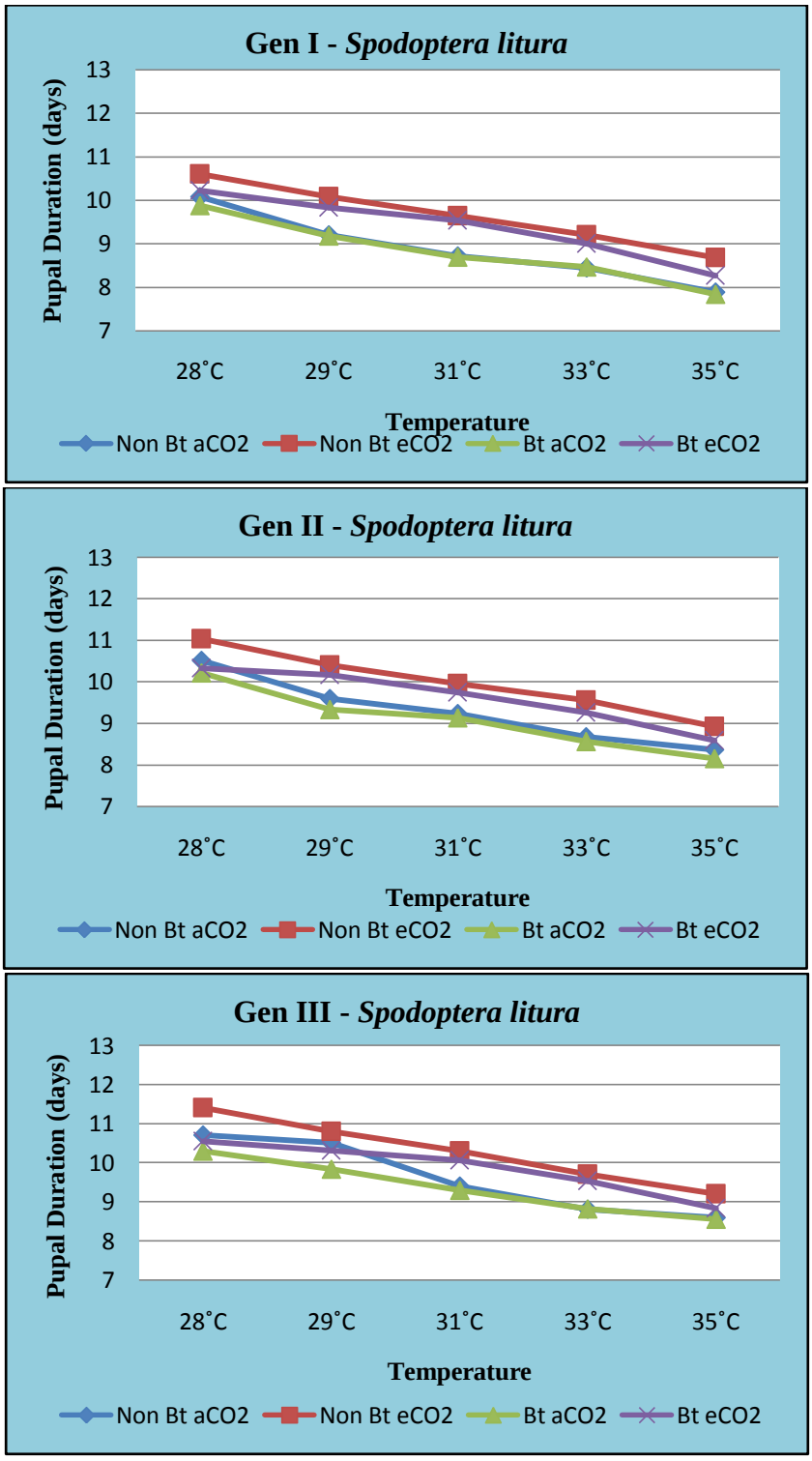


Fig. 4.20. Effect of *e*CO_2 and *e*Temp on pupal duration of *S. litura* on non-Bt and Bt cotton in first, second and third generation

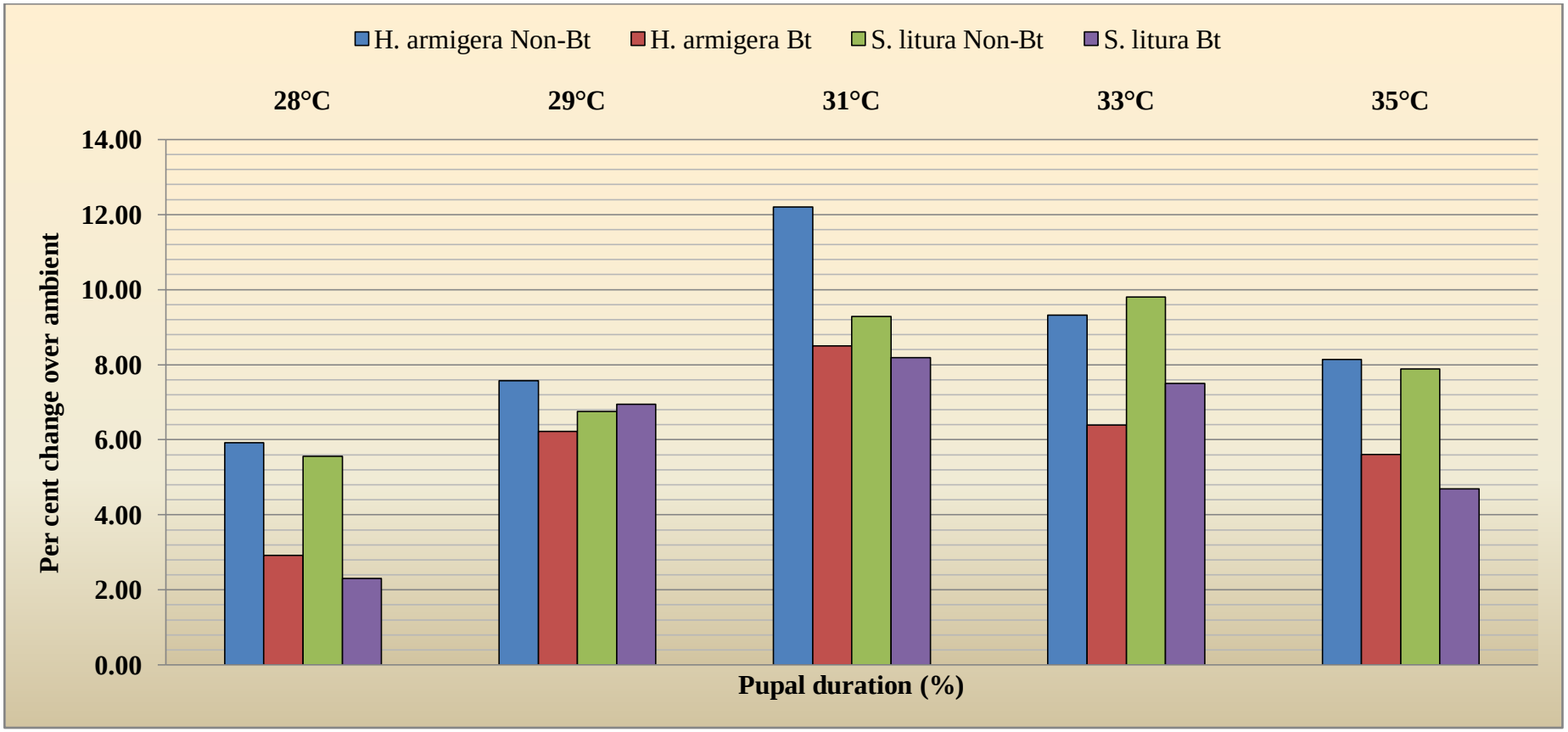


Fig. 4.21. Effect of *e*CO_2 and *e*Temp on mean pupal duration of *H. armigera* and *S. litura* on non-Bt and Bt cotton

temperatures was less predominant under *e*CO_2 compared to that of *a*CO_2 under similar temperatures. Further, the pupal duration under *e*CO_2 was higher (2.30, 6.95, 8.19, 7.50 and 4.69 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The data obtained suggests that, mean pupal duration of *S. litura* in Bt cotton increased with increase in CO_2 (by 2 %) and decreased with temperature and ultimately decreased with *e*CO_2 + *e*Temp by 15 %. Rajesh *et al.* (2007) reported that application of *Bacillus thuringiensis* var. kurstaki 0.2 % in various treatment combinations with 5 per cent each of of neem oil, citronella oil, karanj oil, cottonseed oil and sesamum oil on *S. litura* resulted in significant extension of pupal period (12.0 to 10.3 days,) over untreated check. Ranga Rao *et al*. (1989) reported that pupal duration of *S. litura* decreased from 10.9 (25 °C) to 6.3 days (35 °C) in groundnut. In the study, with every generation, pupal period increased to some extent (9.88, 10.22 and 10.3 days; 10.22, 10.33 and 10.55 days; and 8.27, 8.59 and 8.83 days), in the major test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp, respectively. It can be iterated from the increase in the third value in the third set of values, that with extreme climate stress also (*e*CO_2 +*e*Temp), *S. litura* can improve fitness by extending pupal duration and is definitely an indication for adaptation.

4.1.8 Pre-ovipositional duration: The data regarding the effect of *e*CO_2 and *e*Temp conditions on the pre-ovipositional duration of *H. armigera* and *S. litura* was presented in the Tables 4.29 - 4.32 and Fig. 4.22 - 4.23. With increase in test temperatures (upto 35 °C), under both *a*CO_2 (380$\pm$25 ppm) and *e*CO_2 (550$\pm$ 25 ppm) conditions, the pre-ovipositional duration of test insects gradually decreased. Comparative performance of both the test insects was presented in Fig. 4.24.

4.1.8.1 Effect on pre-ovipositional duration of *H. armigera* in non-Bt cotton

The data pertaining to pre-ovipositional duration of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.29 and Fig. 4.22.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on pre-ovipositional duration. At *a*CO_2, it varied from 2.85 to 2.09 days with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in pre-ovipositional duration was recorded with increase in temperature from 28 ºC to 35 ºC with pre-ovipositional duration of 2.00 and 1.55 days, respectively. The interaction

effect of *e*CO_2 and *e*Temp showed non-significant influence on pre-ovipositional duration.

Second generation: At *a*CO_2, pre-ovipositional duration decrease from 3.07 to 2.31 days corresponding to increase in temperatures from 28 to 35 ºC. At *e*CO_2, decrease in pre-ovipositional duration was recorded ranging from 2.21 to 1.64 days with highest and lowest at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-ovipositional duration.

Third generation: In non-Bt cotton, pre-ovipositional duration ranged between 3.24 to 2.36 days with highest at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, decrease in pre-ovipositional duration was recorded in the range of 2.40 to 1.71 days with corresponding increase in temperature from 28 ºC to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-ovipositional duration.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28-35 ºC) the pre-ovipositional duration has decreased significantly (3.05, 2.85, 2.65, 2.45 and 2.25 days, respectively) corresponding to 6.66, 13.10, 19.76 and 26.20 %. Similarly, under *e*CO_2 also the pre-ovipositional duration decreased significantly (2.20, 2.02, 1.85, 1.79 and 1.63 days) with increase in temperature (28-35 °C, respectively) which has corresponded to a decrease of 8.32, 16.19, 18.91 and 25.87 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on pre-ovipositional duration. And among *a*CO_2 and *e*CO_2, pre-ovipositional duration was higher in *a*CO_2. Further, the pre-ovipositional duration under *e*CO_2 was lower (27.84, 29.12, 30.40, 27.07 and 27.51 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These results infer that, mean pre-ovipositional duration of *H. armigera* decreased with increase in both CO_2 (by 28 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 47 %. Chilton and Bull (1994) reported that pre-ovipositional period of females of ixodid ticks *Amblyomma limbatum* and *Aponomma hydrosauri* decreased with increasing temperature. From the results, pre-ovipositional

Table 4.29. Effect of *e*CO_2 and *e*Temp on pre-ovipositional duration of *H. armigera* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	2.85	2.00	**2.42**	3.07	2.21	**2.64**	3.24	2.40	**2.82**	3.05	2.20	**2.63**
29 ± 1°C	2.67	1.83	**2.25**	2.80	2.00	**2.40**	3.08	2.23	**2.66**	2.85	2.02	**2.44**
31 ± 1°C	2.50	1.73	**2.12**	2.67	1.83	**2.25**	2.79	1.98	**2.39**	2.65	1.85	**2.25**
33 ± 1°C	2.31	1.64	**1.97**	2.50	1.79	**2.14**	2.54	1.93	**2.23**	2.45	1.79	**2.12**
35 ± 1°C	2.09	1.55	**1.82**	2.31	1.64	**1.97**	2.36	1.71	**2.04**	2.25	1.63	**1.94**
Mean	**1.75**	**2.48**		**1.89**	**2.66**		**2.05**	**2.80**		**1.89**	**2.65**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	165.723*	0.04	0.11	138.994*	0.05	0.13	126.113*	0.05	0.13	391.75*	0.03	0.08
Temperature (°C)	13.690*	0.06	0.18	12.005*	0.07	0.21	17.743*	0.08	0.21	39.58*	0.04	0.12
Interaction (CO_2 + Temp(°C))	0.97	0.09	NS	0.28	0.10	NS	0.56	0.11	NS	1.42	0.06	NS
CV	5.26 %			5.76 %			5.75 %			3.21 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

period increased along the three generations as follows: 2.85, 3.07 and 3.24 days; 2, 2.21 and 2.4 days; and 1.55, 1.64 and 1.71 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp). Similar to the observation, Abdul (2017) recorded pre-ovipositional duration of *H. armigera* to lie in the range 2.57-3.14 days across CO_2 and generations on chickpea based semi-synthetic diet, and was highest in the third generation.

4.1.8.2 Effect on pre-ovipositional duration of *H. armigera* in Bt cotton

The data pertaining to pre-ovipositional duration of *H. armigera* in Bt cotton for all three generations was presented in Table 4.30 and Fig. 4.22.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on pre-ovipositional duration ranging between 2.50 and 1.90 days with highest at temperature 28 ºC and lowest at 35 ºC under *a*CO_2. At *e*CO_2, decrease in pre-ovipositional duration was recorded with increase in temperature with highest at 28 ºC with 2.00 days and lowest at 35 ºC with 1.52 days, respectively. The interaction effect of *e*CO_2 and *e*Temp showed significant impact on pre-ovipositional duration.

Second generation: In Bt cotton, pre-ovipositional duration varied from 2.50 to 2.00 days with highest and lowest at temperatures 28 ºC and 35 ºC, respectively. At *e*CO_2, decreased pre-ovipositional duration was recorded with increase in temperature from 28 ºC (2.25 days) to 35 ºC (1.56 days). The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant influence on pre-ovipositional duration.

Third generation: A decrease in pre-ovipositional duration with increase in temperature was recorded with highest duration of 2.75 days at 28 ºC and lowest duration of 2.15 days at 35 ºC. At *e*CO_2, pre-ovipositional duration ranged from 2.50 to 1.73 days with highest and lowest at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant influence on pre-ovipositional duration.

Mean of generations: The mean of three generations also indicated that at *a*CO_2 with increase in temperature (28-35 ºC) the pre-ovipositional duration decreased significantly (2.58, 2.52, 2.36, 2.10 and 2.02 days, respectively) corresponding to 2.32, 8.52, 18.58 and 21.94 %. Similarly, under *e*CO_2 also the pre-ovipositional duration decreased significantly (2.25, 1.85, 1.72, 1.64 and 1.60 days) with increase in

Table 4.30. Effect of *e*CO_2 and *e*Temp on pre-ovipositional duration of *H. armigera* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	2.50	2.00	**2.25**	2.50	2.25	**2.38**	2.75	2.50	**2.63**	2.58	2.25	**2.42**
29 ± 1°C	2.40	1.75	**2.08**	2.50	1.80	**2.15**	2.67	2.00	**2.33**	2.52	1.85	**2.19**
31 ± 1°C	2.20	1.60	**1.90**	2.33	1.67	**2.00**	2.56	1.88	**2.22**	2.36	1.72	**2.04**
33 ± 1°C	2.00	1.50	**1.75**	2.11	1.63	**1.87**	2.20	1.78	**1.99**	2.10	1.64	**1.87**
35 ± 1°C	1.90	1.52	**1.71**	2.00	1.56	**1.78**	2.15	1.73	**1.94**	2.02	1.60	**1.80**
Mean	**1.67**	**2.20**		**1.77**	**2.28**		**1.97**	**2.46**		**1.81**	**2.31**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	303.359*	0.02	0.06	132.065*	0.03	0.08	131.928*	0.03	0.08	474.77*	0.02	0.05
Temperature (°C)	44.859*	0.03	0.09	26.781*	0.05	0.13	34.001*	0.05	0.13	93.71*	0.03	0.07
Interaction (CO_2 + Temp(°C))	2.466*	0.05	0.13	4.047*	0.07	0.19	3.686*	0.07	0.19	8.73*	0.04	0.10
CV	4.38 %			4.72 %			5.16 %			5.73 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

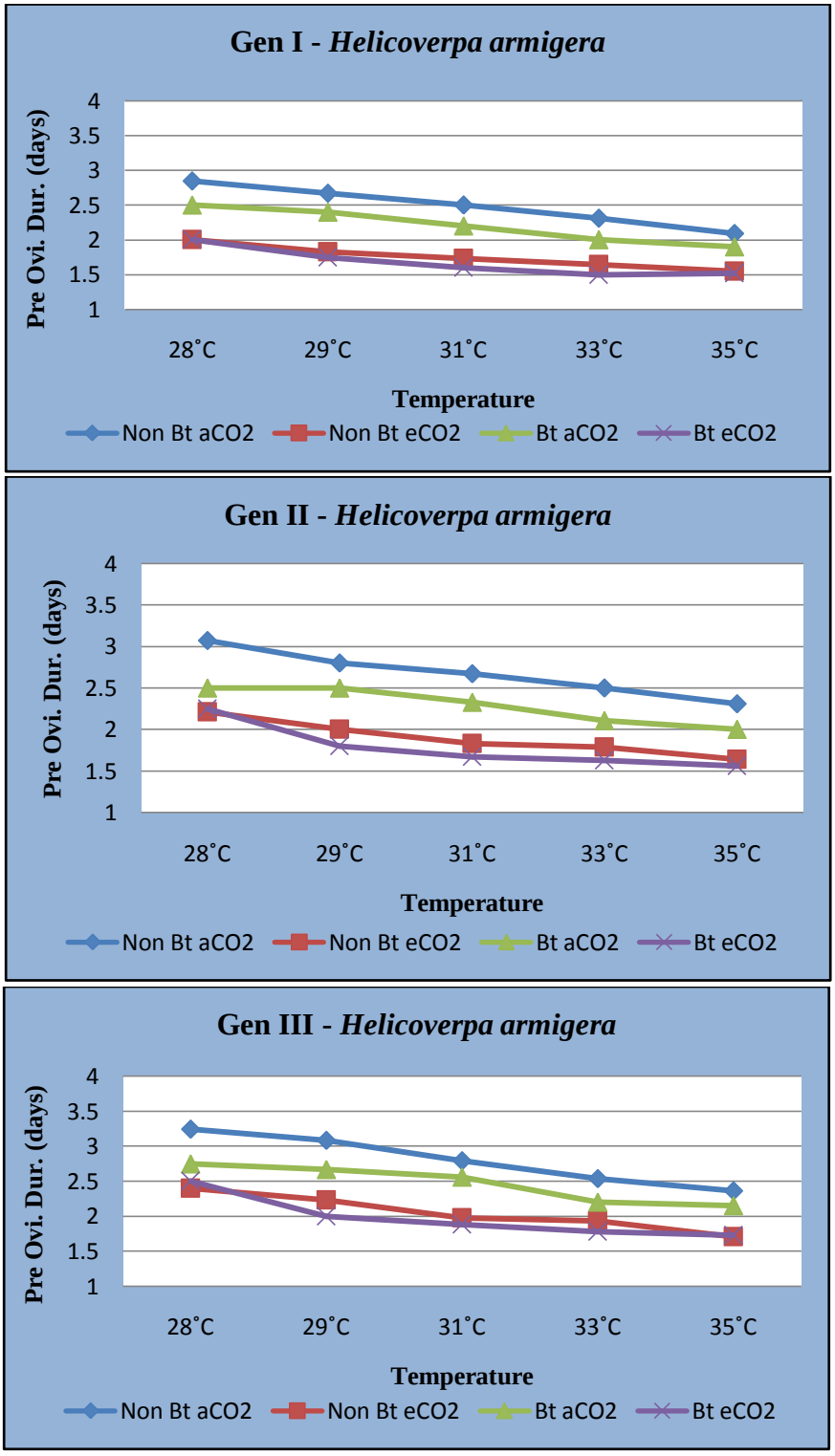


Fig. 4.22. Effect of eCO_2 and *e*Temp on pre-ovipositional duration of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

temperatures (28-35 ºC, respectively) which has corresponded to a decrease of 17.78, 23.70, 27.26 and 28.74 %. The interaction effect of *e*CO_2 and *e*Temp showed significant impact on pre-ovipositional duration. And among *a*CO_2 and *e*CO_2, pre-ovipositional duration was higher in *a*CO_2. Further, the pre-ovipositional duration under *e*CO_2 was lower (12.90, 26.68, 27.36, 22.19 and 20.20 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These results infer that, mean pre-ovipositional duration of *H. armigera* decreased with increase in both CO_2 (by 13 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 38 %. Pre-ovipositional period increased along the three generations as follows: 2.5, 2.5 and 2.75 days; 2, 2.25 and 2.50 days; and 1.52, 1.56 and 1.73 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp).

4.1.8.3 Effect on pre-ovipositional duration of *S. litura* in non-Bt cotton

The data pertaining to pre-ovipositional duration of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.31 and Fig. 4.23.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on pre-ovipositional duration ranging between 2.71 and 2.09 days with highest and lowest at temperatures 28 and 35 ºC, respectively *a*CO_2. At *e*CO_2, decrease in pre-ovipositional duration was recorded with increase in temperature from 28 to 35 ºC with 2.20 and 1.71 days as highest and lowest durations, respectively. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on pre-ovipositional duration.

Second generation: Highest pre-ovipositional duration was recorded at 28 ºC with 2.85 days and lowest at 35 ºC with 2.18 days. At *e*CO_2, decrease in pre-ovipositional duration was recorded with increase in temperature with highest pre-ovipositional duration of 2.31 days at 28 ºC temperature and 1.91 days at 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-ovipositional duration.

Third generation: A decrease in pre-ovipositional duration was recorded with increase in temperature with highest at 28 ºC (3.07 days) and lowest at 35 ºC (2.31 days). At *e*CO_2, pre-ovipositional duration ranged between 2.21 and 1.64 days with highest and lowest at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-ovipositional duration.

Mean of generations: The mean of three generations in non-Bt cotton also indicated that the pre-ovipositional duration has decreased significantly (2.88, 2.68, 2.53, 2.45 and 2.19 days, respectively) with increase in temperature (28- 35 ºC) corresponding to a decrease of 6.72, 11.94, 14.95 and 23.75 %. Similarly, under *e*CO_2, the pre-ovipositional duration decreased significantly (2.24, 2.11, 1.89, 1.83 and 1.75 days) with increase in temperatures (28- 35ºC, respectively) which has corresponded to a decrease of 5.80, 15.48, 18.30 and 21.73 %. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on pre-ovipositional duration. And pre-ovipositional duration was higher in *a*CO_2 than *e*CO_2. Further, the pre-ovipositional duration under *e*CO_2 was lower (22.13, 21.37, 25.26, 25.20 and 20.06 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These results infer that, mean pre-ovipositional duration of *S. litura* decreased with increase in both CO_2 (by 22 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 39 %. Findings of Ranga Rao *et al*. (1989) supports the present results where pre-ovipositional duration of *S. litura* in groundnut decreased from 3.3 (20 °C) to 1.6 days (30 °C), after which the moths did not survive 35 °C. Srinivasa Rao *et al*. (2014a) recorded that pre-ovipositional duration of *S. litura* on peanut decreased with both *e*CO_2 and high temperature from 5 days @ *a*CO_2 + 25 °C to 2.5 days @ *e*CO_2 + 35 °C. Liu *et al* (2017) observed no significant difference in the pre-ovipositional periods of female moths of *H. armigera* under three CO_2 concentrations (380, 550 and 750 ppm), although the longest total pre-ovipositional period was observed at 750 ppm. *S. litura* pre-ovipositional period decreased along the three generations as follows: 2.71, 2.85 and 3.07 days; 2.20, 2.31 and 2.21 days; and 1.71, 1.91 and 1.64 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp).

4.1.8.4 Effect on pre-ovipositional duration of *S. litura* in Bt cotton

The data pertaining to pre-ovipositional duration of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.32 and Fig. 4.23.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on pre-ovipositional duration, ranging from 2.25 to 1.85 days with highest and lowest

Table 4.31. Effect of *e*CO_2 and *e*Temp on pre-ovipositional duration of *S. litura* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	2.71	2.20	**2.45**	2.85	2.31	**2.58**	3.07	2.21	**2.64**	2.88	2.24	**2.56**
29 ± 1°C	2.58	2.08	**2.33**	2.67	2.25	**2.46**	2.80	2.00	**2.40**	2.68	2.11	**2.40**
31 ± 1°C	2.43	1.85	**2.14**	2.50	2.00	**2.25**	2.67	1.83	**2.25**	2.53	1.89	**2.21**
33 ± 1°C	2.38	1.79	**2.09**	2.46	1.91	**2.19**	2.50	1.79	**2.14**	2.45	1.83	**2.14**
35 ± 1°C	2.09	1.71	**1.90**	2.18	1.91	**2.05**	2.31	1.64	**1.97**	2.19	1.75	**1.97**
Mean	**1.92**	**2.43**		**2.07**	**2.53**		**1.89**	**2.66**		**1.96**	**2.54**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	84.447*	0.04	0.11	76.598*	0.04	0.10	138.994*	0.05	0.13	336.22*	0.02	0.06
Temperature (°C)	11.820*	0.06	0.17	13.416*	0.06	0.16	12.005*	0.07	0.21	40.95*	0.04	0.10
Interaction (CO_2 + Temp(°C))	0.49	0.09	NS	0.98	0.08	NS	0.28	0.10	NS	1.36	0.05	NS
CV	5.26 %			5.89 %			5.76 %			5.11 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Table 4.32. Effect of *e*CO_2 and *e*Temp on pre-ovipositional duration of *S. litura* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	2.25	2.00	**2.13**	2.50	2.20	**2.35**	2.50	2.40	**2.45**	2.42	2.20	**2.31**
29 ± 1°C	2.17	1.75	**1.96**	2.20	2.00	**2.10**	2.29	2.14	**2.21**	2.22	1.96	**2.09**
31 ± 1°C	2.00	1.67	**1.83**	2.13	1.88	**2.00**	2.25	2.00	**2.13**	2.13	1.85	**1.99**
33 ± 1°C	1.88	1.67	**1.77**	1.90	1.70	**1.80**	1.90	1.90	**1.90**	1.89	1.76	**1.82**
35 ± 1°C	1.85	1.58	**1.71**	1.85	1.64	**1.74**	1.92	1.75	**1.84**	1.87	1.66	**1.76**
Mean	**1.73**	**2.02**		**1.88**	**2.11**		**2.03**	**2.17**		**1.88**	**2.10**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	45.293*	0.03	0.09	24.029*	0.03	0.09	5.171*	0.04	0.12	62.66*	0.02	0.06
Temperature (°C)	11.249*	0.05	0.14	21.386*	0.05	0.15	14.310*	0.07	0.18	49.29*	0.03	0.09
Interaction (CO_2 + Temp(°C))	0.69	0.07	NS	0.17	0.08	NS	0.50	0.09	NS	0.74	0.04	NS
CV	5.40 %			5.72 %			5.07 %			5.01 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

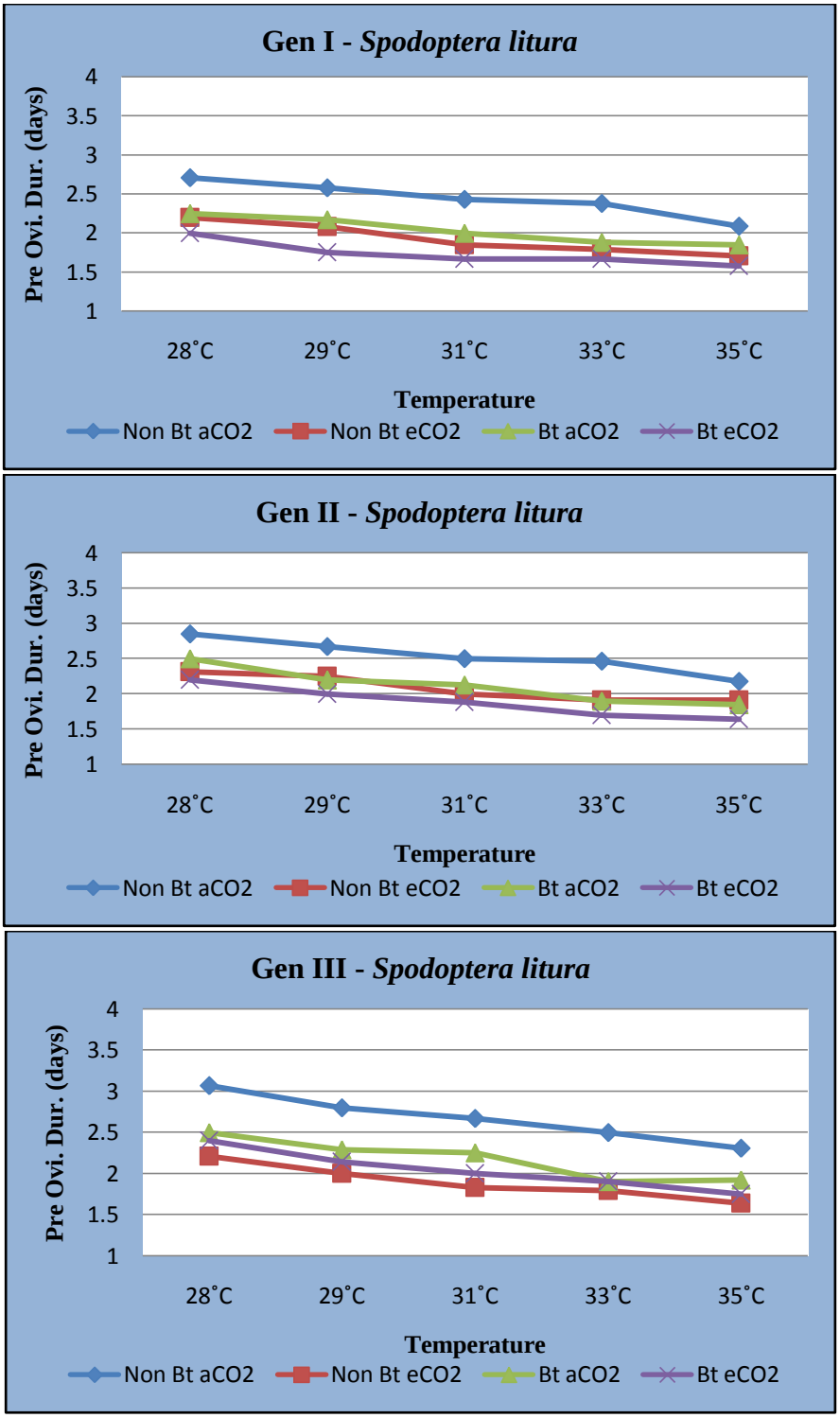


Fig. 4.23. Effect of *e*CO_2 and *e*Temp on pre-ovipositional duration of *S. litura* on non-Bt and Bt cotton in first, second and third generation

at temperatures 28 and 35 ºC, respectively under *a*CO_2. At *e*CO_2, a decrease in pre-ovipositional duration was recorded with increase in temperature with highest at 28 ºC with 2.00 days and lowest at 35 ºC with 1.58 days. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on pre-ovipositional duration.

Second generation: A decrease in pre-ovipositional duration with increase in temperature from 28 to 35 ºC with 2.50 to 1.85 days as highest and lowest pre-ovipositional duration, respectively. At *e*CO_2, pre-ovipositional duration ranged between 2.20 to 1.64 days with highest and lowest duration at temperatures 28 and 35 ºC, respectively.The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-ovipositional duration.

Third generation: Pre-ovipositional duration varied from 2.50 to 1.92 days with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in pre-ovipositional duration was recorded with increase in temperature from 28 to 35 ºC with 2.40 to 1.75 days, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on pre-ovipositional duration.

Mean of generations: The mean pre-ovipositional duration has decreased significantly (2.42, 2.22, 2.13, 1.89 and 1.87 days, respectively) with increase in temperature (28- 35 ºC) corresponding to a decrease of 8.14, 12.00, 21.66 and 22.48 %. Similarly, under *e*CO_2 also the pre-ovipositional duration decreased significantly (2.20, 1.96, 1.85, 1.76 and 1.66 days) with increase in temperatures (28- 35ºC, respectively) which has corresponded to a decrease of 10.76, 15.91, 20.15 and 24.70 %. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on pre-ovipositional duration in Bt cotton. And pre-ovipositional duration was higher in *a*CO_2 than *e*CO_2. Further, the pre-ovipositional duration under *e*CO_2 was lower (8.97, 11.56, 13.01, 7.22 and 11.57 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2. Finally it can be inferred that, mean pre-ovipositional duration of *S. litura* in Bt cotton decreased with increase in both CO_2 (by 9 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 31 %. Pre-ovipositional period increased along the three generations as follows: 2.25, 2.5 and 2.5 days; 2.0, 2.2 and 2.40 days; and 1.58, 1.64 and 1.75 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp.

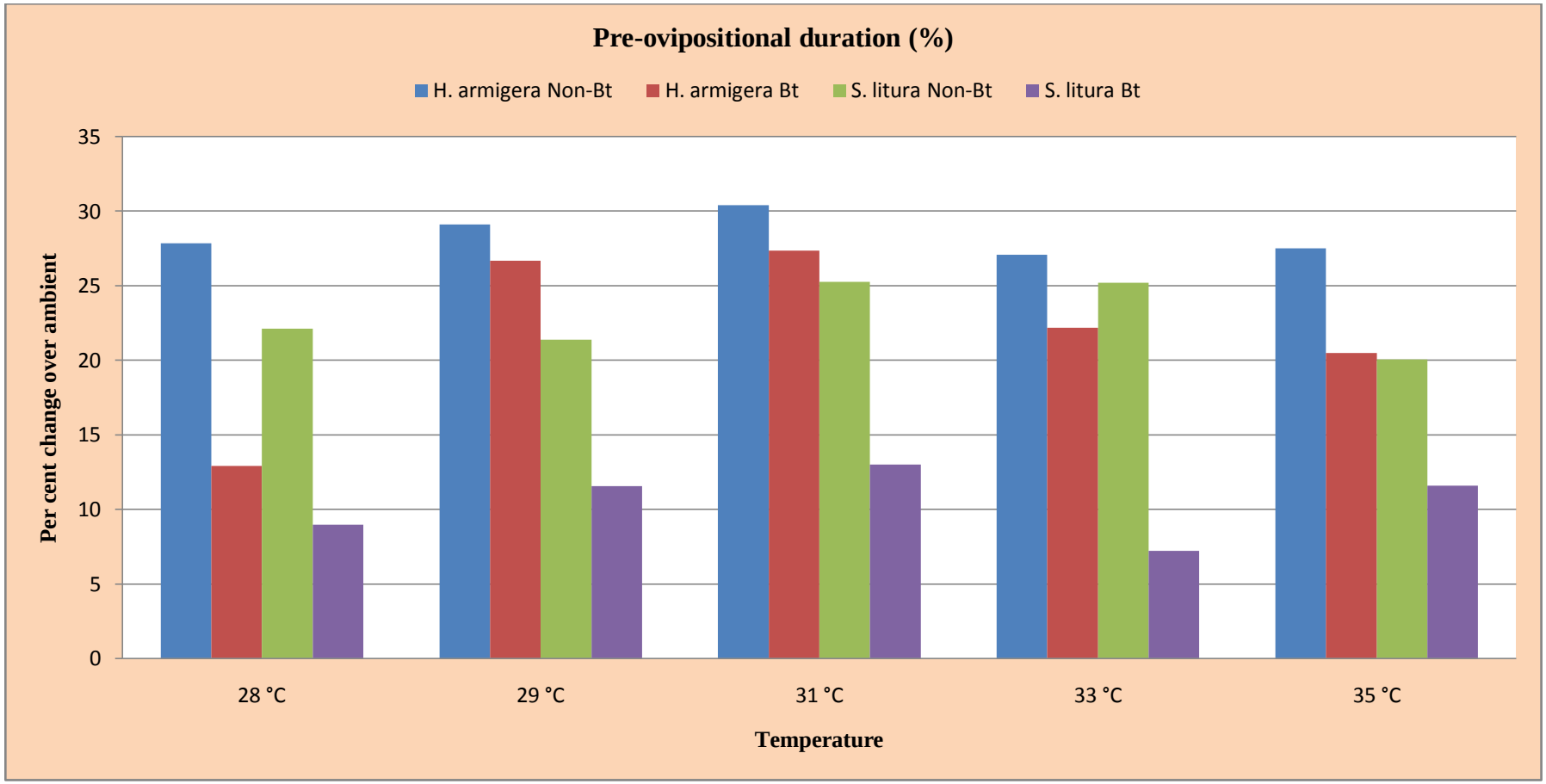


Fig. 4.24. Effect of *e*CO_2 and *e*Temp on mean pre-ovipositional duration of *H. armigera* and *S. litura* on non-Bt and Bt cotton

4.1.9 Ovipositional duration: The perusal of results provided in Tables 4.33 - 4.36 and Fig. 4.25 - 4.26 indicate that, with increase in test temperatures (28, 29, 31, 33 and 35 °C), under both *a*CO_2 (380±25 ppm) and *e*CO_2 (550± 25 ppm) conditions, ovipositional duration of *H. armigera* and *S. litura* gradually decreased. Comparative performance of both the test insects was presented in Fig. 4.27.

4.1.9.1 Effect on ovipositional duration of *H. armigera* in non-Bt cotton

The data pertaining to ovipositional duration of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.33 and Fig. 4.25.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on ovipositional duration varying from 7.46 to 6.82 days with highest and lowest at temperatures 28 and 35 ºC, respectively under *a*CO_2. At *e*CO_2, decrease in ovipositional duration was recorded with increase in temperature with highest and lowest ovipositional duration at temperatures 28 and 35 ºC with 6.77 and 6.09 days, respectively. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on ovipositional duration.

Second generation: At *a*CO_2, ovipositional duration varied from 7.60 to 6.92 days with highest and lowest at temperatures 28 ºC and 35 ºC, respectively. At *e*CO_2, decrease in ovipositional duration was recorded with increase in temperature with highest and lowest ovipositional duration of 6.86 and 6.27 days at temperatures 28 ºC and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on ovipositional duration.

Third generation: Ovipositional duration ranged between 7.76 to 7.00 days with highest at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, decrease in ovipositional duration was recorded in the range of 7.13 to 6.43 days with corresponding increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on ovipositional duration.

Mean of generations: The mean of three generations in non-Bt cotton also indicated that with increase in temperature (28-35 °C) the ovipositional duration of *H. armigera* has decreased significantly (7.61, 7.50, 7.32, 7.08 and 6.91 days, respectively)

corresponding to 1.36, 3.81, 6.97 and 9.11 %. Similarly, under *e*CO_2 also the ovipositional duration decreased significantly (6.92, 6.77, 6.73, 6.52 and 6.26 days) with increase in temperatures (28- 35 ºC, respectively) which has corresponded to a decrease of 2.12, 2.79, 5.73 and 9.49 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on ovipositional duration. And among *a*CO_2 and *e*CO_2, ovipositional duration was higher in *a*CO_2. Further, the ovipositional duration under *e*CO_2 was lower (9.03, 9.73, 8.06, 7.82 and 9.40 %, at 28, 29, 31, 33 and 35 ºC, respectively) compared to that of *a*CO_2.

These results infer that, mean the ovipositional duration of *H. armigera* decreased with increase in both CO_2 (by 9 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 18 %. The ovipositional period increased along the three generations as follows: 7.4, 7.6 and 7.7 days; 6.7, 6.8 and 7.1 days; and 6, 6.2 and 6.4 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp. Similarly, Abdul (2017) recorded decrease in the ovipositional duration of *H. armigera* on chickpea diet from first to fourth generations (6.71, 6.5, 6.35 and 6.28 days) under *e*CO_2 condition compared to *a*CO_2 (7.64, 7.35, 7.64 and 7.35 days).

4.1.9.2 Effect on ovipositional duration of *H. armigera* in Bt cotton

The data pertaining to ovipositional duration of *H. armigera* in Bt cotton for all three generations was presented in Table 4.34 and Fig. 4.25.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on ovipositional duration. At *a*CO_2, ovipositional duration decreased from 7.25 to 6.60 days with highest duration at temperature 28 ºC and lowest 35 ºC. At *e*CO_2, decrease in ovipositional duration was recorded from 6.67 to 5.89 days with increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on ovipositional duration.

Second generation: Highest ovipositional duration was recorded at 28 ºC with 7.50 days and lowest at 35 ºC with 6.82 days. At *e*CO_2, decreased ovipositional duration was recorded with increase in temperature with highest duration of 6.75 days at 28 ºC and lowest 6.11 days at 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on ovipositional duration.

Table 4.33. Effect of *e*CO_2 and *e*Temp on ovipositional duration of *H. armigera* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	7.46	6.77	**7.12**	7.60	6.86	**7.23**	7.76	7.13	**7.45**	7.61	6.92	**7.26**
29 ± 1°C	7.40	6.50	**6.95**	7.53	6.82	**7.18**	7.58	7.00	**7.29**	7.50	6.77	**7.14**
31 ± 1°C	7.19	6.53	**6.86**	7.33	6.67	**7.00**	7.43	6.98	**7.20**	7.32	6.73	**7.02**
33 ± 1°C	7.00	6.36	**6.68**	7.08	6.50	**6.79**	7.15	6.71	**6.93**	7.08	6.52	**6.80**
35 ± 1°C	6.82	6.09	**6.45**	6.92	6.27	**6.60**	7.00	6.43	**6.71**	6.91	6.26	**6.59**
Mean	**6.45**	**7.17**		**6.62**	**7.29**		**6.85**	**7.38**		**6.64**	**7.28**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	62.358*	0.07	0.18	65.358*	0.06	0.16	34.992*	0.06	0.18	155.66*	0.04	0.10
Temperature (°C)	6.176*	0.10	0.29	8.105*	0.09	0.26	8.407*	0.10	0.28	21.91*	0.06	0.16
Interaction (CO_2 + Temp(°C))	0.27	0.15	NS	0.11	0.13	NS	0.18	0.14	NS	0.39	0.08	NS
CV	3.61 %			4.45 %			5.05 %			5.85 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Table 4.34. Effect of *e*CO_2 and *e*Temp on ovipositional duration of *H. armigera* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	7.25	6.67	**6.96**	7.50	6.75	**7.13**	7.75	7.00	**7.38**	7.50	6.81	**7.15**
29 ± 1°C	7.20	6.50	**6.85**	7.50	6.60	**7.05**	7.67	6.80	**7.23**	7.46	6.63	**7.04**
31 ± 1°C	7.20	6.40	**6.80**	7.33	6.50	**6.92**	7.44	6.63	**7.03**	7.32	6.51	**6.92**
33 ± 1°C	6.90	6.33	**6.62**	7.00	6.38	**6.69**	7.20	6.44	**6.82**	7.03	6.38	**6.71**
35 ± 1°C	6.60	5.89	**6.24**	6.82	6.11	**6.46**	7.08	6.36	**6.72**	6.83	6.12	**6.43**
Mean	**6.35**	**7.03**		**6.46**	**7.23**		**6.64**	**7.42**		**6.49**	**7.21**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	166.383*	0.04	0.10	83.382*	0.06	0.15	162.591*	0.04	0.12	333.56*	0.03	0.08
Temperature (°C)	23.088*	0.06	0.16	13.772*	0.09	0.24	15.953*	0.07	0.19	42.33*	0.04	0.12
Interaction (CO_2 + Temp(°C))	0.69	0.08	NS	1.13	0.12	NS	0.20	0.10	NS	1.14	0.06	NS
CV	6.15 %			5.99 %			6.88 %			4.55 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

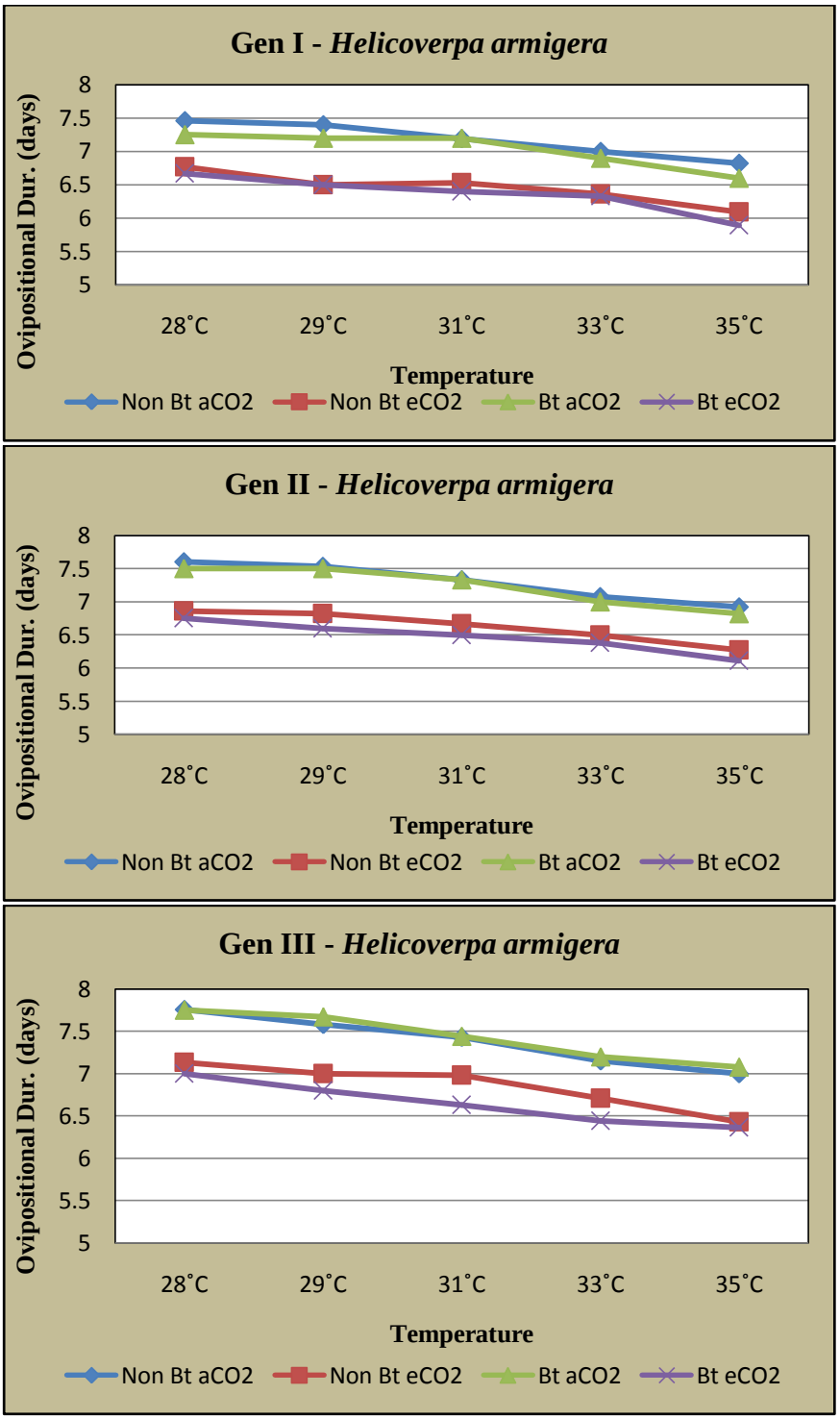


Fig. 4.25. Effect of eCO_2 and *e*Temp on ovipositional duration of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

Third generation: At *a*CO_2, decrease in ovipositional duration from 7.75 to 7.08 days was recorded with increase in temperature from 28 to 35 ºC. At *e*CO_2, ovipositional duration decreased from 7.00 and 6.36 days with increase in temperature from 28 and 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on ovipositional duration.

Mean of generations: The mean of three generations in Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28-35 °C) the ovipositional duration has decreased significantly (7.50, 7.46, 7.32, 7.03 and 6.83 days, respectively) corresponding to 0.58, 2.36, 6.22 and 8.89 %. Similarly, under *e*CO_2 also the ovipositional duration decreased significantly (6.81, 6.63, 6.51, 6.38 and 6.12 days) with increase in temperatures (28-35 °C, respectively) which has corresponded to a decrease of 2.55, 4.36, 6.22 and 10.09 %. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on ovipositional duration in Bt cotton. And among *a*CO_2 and *e*CO_2, ovipositional duration was higher in *a*CO_2. Further, the ovipositional duration under *e*CO_2 was lower (9.24, 11.04, 11.11, 9.24 and 10.44 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These results infer that, mean ovipositional duration of *H. armigera* decreased with increase in both CO_2 (by 9 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 18 %. The ovipositional period increased along the three generations as follows: 7.2, 7.5 and 7.75 days; 6.67, 6.75 and 7.00 days; and 5.89, 6.11 and 6.36 days respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp.

4.1.9.3 Effect on ovipositional duration of *S. litura* in non-Bt cotton

The data pertaining to ovipositional duration of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.35 and Fig. 4.26.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on ovipositional duration. And under *a*CO_2 decreased from 7.41 and 6.91 days with increase in temperatures from 28 and 35 ºC. At *e*CO_2, decrease in ovipositional duration was recorded with increase in temperature with highest ovipositional duration of 7.07 days at 28 ºC and lowest 6.29 days at 35 ºC. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on ovipositional duration.

Second generation: At *a*CO_2, ovipositional duration ranged between 7.62 and 6.91 days with highest and lowest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in ovipositional duration was recorded with increase in temperature with highest at temperature 28 ºC (7.31 days) and lowest at 35 ºC (6.45 days).The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on ovipositional duration.

Third generation: In non-Bt cotton, decrease in ovipositional duration was recorded with increase in temperature from 28 to 35 ºC with 7.80 and 7.08 days of ovipositional duration as highest and lowest, respectively. At *e*CO_2, ovipositional duration ranged from 7.57 to 6.64 days with corresponding increase in temperature from 28 and 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on ovipositional duration.

Mean of generations: The mean of three generations also indicated that the ovipositional duration has decreased significantly (7.61, 7.52, 7.34, 7.16 and 6.97 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 1.18, 3.55, 5.87 and 8.45 %. Similarly, under *e*CO_2, the ovipositional duration decreased significantly (7.32, 7.09, 7.01, 6.80 and 6.46 days) with increase in temperatures (28-35 ºC, respectively) which has corresponded to a decrease of 3.10, 4.24, 7.11 and 11.71 %. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on ovipositional duration. And ovipositional duration was significantly higher in *a*CO_2 than *e*CO_2. Further, the ovipositional duration under *e*CO_2 was lower (3.85, 5.72, 4.54, 5.12 and 7.27 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These results infer that, mean ovipositional duration of *S. litura* decreased with increase in both CO_2 (by 4 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 15 %. Analogously, Farahani *et al*. (2011) reported that ovipositional duration of *S. exigua* was least in cotton (6.55), followed by maize (8.0) and rapeseed (8.5 days). The ovipositional period increased along the three generations as follows: 7.41, 7.62 and 7.8 days; 7.07, 7.31 and 7.57 days; and 6.29, 6.45 and 6.64 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp).

Table 4.35. Effect of *e*CO_2 and *e*Temp on ovipositional duration of *S. litura* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	7.41	7.07	**7.24**	7.62	7.31	**7.46**	7.80	7.57	**7.69**	7.61	7.32	**7.46**
29 ± 1°C	7.42	6.92	**7.17**	7.47	7.08	**7.28**	7.67	7.27	**7.47**	7.52	7.09	**7.30**
31 ± 1°C	7.21	6.78	**6.99**	7.31	7.07	**7.19**	7.50	7.17	**7.33**	7.34	7.01	**7.17**
33 ± 1°C	7.08	6.57	**6.82**	7.08	6.82	**6.95**	7.33	7.00	**7.17**	7.16	6.80	**6.98**
35 ± 1°C	6.91	6.29	**6.60**	6.91	6.45	**6.68**	7.08	6.64	**6.86**	6.97	6.46	**6.71**
Mean	**6.72**	**7.20**		**6.94**	**7.27**		**7.12**	**7.47**		**6.93**	**7.31**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	29.420*	0.06	0.18	14.199*	0.06	0.17	18.291*	0.06	0.16	64.98*	0.03	0.09
Temperature (°C)	6.934*	0.10	0.28	9.565*	0.10	0.27	12.013*	0.09	0.25	29.69*	0.05	0.15
Interaction (CO_2 + Temp(°C))	0.26	0.14	NS	0.20	0.14	NS	0.19	0.13	NS	0.58	0.08	NS
CV	4.07 %			4.74 %			4.76 %			5.31 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

4.1.9.4 Effect on ovipositional duration of *S. litura* in Bt cotton

The data pertaining to ovipositional duration of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.36 and Fig. 4.26.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on ovipositional duration. And under *a*CO_2, it decreased from 7.25 to 6.54 days with highest and lowest duration at temperatures 28 and 35 ºC, respectively. At *e*CO_2, ovipositional duration decreased with increase in temperature with highest and lowest ovipositional duration at 28 ºC with 6.75 days and lowest at 35 ºC with 6.08 days. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on ovipositional duration.

Second generation: At *a*CO_2, decrease in ovipositional duration was recorded from 7.50 to 6.46 days with increase in temperature from 28 to 35 ºC. At *e*CO_2, ovipositional duration ranged between 7.00 and 6.18 days with highest and lowest ovipositional duration at temperatures 28 and 35ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on ovipositional duration.

Third generation: At *a*CO_2, ovipositional duration varied from 7.67 to 6.77 days with highest duration at 28 ºC and lowest at 35 ºC. At *e*CO_2, a decrease in ovipositional duration was recorded from 7.20 to 6.33 days with corresponding increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on ovipositional duration.

Mean of generations: The mean of three generations on Bt cotton, also indicated that the ovipositional duration by larvae has decreased significantly (7.47, 7.21, 7.00, 6.78 and 6.59 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to decrease of 3.52, 6.29, 9.23, 11.82 %. Similarly, under *e*CO_2 also the ovipositional duration decreased significantly (6.98, 6.77, 6.60, 6.48 and 6.20 days) with increase in temperatures (28-35 °C, respectively) which has corresponded to a decrease of 3.10, 5.54, 7.16 and 11.26 %. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on ovipositional duration. And ovipositional duration was significantly higher in *a*CO_2 than *e*CO_2. Further, the ovipositional duration under

Table 4.36. Effect of *e*CO_2 and *e*Temp on ovipositional duration of *S. litura* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	7.25	6.75	**7.00**	7.50	7.00	**7.25**	7.67	7.20	**7.43**	7.47	6.98	**7.23**
29 ± 1°C	7.00	6.50	**6.75**	7.20	6.80	**7.00**	7.43	7.00	**7.21**	7.21	6.77	**6.99**
31 ± 1°C	6.88	6.33	**6.60**	6.88	6.63	**6.75**	7.25	6.83	**7.04**	7.00	6.60	**6.80**
33 ± 1°C	6.75	6.45	**6.60**	6.60	6.40	**6.50**	7.00	6.60	**6.80**	6.78	6.48	**6.63**
35 ± 1°C	6.54	6.08	**6.31**	6.46	6.18	**6.32**	6.77	6.33	**6.55**	6.59	6.20	**6.39**
Mean	**6.42**	**6.88**		**6.60**	**6.92**		**6.79**	**7.22**		**6.60**	**7.01**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	65.316*	0.04	0.11	38.852*	0.04	0.10	39.793*	0.05	0.13	147.67*	0.02	0.07
Temperature (°C)	15.552*	0.06	0.18	40.771*	0.06	0.16	20.550*	0.08	0.21	73.79*	0.04	0.10
Interaction (CO_2 + Temp(°C))	0.52	0.09	NS	1.09	0.08	NS	0.03	0.11	NS	0.87	0.05	NS
CV	6.77 %			6.11 %			5.68 %			3.87 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

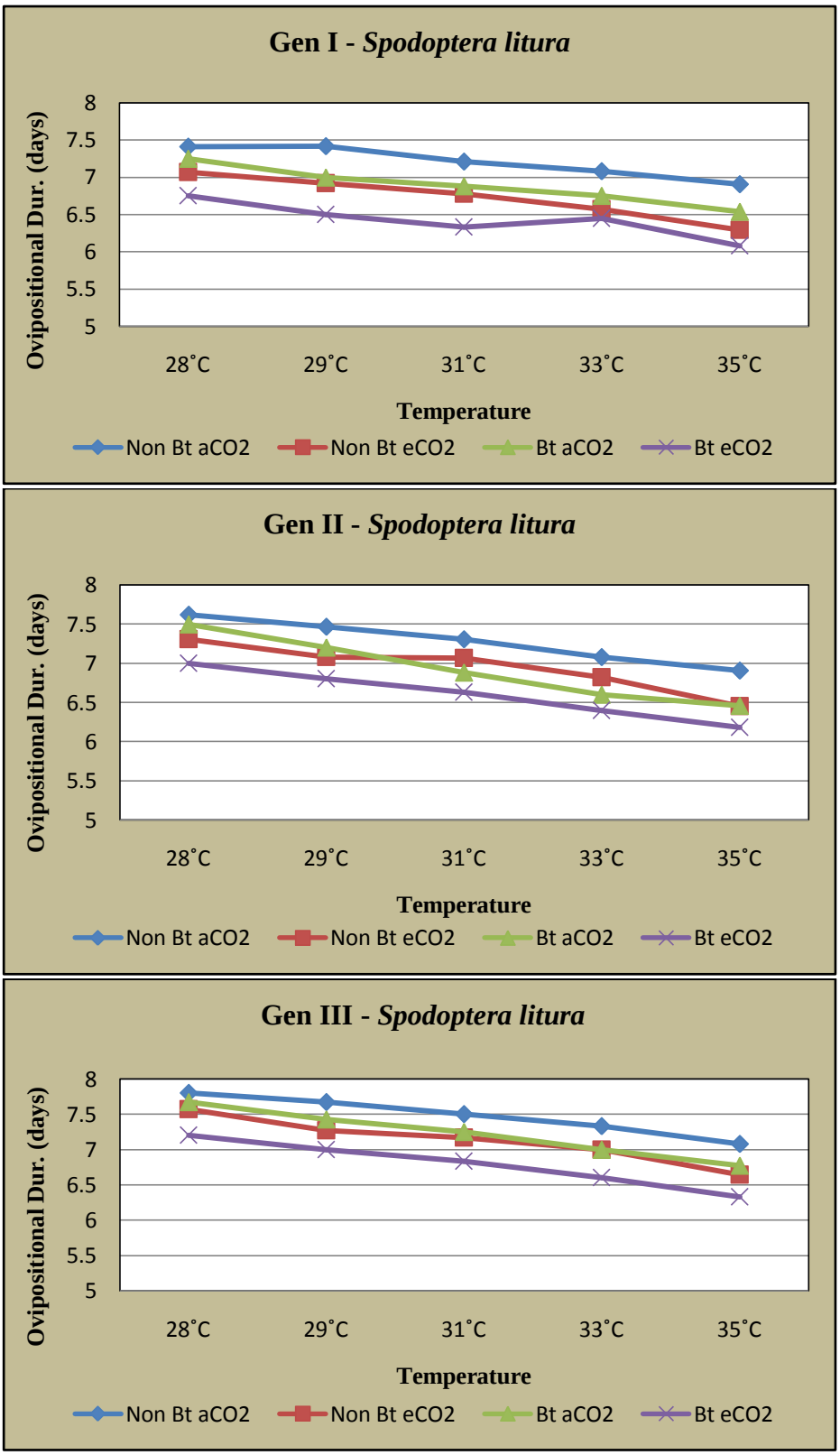


Fig. 4.26. Effect of eCO_2 and *e*Temp on ovipositional duration of *S. litura* on non-Bt and Bt cotton in first, second and third generation

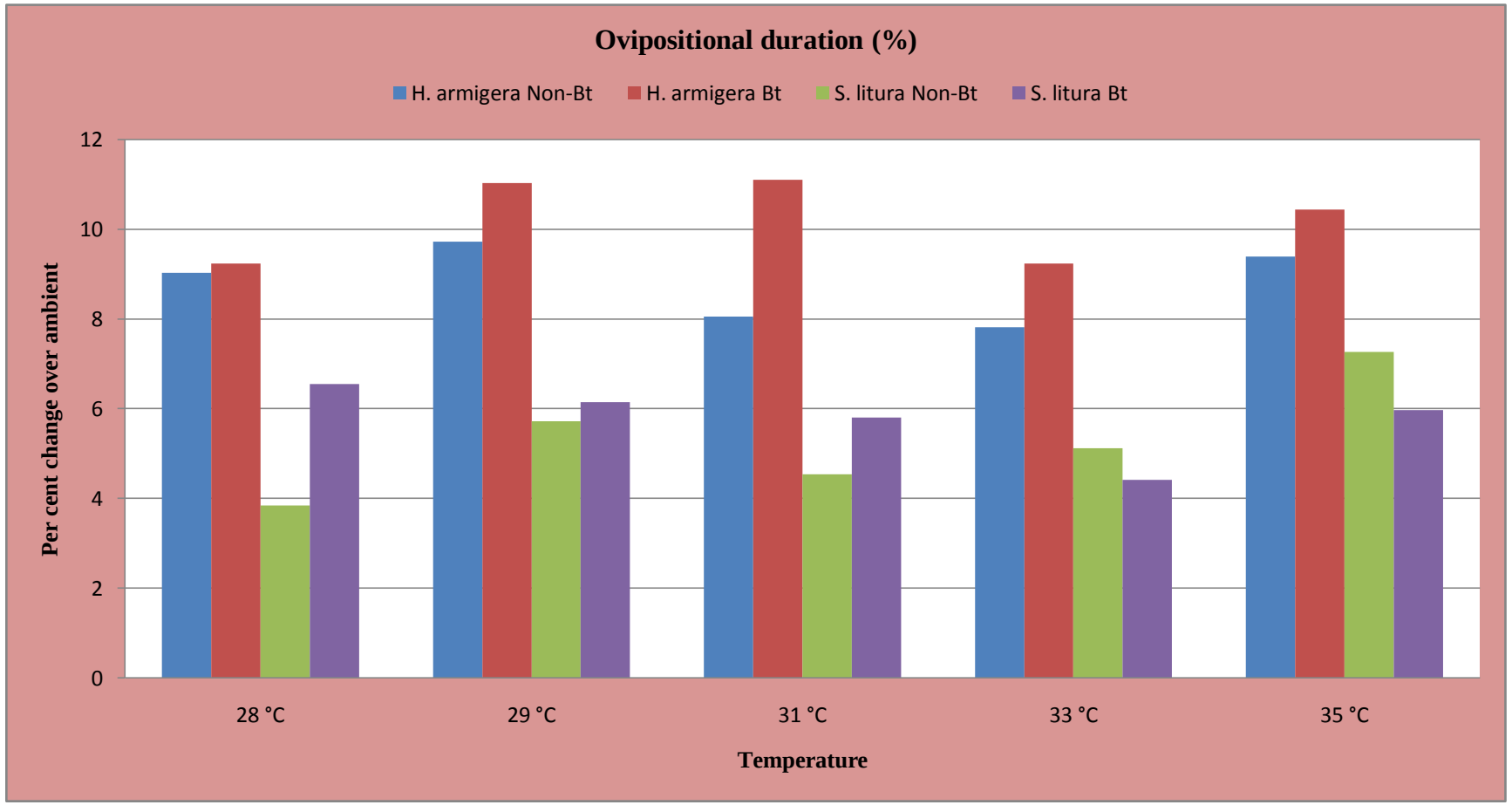


Fig. 4.27. Effect of *e*CO_2 and *e*Temp on mean ovipositional duration of *H. armigera* and *S. litura* on non-Bt and Bt cotton

eCO_2 was lower (6.56, 6.15, 5.81, 4.42 and 5.97 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.These results infer that, mean ovipositional duration of *S. litura* in Bt cotton decreased with increase in both CO_2 (by 7 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 17 %. The ovipositional period increased along the three generations as follows: 7.25, 7.5 and 7.67 days; 6.75, 7.00 and 7.20 days; and 6.08, 6.18 and 6.33 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp).

4.1.10 Post-ovipositional duration: The data on the effect of *e*CO_2 and *e*Temp conditions on the post-ovipositional duration of *H. armigera* and *S. litura* was presented in the Tables 4.37 - 4.40 and Fig. 4.28 - 4.29 With increase in CO_2 and test temperatures (28 - 35 ºC), the post-ovipositional duration of test insects gradually decreased. Comparative performance of both the test insects was presented in Fig. 4.30.

4.1.10.1 Effect on post-ovipositional duration of *H. armigera* in non-Bt cotton

The data pertaining to ovipositional duration of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.37 and Fig. 4.28.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on post-ovipositional duration. At *a*CO_2, the post-ovipositional duration varied from 3.23 to 2.27 days with highest and lowest duration at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in post-ovipositional duration was recorded from 2.08 to 1.45 days with corresponding increase in temperature from 28 to 35 ºC. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on post-ovipositional duration.

Second generation: At *a*CO_2, post-ovipositional duration ranged between 3.40 to 2.46 days with highest temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, decrease in post-ovipositional duration was recorded with increase in temperature from 28 to 35 ºC with highest and lowest post-ovipositional duration as 2.21 and 1.45 days, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on post-ovipositional duration.

Table 4.37. Effect of *e*CO_2 and *e*Temp on post-ovipositional duration of *H. armigera* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	3.23	2.08	**2.65**	3.40	2.21	**2.81**	3.53	2.33	**2.93**	3.39	2.21	**2.80**
29 ± 1°C	3.00	1.75	**2.38**	3.20	1.91	**2.55**	3.33	2.00	**2.67**	3.18	1.89	**2.53**
31 ± 1°C	2.75	1.60	**2.18**	2.92	1.75	**2.33**	3.07	1.78	**2.43**	2.91	1.71	**2.31**
33 ± 1°C	2.54	1.55	**2.04**	2.75	1.57	**2.16**	2.92	1.71	**2.32**	2.74	1.61	**2.17**
35 ± 1°C	2.27	1.45	**1.86**	2.46	1.45	**1.96**	2.73	1.57	**2.15**	2.49	1.49	**1.99**
Mean	**1.68**	**2.75**		**1.78**	**2.94**		**1.88**	**3.11**		**1.78**	**2.94**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	230.429*	0.05	0.14	299.478*	0.05	0.13	303.881*	0.05	0.14	875.92	0.03	0.08
Temperature (°C)	14.928*	0.08	0.22	19.381*	0.08	0.21	14.898*	0.08	0.22	51.49*	0.04	0.12
Interaction (CO_2 + Temp(°C))	1.15	0.11	NS	0.46	0.11	NS	0.21	0.11	NS	1.57	0.06	NS
CV	5.15 %			4.54 %			5.46 %			3.11 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Third generation: At *a*CO_2, post-ovipositional duration varied from 3.53 to 2.73 days with highest and lowest at temperatures 28 ºC and 35 ºC, respectively. At *e*CO_2, decrease in post-ovipositional duration was recorded with increase in temperature with highest at 28 ºC with 2.33 days and lowest at 35 ºC with 1.57 days. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on post-ovipositional duration.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28-35 ºC) the post-ovipositional duration has decreased significantly (3.39, 3.18, 2.91, 2.74 and 2.49 days, respectively) corresponding to 6.20, 13.98, 19.19 and 26.57 %. Similarly, under *e*CO_2 also the post-ovipositional duration decreased significantly (2.21, 1.89, 1.71, 1.61 and 1.49 days) with increase in temperature (28- 35 ºC) which has corresponded to a decrease of 14.50, 22.51, 27.04 and 32.48 %. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on post-ovipositional duration. And post-ovipositional duration was higher in *a*CO_2 than *e*CO_2. Further, the post-ovipositional duration under *e*CO_2 was lower (34.84, 40.61, 41.30, 41.17 and 40.08 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

Finally it can be inferred that, mean post-ovipositional duration of *H. armigera* decreased with increase in both CO_2 (by 35 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 56 %. Abdul (2017) recorded post-ovipositional duration of *H. armigera* to lie in the range 2.71-3.28 days across the CO_2 conditions on chickpea semi-synthetic diet. Post-ovipositional period increased along the three generations as follows: 3.23, 3.40 and 3.53 days; 2.08, 2.21 and 2.33 days; and 1.45, 1.45 and 1.57 days respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp.

4.1.10.2 Effect on post-ovipositional duration of *H. armigera* in Bt cotton

The data pertaining to ovipositional duration of *H. armigera* in Bt cotton for all three generations was presented in Table 4.38 and Fig. 4.28.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on post-ovipositional duration. At *a*CO_2, post-ovipositional duration decreased from 3.00 to 2.30 days with corresponding increase in temperature from 28 to 35 ºC. At

Table 4.38. Effect of *e*CO_2 and *e*Temp on post-ovipositional duration of *H. armigera* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	3.00	2.00	**2.50**	2.75	2.25	**2.50**	3.00	2.50	**2.75**	2.92	2.25	**2.58**
29 ± 1°C	2.80	1.75	**2.28**	2.67	2.00	**2.33**	2.83	2.20	**2.52**	2.77	1.98	**2.38**
31 ± 1°C	2.60	1.60	**2.10**	2.50	1.83	**2.17**	2.56	2.13	**2.34**	2.55	1.85	**2.20**
33 ± 1°C	2.50	1.50	**2.00**	2.33	1.63	**1.98**	2.50	1.89	**2.19**	2.44	1.67	**2.06**
35 ± 1°C	2.30	1.44	**1.87**	2.18	1.56	**1.87**	2.31	1.64	**1.97**	2.26	1.55	**1.89**
Mean	**1.65**	**2.64**		**1.85**	**2.48**		**2.07**	**2.63**		**1.86**	**2.58**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	447.403*	0.03	0.09	144.268*	0.04	0.10	104.685*	0.04	0.11	623.40*	0.02	0.06
Temperature (°C)	22.337*	0.05	0.14	22.086*	0.06	0.16	22.924*	0.06	0.17	69.73*	0.03	0.09
Interaction (CO_2 + Temp(°C))	0.50	0.07	NS	0.63	0.08	NS	0.65	0.09	NS	0.66	0.05	NS
CV	5.06 %			6.38 %			5.68 %			4.29 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

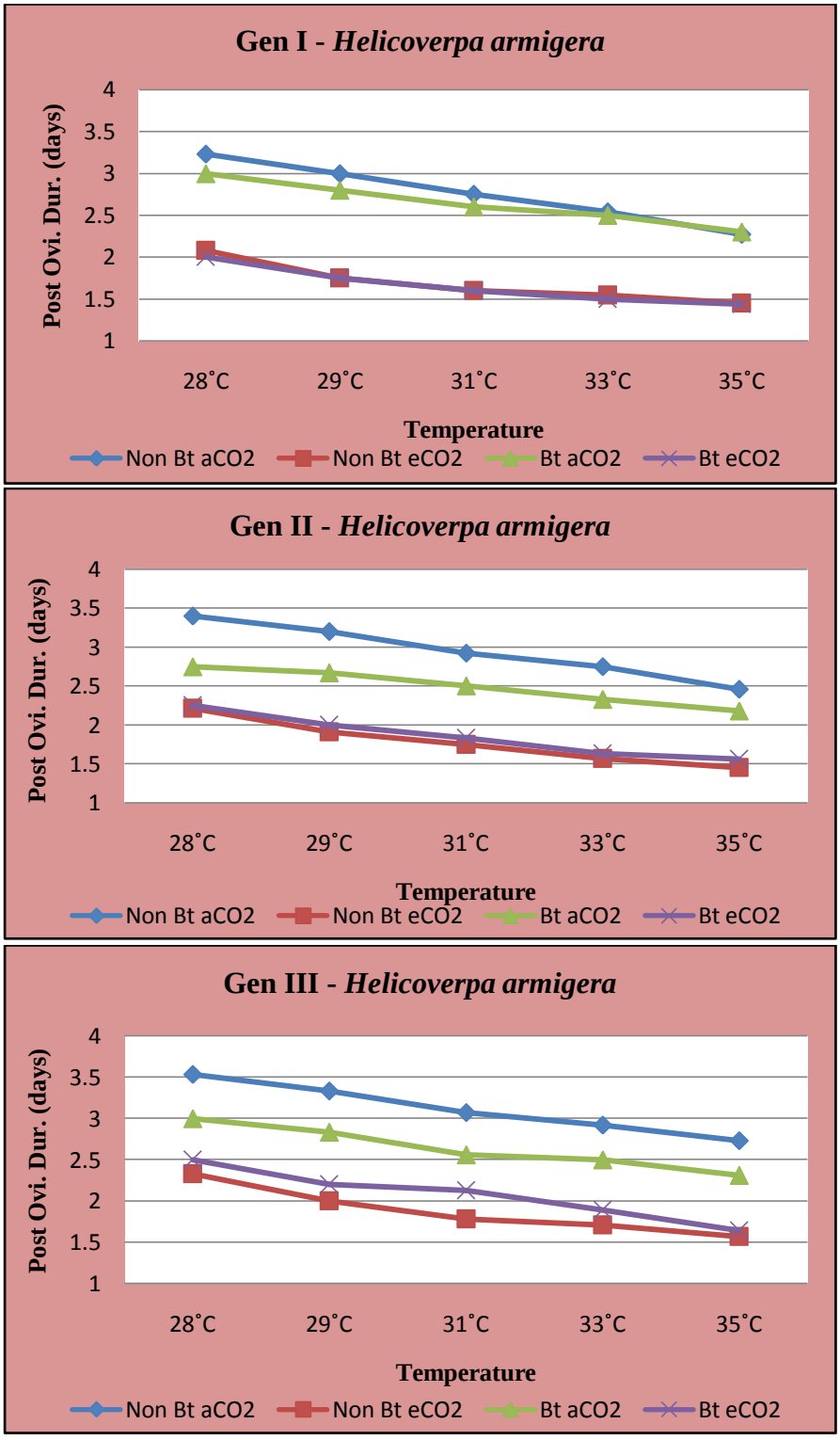


Fig. 4.28. Effect of eCO_2 and *e*Temp on post-ovipositional duration of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

eCO_2, decrease in post-ovipositional duration was recorded with increase in temperature with highest at 28 ºC with 2.00 days and lowest at 35 ºC with 1.44 days. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on post-ovipositional duration.

Second generation: Post-ovipositional duration varied from 2.75 to 2.18 days with highest and lowest duration at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decreased post-ovipositional duration was recorded with increase in temperature with highest duration at 28 ºC with 2.25 days and lowest at 35 ºC with 1.56 days. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on post-ovipositional duration.

Third generation: In Bt cotton, decrease in post-ovipositional duration with increase in temperature with highest and lowest post-ovipositional duration as 3.00 and 2.31 days at temperatures 28 and 35 ºC, respectively. At *e*CO_2, post-ovipositional duration ranged between 2.50 to 1.64 days with highest and lowest durations at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on post-ovipositional duration.

Mean of generations: The mean of three generations in Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the post-ovipositional duration decreased significantly (2.92, 2.77, 2.55, 2.44 and 2.26 days, respectively) corresponding to 5.14, 12.46, 16.23 and 22.40 %. Similarly, under *e*CO_2 also the post-ovipositional duration decreased significantly (2.25, 1.98, 1.85, 1.67 and 1.55 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 11.85, 17.63, 25.63 and 31.26 %. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on post-ovipositional duration. And post-ovipositional duration was higher in *a*CO_2 than *e*CO_2. Further, the post-ovipositional duration under *e*CO_2 was lower (22.86, 28.31, 27.42, 31.51 and 31.66 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred that, mean post-ovipositional duration of *H. armigera* in Bt cotton also decreased with increase in both CO_2 (by 23 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 47 %. Post-ovipositional period

increased along the three generations as follows: 3.00, 2.75 and 3.00 days; 2.00, 2.25 and 2.50 days; and 1.44, 1.56 and 1.64 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp.

4.1.10.3 Effect on post-ovipositional duration of *S. litura* in non-Bt cotton

The data pertaining to post-ovipositional duration of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.39 and Fig. 4.29.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on post-ovipositional duration. At *a*CO_2, post-ovipositional duration decreased from 3.35 to 2.55 days with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, decrease in post-ovipositional duration was recorded with increase in temperature with 2.27 days at 28 ºC as highest and 1.57 days as lowest at 35 ºC. The interaction effect of *e*CO_2 and *e*Temp has shown a non-significant impact on post-ovipositional duration.

Second generation: At *a*CO_2, post-ovipositional duration decreased from 3.38 to 2.55 days with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, decrease in post-ovipositional duration was recorded with increase in temperature with highest as 2.38 days at 28 ºC and lowest as 1.55 days at 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on post-ovipositional duration. And post-ovipositional duration was higher in *a*CO_2 than *e*CO_2.

Third generation: At *a*CO_2, decrease in post-ovipositional duration was recorded from 3.53 to 2.62 days with increase in corresponding temperature from 28 to 35 ºC. At *e*CO_2, post-ovipositional duration ranged between 2.50 to 1.73 days with highest duration at 28 ºC and lowest at 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on post-ovipositional duration.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the post-ovipositional duration has decreased significantly (3.42, 3.15, 2.92, 2.76 and 2.57 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 7.80, 14.72, 19.20 and 24.76 %. Similarly, under *e*CO_2, the post-ovipositional duration decreased significantly (2.38, 2.17, 1.95, 1.77 and 1.62 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively)

Table 4.39. Effect of *e*CO_2 and *e*Temp on post-ovipositional duration of *S. litura* in non-Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	3.35	2.27	**2.81**	3.38	2.38	**2.88**	3.53	2.50	**3.02**	3.42	2.38	**2.90**
29 ± 1°C	3.00	2.08	**2.54**	3.13	2.17	**2.65**	3.33	2.27	**2.80**	3.15	2.17	**2.66**
31 ± 1°C	2.86	1.78	**2.32**	2.81	2.00	**2.41**	3.08	2.08	**2.58**	2.92	1.95	**2.44**
33 ± 1°C	2.69	1.64	**2.17**	2.77	1.82	**2.29**	2.83	1.86	**2.35**	2.76	1.77	**2.27**
35 ± 1°C	2.55	1.57	**2.06**	2.55	1.55	**2.05**	2.62	1.73	**2.17**	2.57	1.62	**2.09**
Mean	**1.86**	**2.89**		**1.98**	**2.92**		**2.08**	**3.08**		**1.97**	**2.96**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	127.936*	0.06	0.18	146.898*	0.06	0.15	130.280*	0.06	0.17	430.92*	0.03	0.09
Temperature (°C)	8.866*	0.10	0.28	13.745*	0.09	0.24	12.254*	0.10	0.27	36.32*	0.05	0.15
Interaction (CO_2 + Temp(°C))	0.12	0.14	NS	0.20	0.12	NS	0.12	0.14	NS	0.10	0.08	NS
CV	5.05 %			5.41 %			5.37 %			5.20 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

which has corresponded to a decrease of 8.81, 18.04, 25.59 and 32.17 %. The interaction effect of *e*CO_2 and *e*Temp has shown a non-significant impact on post-ovipositional duration. And post-ovipositional duration was higher in *a*CO_2 than *e*CO_2. Further, the post-ovipositional duration under *e*CO_2 was lower (30.31, 31.08, 33.03, 35.83 and 37.18 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be iterated that, mean post-ovipositional duration of *S. litura* decreased with increase in both CO_2 (by 30 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 53 %. Post-ovipositional period increased along the three generations as follows: 3.35, 3.38 and 3.53 days; 2.27, 2.38 and 2.50 days; and 1.57, 1.55 and 1.73 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp.

4.1.10.4 Effect on post-ovipositional duration of *S. litura* in Bt cotton

The data pertaining to post-ovipositional duration of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.40 and Fig. 4.29.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on post-ovipositional duration. It decreased under *a*CO_2 from 3.25 to 2.38 days with corresponding increase in temperature from 28 and 35 ºC. At *e*CO_2, a decrease in post-ovipositional duration with increase in temperature was recorded with highest duration at 28 ºC with 2.25 days and lowest at 35 ºC with 1.42 days. The interaction effect of *e*CO_2 and *e*Temp has shown a non-significant impact on post-ovipositional duration.

Second generation: In Bt cotton, decrease in post-ovipositional duration from 3.25 to 2.31 days was recorded with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, post-ovipositional duration ranged between 2.40 and 1.64 days with highest and lowest duration at temperature 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on post-ovipositional duration.

Third generation: At *a*CO_2, post-ovipositional duration varied from 3.33 to 2.46 days with highest post-ovipositional duration at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, post-ovipositional durations were recorded in the range of 2.60 to 1.83 days with highest and lowest duration at temperatures 28 and 35 ºC, respectively.

Table 4.40. Effect of *e*CO_2 and *e*Temp on post-ovipositional duration of *S. litura* in Bt cotton

Temperature	First generation (days)			Second generation (days)			Third generation (days)			Mean (days)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	3.25	2.25	**2.75**	3.25	2.40	**2.83**	3.33	2.60	**2.97**	3.28	2.42	**2.85**
29 ± 1°C	3.17	2.00	**2.58**	3.20	2.20	**2.70**	3.29	2.43	**2.86**	3.22	2.21	**2.71**
31 ± 1°C	2.75	1.83	**2.29**	2.88	2.00	**2.44**	3.00	2.33	**2.67**	2.88	2.05	**2.47**
33 ± 1°C	2.50	1.67	**2.08**	2.50	1.80	**2.15**	2.70	2.10	**2.40**	2.57	1.86	**2.21**
35 ± 1°C	2.38	1.42	**1.90**	2.31	1.64	**1.97**	2.46	1.83	**2.15**	2.38	1.63	**2.01**
Mean	**1.83**	**2.81**		**2.00**	**2.82**		**2.25**	**2.95**		**2.03**	**2.86**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	418.319*	0.03	0.09	202.007*	0.04	0.01	108.936*	0.05	0.13	707.74*	0.02	0.06
Temperature (°C)	42.689*	0.05	0.15	31.075*	0.06	0.18	20.157*	0.08	0.21	98.57*	0.04	0.10
Interaction (CO_2 + Temp(°C))	1.32	0.08	NS	1.10	0.09	NS	0.47	0.11	NS	2.67*	0.05	0.14
CV	4.27 %			5.85 %			5.25 %			5.09 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

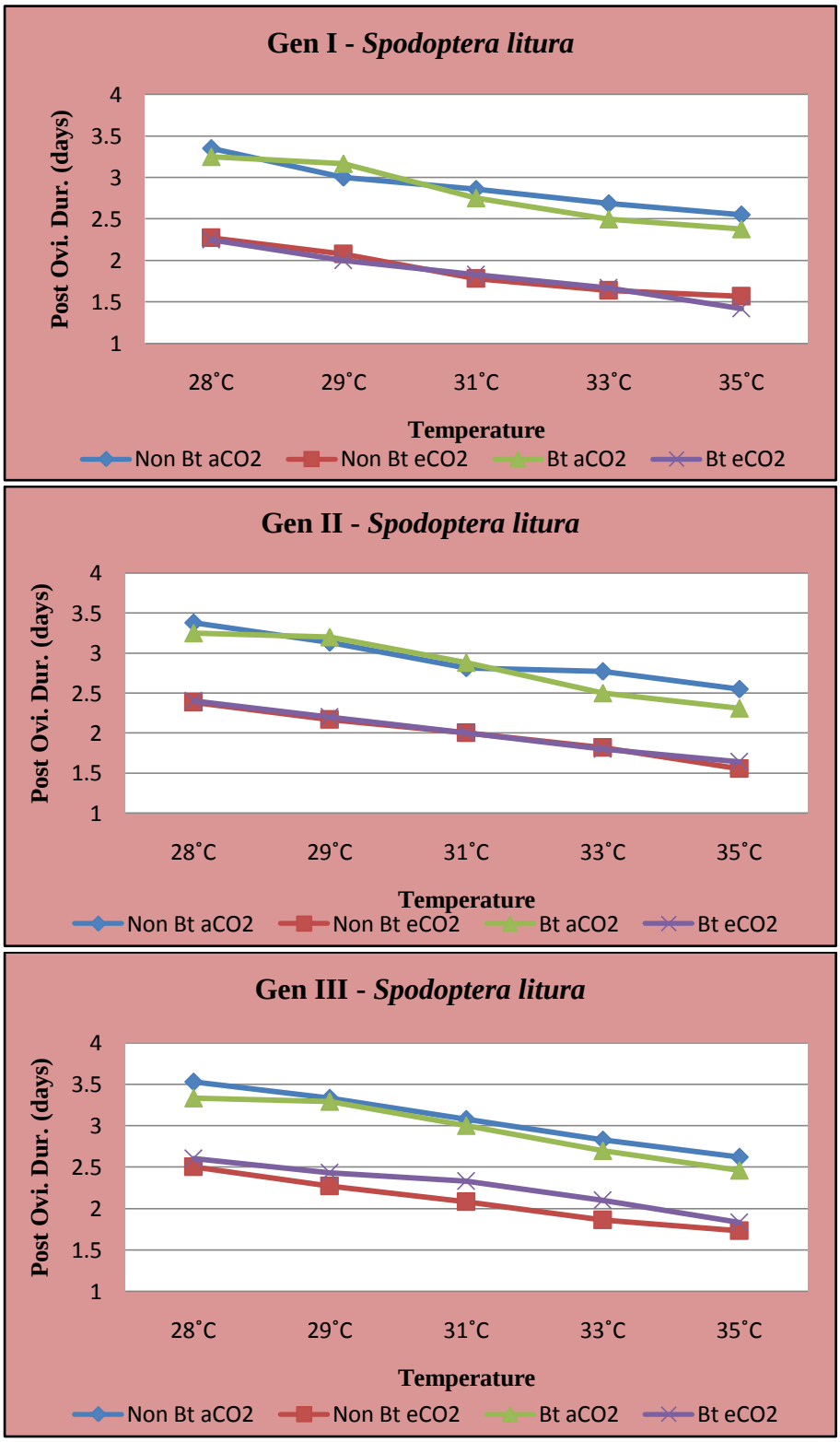


Fig. 4.29. Effect of *e*CO_2 and *e*Temp on post-ovipositional duration of *S. litura* on non-Bt and Bt cotton in first, second and third generation

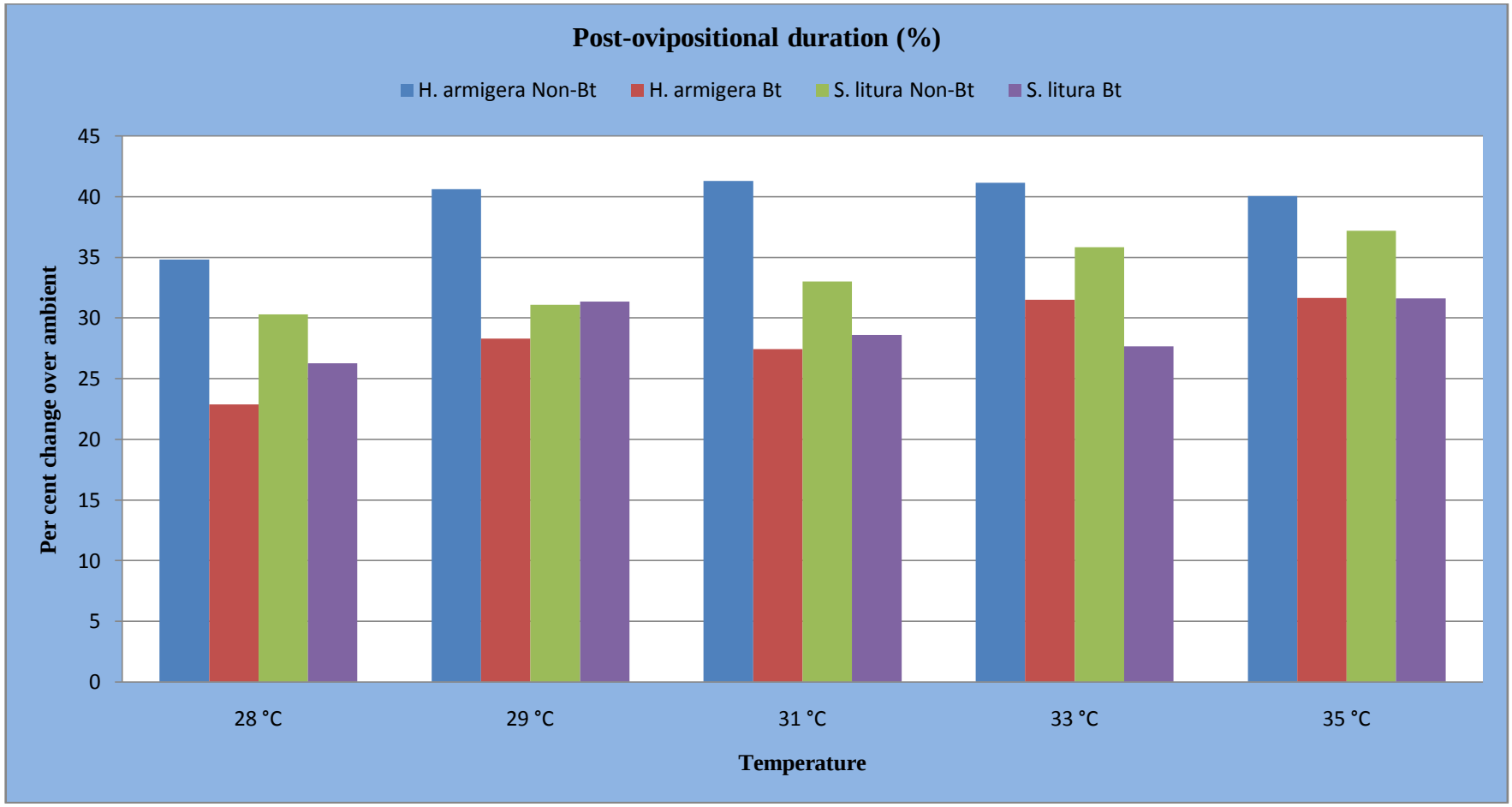


Fig. 4.30. Effect of *e*CO_2 and *e*Temp on mean post-ovipositional duration of *H. armigera* and *S. litura* on non-Bt and Bt cotton

The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on post-ovipositional duration.

Mean of generations: The mean of three generations in Bt cotton, indicated that the post-ovipositional duration has decreased significantly (3.28, 3.22, 2.88, 2.57 and 2.38 days, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 1.73, 12.21, 21.67 and 27.26 %. Similarly, under *e*CO_2 also the post-ovipositional duration decreased significantly (2.42, 2.21, 2.05, 1.86 and 1.63 days) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 8.55, 15.03, 23.17 and 32.55 %. The interaction effect of *e*CO_2 and *e*Temp has shown a non-significant impact on post-ovipositional duration. And post-ovipositional duration was higher in *a*CO_2 than *e*CO_2. Further, the post-ovipositional duration under *e*CO_2 was lower (26.25, 31.37, 28.62, 27.66 and 31.61 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be iterated that, mean post-ovipositional duration of *S. litura* in Bt cotton decreased with increase in both CO_2 (by 26 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 50 %. Post-ovipositional period increased along the three generations as follows: 3.25, 3.25 and 3.33 days; 2.25, 2.40 and 2.60 days; and 1.42, 1.64 and 1.83 days, respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp.

4.1.11 Fecundity: The data on the effect of *e*CO_2 and *e*Temp conditions on the fecundity of *H. armigera* and *S. litura* was presented in the Tables 4.41 - 4.44 and Fig 4.31 - 4.32. Fecundity under *e*CO_2 was lower than that of *a*CO_2. With increase in test temperatures also, fecundity of test insects decreased. Comparative performance of both the test insects was presented in Fig. 4.33.

4.1.11.1. Effect on fecundity of *H. armigera* in non-Bt cotton

The data pertaining to fecundity of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.41 and Fig. 4.31.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on fecundity. At *a*CO_2, fecundity varied from 860.69 to 752.36 eggs per female with highest and lowest fecundity recorded at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in fecundity was recorded with increase in temperature with highest

and lowest fecundity at 28 ºC (758.77 eggs per female) and 35 ºC (666.36 eggs per female), respectively. The interaction effect of *e*CO_2 and *e*Temp showed a non-significant influence on fecundity.

Second generation: In non-Bt cotton, at *a*CO_2, fecundity ranged between 885.86-768.27 eggs per female with highest fecundity at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, decrease in fecundity was recorded from 807.24 to 713.18 eggs per female with corresponding increase in temperature from 28 to 35 ºC. And among *a*CO_2 and *e*CO_2, fecundity was higher in *a*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on fecundity.

Third generation: At *a*CO_2, fecundity varied from 922.00 to 782.43 eggs per female with a corresponding temperature range of 28 to 35 ºC. At *e*CO_2, decrease in fecundity was recorded with increase in temperature with highest fecundity at 28 ºC with 781.13 eggs per female and lowest at 35 ºC with 689.23 eggs per female. And among *a*CO_2 and *e*CO_2, fecundity was higher in *a*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on fecundity.

Mean of generations: The mean of three generations in non-Bt cotton, indicated that with increase in temperature (28, 29, 31, 33 and 35 °C) the fecundity has decreased significantly (889.52, 854.86, 825.95, 794.96 and 767.69 eggs per female, respectively) corresponding to 3.90, 7.15, 10.63 and 13.70 %. Similarly, under *e*CO_2 also the fecundity decreased significantly (782.38, 754.57, 725.57, 707.81 and 689.59 eggs per female) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 3.55, 7.26, 9.53 and 11.86 %. The interaction effect of *e*CO_2 and *e*Temp showed a non-significant influence on fecundity. And among *a*CO_2 and *e*CO_2, fecundity was higher in *a*CO_2. Further, the fecundity under *e*CO_2 was lower (12.04, 11.73, 12.15, 10.96 and 10.17 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

These findings suggest that, mean fecundity of *H. armigera* decreased with increase in both CO_2 (by 12 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 22 %. Stange (1997) reported that at elevated CO_2, females of

Table 4.41. Effect of *e*CO_2 and *e*Temp on fecundity of *H. armigera* in non-Bt cotton

Temperature	First generation (eggs/female)			Second generation (eggs/female)			Third generation (eggs/female)			Mean (eggs/female)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	860.69	758.77	**809.73**	885.86	807.24	**846.55**	922.00	781.13	**851.57**	889.52	782.38	**835.95**
29 ± 1°C	828.83	734.20	**781.52**	857.45	775.58	**816.52**	878.31	753.93	**816.12**	854.86	754.57	**804.72**
31 ± 1°C	839.51	706.88	**773.19**	817.25	741.50	**779.38**	821.10	728.33	**774.72**	825.95	725.57	**774.85**
33 ± 1°C	776.09	683.69	**729.89**	792.36	731.92	**762.14**	816.43	707.83	**762.13**	794.96	707.81	**751.39**
35 ± 1°C	752.36	666.36	**709.36**	768.27	713.18	**740.73**	782.43	689.23	**735.83**	767.69	689.59	**728.64**
Mean	**811.49**	**709.98**		**824.23**	**753.88**		**844.05**	**732.09**		**826.23**	**731.98**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	113.159*	6.30	17.56	330.646*	3.58	9.98	267.870*	3.90	10.85	793.56*	2.37	6.59
Temperature (°C)	16.079*	9.96	27.76	55.124*	5.67	15.78	56.911*	6.16	17.16	129.01*	3.74	10.42
Interaction (CO_2 + Temp(°C))	0.10	14.09	NS	1.03	8.01	NS	2.21	8.71	NS	1.38	5.29	NS
CV	5.30%			5.15 %			5.45 %			3.39 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
*Significant @ 5 per cent level of significance

Cactoblastis cactorum laid very few eggs on *Opuntia stricta*. Chen *et al*. (2004) found that *H. armigera* fecundity in wheat reduced from 595 to 518 eggs per female with increase in CO_2 (738 µL L^{-1}). Wu *et al*. (2006) recorded decrease in fecundity with CO_2 from 446-453 eggs per female (*a*CO_2) to 369-413 eggs per female (*e*CO_2) in *H. armigera* on spring wheat. Mallikarjuna *et al*. (2020) observed that *H. armigera* fecundity reduced by 37.8 % from 238 at ambient to 148 eggs per female at *e*CO_2+ *e*Temp. On the contrary, Akbar *et al*. (2016) reported increased fecundity by 1.5 fold with increase in CO_2 (750 ppm), from 831 to 1293 eggs per female. Similarly O'Neill *et al*. (2008) recorded that Japanese beetle, *Popillia japonica* females under eCO_2 laid approximately twice as many eggs as compared to females fed on soybean foliage grown under normal ambient conditions. From the present investigation, fecundity was found to increase with every generation as follows: 861, 886 and 922 eggs; 759, 807 and 781 eggs; and 666, 713 and 689 eggs per female moth respectively at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp. Abdul *et al*. (2014) observed that there was significant reduction in fecundity of *H. armigera* under elevated CO_2 conditions compared with ambient by 12-16 % along the four generations.Srinivasa Rao *et al*. (2013) reported reduced fecundity of *A. janata* by 2-4 % in first generation, 3 % in second generation, 8 % in third generation and 10 % in fourth generation under *e*CO_2.

4.1.11.2 Effect on fecundity of *H. armigera* in Bt cotton

The data pertaining to fecundity of *H. armigera* in Bt cotton for all three generations was presented in Table 4.42 and Fig. 4.31.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on fecundity. At *a*CO_2, it decreased with increase in temperature with highest fecundity of 770.67 eggs per female at 28 ºC and lowest 679.25 eggs per female at 35 ºC. At *e*CO_2, decrease in fecundity was recorded from 713.00 to 618.00 eggs per female withcorresponding increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed a non-significant influence on fecundity. And among *a*CO_2 and *e*CO_2, fecundity was higher in *a*CO_2.

Second generation: In Bt cotton, at *a*CO_2, fecundity varied from 801.50 to 671.11 eggs per female with highest and lowest fecundity at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decreased fecundity was recorded with increase in temperature with 766.75 eggs per female at 28 ºC and 658.46 eggs per female at 35 ºC.

Table 4.42. Effect of *e*CO_2 and *e*Temp on fecundity of *H. armigera* in Bt cotton

Temperature	First generation (eggs/female)			Second generation (eggs/female)			Third generation (eggs/female)			Mean (eggs/female)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	770.67	713.00	**741.83**	801.50	766.75	**784.13**	823.00	740.75	**781.88**	798.39	740.17	**769.28**
29 ± 1°C	752.75	670.80	**711.78**	776.00	729.67	**752.84**	783.80	703.33	**743.57**	770.85	701.27	**736.06**
31 ± 1°C	722.20	649.60	**685.90**	739.17	689.89	**714.53**	762.25	671.17	**716.71**	741.21	670.22	**705.71**
33 ± 1°C	698.33	627.40	**662.87**	724.38	673.30	**698.84**	749.11	655.56	**702.34**	723.94	652.09	**688.01**
35 ± 1°C	679.25	618.00	**648.63**	671.11	658.46	**664.79**	696.45	639.64	**668.05**	682.27	638.70	**656.22**
Mean	**724.61**	**655.76**		**742.43**	**703.61**		**762.92**	**682.09**		**743.33**	**678.78**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	439.502*	2.32	6.47	124.086*	4.16	11.58	310.088*	2.38	6.64	636.65*	1.81	5.04
Temperature (°C)	104.220*	3.67	10.23	55.520*	6.57	18.30	141.032*	3.77	10.49	231.67*	2.86	7.97
Interaction (CO_2 + Temp(°C))	1.73	5.20	NS	0.24	9.29	NS	4.113*	5.33	14.84	1.40	4.05	NS
CV	3.76 %			6.55 %			3.63 %			2.84 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

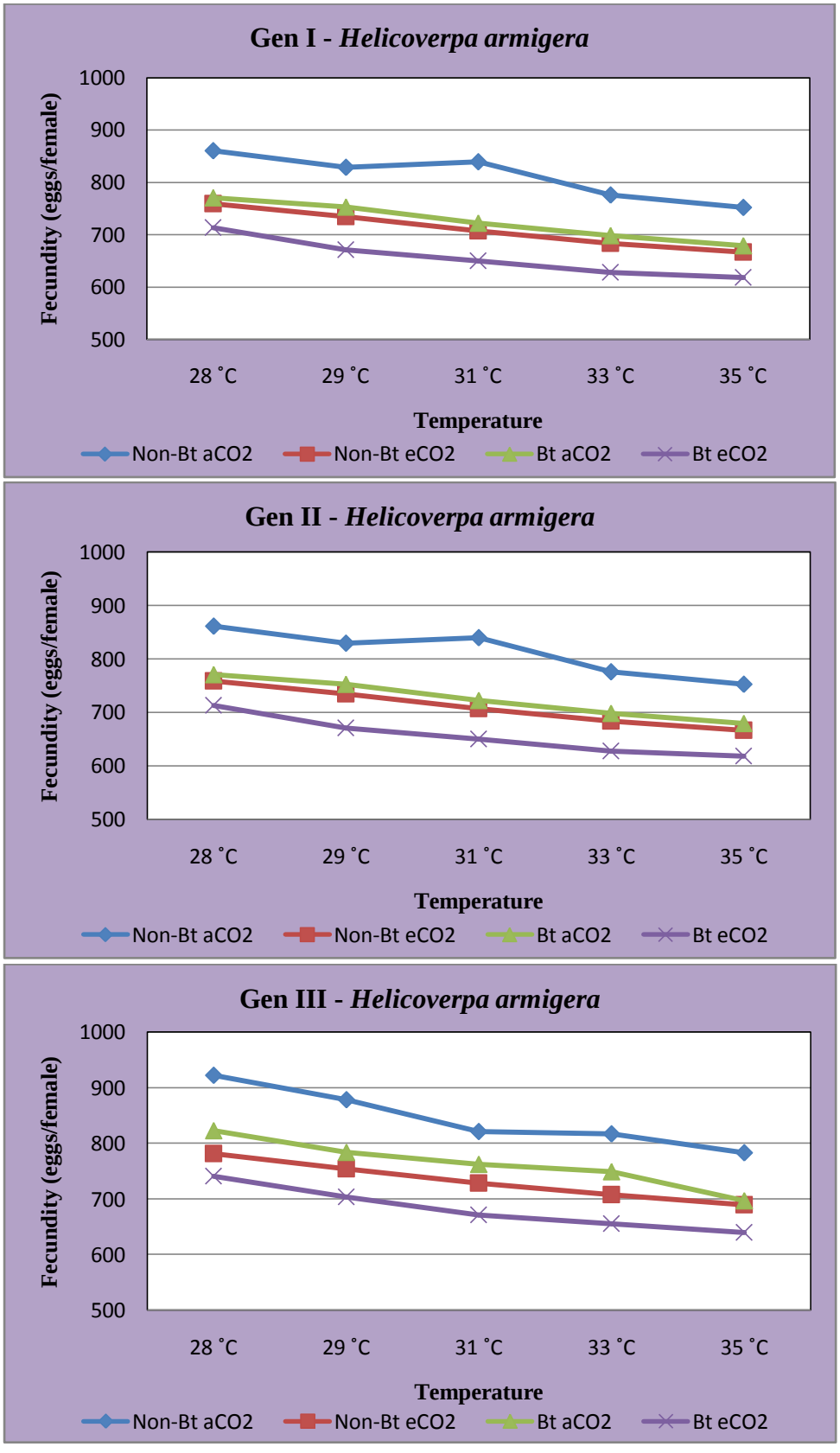


Fig. 4.31. Effect of eCO_2 and *e*Temp on fecundity of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on fecundity.

Third generation: At *a*CO_2, decrease in fecundity was recorded with increase in temperature with highest and lowest fecundity of 823.00 and 696.45 eggs per female at temperatures 28 and 35 ºC, respectively. At *e*CO_2, fecundity ranged between 740.75 and 639.64 eggs per female with highest and lowest at temperatures 28 and 35 ºC, respectively. And among *a*CO_2 and *e*CO_2, fecundity was higher in *a*CO_2. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on fecundity.

Mean of generations: The mean of three generations in Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28, 29, 31, 33 and 35 °C) the fecundity has decreased significantly (798.39, 770.85, 741.21, 723.94 and 682.27 eggs per female, respectively) corresponding to 3.45, 7.16, 9.33 and 14.54 %. Similarly, under *e*CO_2 also the fecundity decreased significantly (740.17, 701.27, 670.22, 652.09 and 638.70 eggs per female) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which has corresponded to a decrease of 5.26, 9.45, 11.90 and 13.71 The interaction effect of *e*CO_2 and *e*Temp showed a non-significant influence on fecundity in Bt cotton. And among *a*CO_2 and *e*CO_2, fecundity was higher in *a*CO_2. Further, the fecundity under *e*CO_2 was lower (7.29, 9.03, 9.58, 9.93 and 6.39 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

Finally it can be inferred that, mean fecundity of *H. armigera* in Bt cotton decreased with increase in both CO_2 (by 7 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 20 %. Analogously Chen *et al*. (2005b) revealed that elevated CO_2 resulted in lower fecundity in the *H. armigera* in transgenic Bt cotton. Similarly Chen *et al.* (2007) observed that *H. armigera* reared on transgenic cotton grown under elevated CO_2 (750 µl L^{-1}) exhibited lower fecundity. From the study, it is understood that fecundity increased with advancement of *H. armigera* generations in ambient conditions (771, 802 and 823 eggs), whereas it was highest in the second generation in *e*CO_2 (713, 767 and 741 eggs) and *e*CO_2 + *e*Temp (618, 658 and 640 eggs per female).

4.1.11.3 Effect on fecundity of *S. litura* in non-Bt cotton

The data pertaining to fecundity of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.43 and Fig. 4.32.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on fecundity. It decreased under *a*CO_2 in the range of 780.73 to 666.64 eggs per female with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, decrease in fecundity was recorded with increase in temperature with highest and lowest fecundity at temperatures 28 ºC (730.71 eggs per female) and 35 ºC (644.91 eggs per female), respectively. A significant interactive effect of *e*CO_2 and *e*Temp was observed on fecundity and was higher in *a*CO_2 than *e*CO_2.

Second generation: At *a*CO_2, fecundity ranged between 807.69 – 685.55 eggs per female with highest and lowest fecundity at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in fecundity was recorded with increase in temperature with highest fecundity at 28 ºC with 772.60 eggs per female and lowest at 35 ºC with 685.62 eggs per female. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant influence on fecundity and was higher in *a*CO_2 than *e*CO_2.

Third generation: At *a*CO_2, decrease in fecundity was recorded with increase in temperature with highest fecundity at 28 ºC with 830.93 eggs per female and lowest at 35 ºC with 705.18 eggs per female, respectively. At *e*CO_2, fecundity decreased in the range of 751.23 to 668.09 eggs per female with increase in corresponding temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant influence on fecundity and was higher in *a*CO_2 than *e*CO_2.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the fecundity has decreased significantly (806.45, 779.30, 750.55, 717.95 and 685.79 eggs per female, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to a decrease of 3.37, 6.93, 10.97 and 14.96 %. Similarly, under *e*CO_2, the fecundity decreased significantly (751.51, 724.54, 712.57, 696.40 and 666.21 eggs per female) with increase in temperatures (28, 29, 31, 33 and 35 °C,

Table 4.43. Effect of *e*CO_2 and *e*Temp on fecundity of *S. litura* in non-Bt cotton

Temperature	First generation (eggs/female)			Second generation (eggs/female)			Third generation (eggs/female)			Mean (eggs/female)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	780.73	730.71	**755.72**	807.69	772.60	**790.15**	830.93	751.23	**791.08**	806.45	751.51	**778.98**
29 ± 1°C	757.69	703.75	**730.72**	778.67	741.13	**759.90**	801.55	728.73	**765.14**	779.30	724.54	**751.92**
31 ± 1°C	712.67	692.57	**702.62**	758.07	732.58	**745.33**	780.92	712.56	**746.74**	750.55	712.57	**731.56**
33 ± 1°C	690.00	674.38	**682.19**	719.91	718.83	**719.37**	743.93	696.00	**719.97**	717.95	696.40	**707.18**
35 ± 1°C	666.64	644.91	**655.78**	685.55	685.62	**685.59**	705.18	668.09	**686.64**	685.79	666.21	**676.00**
Mean	**721.54**	**689.26**		**749.97**	**730.15**		**772.50**	**711.32**		**748.08**	**710.24**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	41.518*	3.54	9.87	78.179*	3.09	8.61	105.533*	2.92	8.12	226.98*	1.77	4.94
Temperature (°C)	49.256*	5.60	15.61	66.442*	4.89	13.62	76.500*	4.61	12.84	201.07*	2.80	7.81
Interaction (CO_2 + Temp(°C))	2.633*	7.92	22.07	3.030*	6.91	19.26	4.220*	6.52	18.16	9.37*	3.96	11.04
CV	5.62 %			4.73 %			4.34%			2.72 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

respectively) which has corresponded to a decrease of 3.59, 5.18, 7.33 and 11.35 %. A significant interactive effect of *e*CO_2 and *e*Temp was observed on fecundity and was higher in *a*CO_2 than *e*CO_2. Further in non-Bt cotton, the fecundity under *e*CO_2 was lower (6.81, 7.03, 5.06, 3.00 and 2.86 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred that, mean fecundity of *S. litura* decreased with increase in both CO_2 (by 7 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 17 %. Shwetha *et al*. (2017) reported a decrease in fecundity by 6.86 % over reference ambient conditions (520.67 eggs per female under aCO_2; 484.94 under *e*CO_2). Divya *et al*. (2020) recoded that fecundity of *Spodoptera exigua* was lower with 207.8 eggs under elevated CO_2 (550 ppm) than ambient CO_2 conditions with 390.5 eggs per female. On the contrary, Kranthi (2014) reported that *S. litura* exhibited higher fecundity on plants exposed to *e*CO_2. Similarly, Viswajyothi *et al*. (2020) reported that fecundity of *S. inferens* in maize increased by 10.8 % at *e*Temp (32 °C) and got reduced by 14.5 % at *e*CO_2 (450 ppm). CO_2 has a major influence on fecundity where interaction with temperature had non significant influence.

In the study, fecundity was found to increase along the three generations as follows: 781, 808 and 831 (aCO_2+ *a*Temp); 731, 773 and 751 eggs (*e*CO_2); and 645, 686 and 668 eggs per female *S. litura* (*e*CO_2 + *e*Temp). The presented results are on par with that of Wu *et al*. (2009) who recorded fecundity of *S. exigua* to be 566 in the first, 583 in the second and 587 eggs per female in the third generations on non-Bt cotton (Simian 3) that were fairly higher than those on Bt cotton (GK-12). Divya (2017) recorded that the fecundity per female of *S. exigua* was significantly less in first (434) over second (463) which declined from third (450) to fourth (383 eggs per female) generations under *e*CO_2 conditions compared to that of *a*CO_2 conditions (460, 506, 519 and 441 eggs per female) respectively.

4.1.11.4 Effect on fecundity of *S. litura* in Bt cotton

The data pertaining to fecundity of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.44 and Fig. 4.32.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on fecundity. It decreased under *a*CO_2 from 762.25 to 649.83 eggs per female with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, highest fecundity

Table 4.44. Effect of *e*CO_2 and *e*Temp on fecundity of *S. litura* in Bt cotton

Temperature	First generation (eggs/female)			Second generation (eggs/female)			Third generation (eggs/female)			Mean (eggs/female)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	762.25	725.50	**743.88**	767.80	771.00	**769.40**	757.40	747.50	**752.45**	762.48	748.00	**755.24**
29 ± 1°C	728.25	683.50	**705.88**	716.80	719.86	**718.33**	725.57	690.40	**707.99**	723.54	697.92	**710.73**
31 ± 1°C	706.50	659.88	**683.19**	690.88	675.88	**683.38**	703.50	656.25	**679.88**	700.29	664.00	**682.15**
33 ± 1°C	663.25	670.00	**666.63**	654.80	623.50	**639.15**	677.30	639.50	**658.40**	665.12	644.33	**654.73**
35 ± 1°C	649.83	650.46	**650.15**	637.82	614.92	**626.37**	656.33	618.77	**637.55**	647.99	628.05	**638.02**
Mean	**702.01**	**677.86**		**693.61**	**681.03**		**704.02**	**670.48**		**699.88**	**676.46**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	86.442*	1.84	5.12	55.494*	2.20	6.12	10.845*	2.36	6.57	127.80*	1.22	3.39
Temperature (°C)	158.170*	2.90	8.09	214.824*	3.47	9.67	150.267*	3.73	10.39	533.20*	1.92	5.35
Interaction (CO_2 + Temp(°C))	19.692*	4.11	11.44	1.19	4.91	NS	4.819*	5.28	14.70	8.66*	2.72	7.57
CV	2.98 %			3.60 %			3.78 %			1.97 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

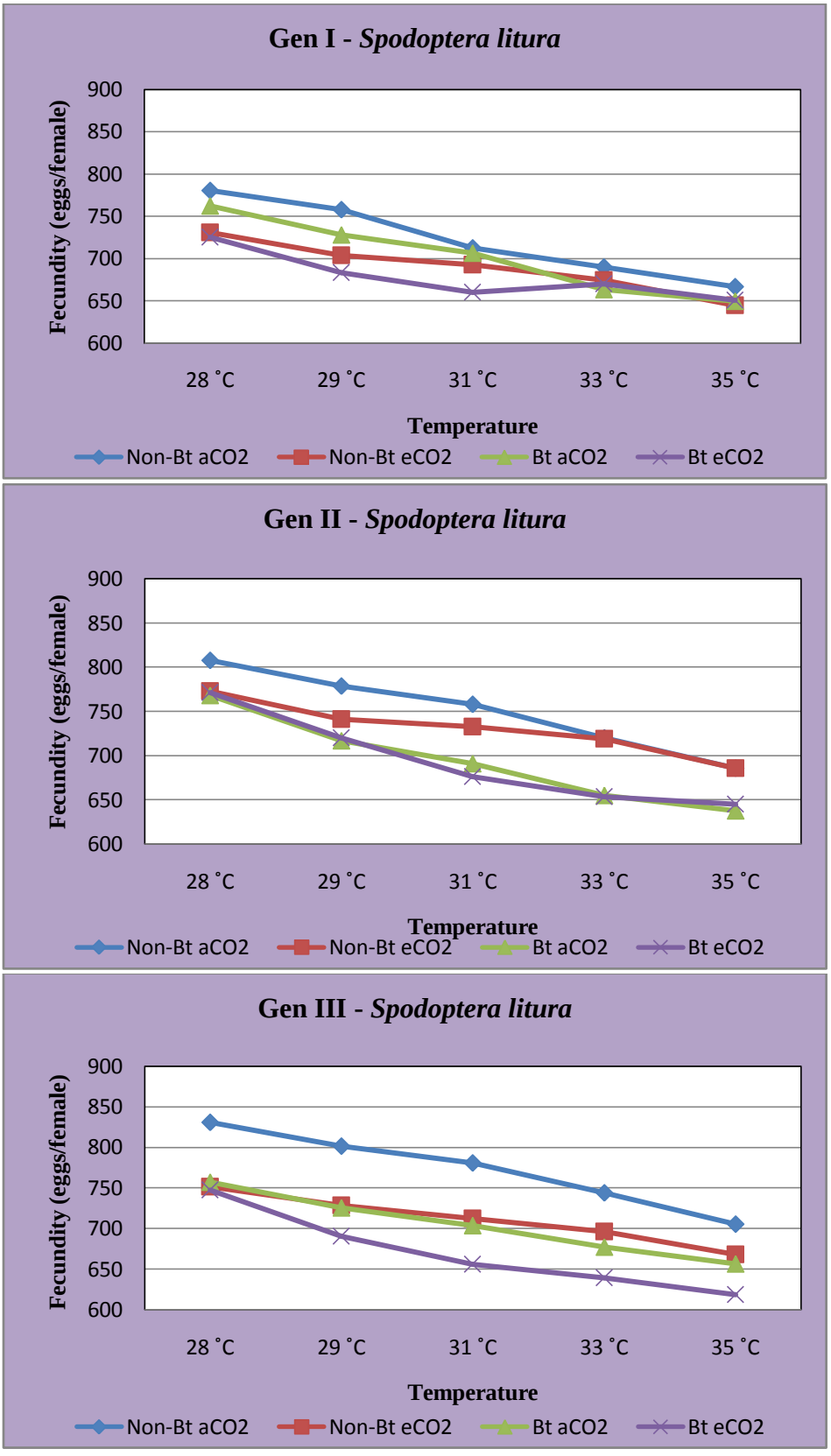


Fig. 4.32. Effect of eCO_2 and *e*Temp on fecundity of *S. litura* on non-Bt and Bt cotton in first, second and third generation

of 725.50 eggs/female was recorded at 28 ºC and lowest at 35 ºC with 650.46 eggs/female. A significant interactive effect of *e*CO_2 and *e*Temp was observed on fecundity and was higher in *a*CO_2 than *e*CO_2.

Second generation: At *a*CO_2, decrease in fecundity with increase in temperature was recorded with highest fecundity of 767.80 eggs/female at 28 ºC and lowest of 637.82 eggs/female at 35 ºC. At *e*CO_2, fecundity ranged from 771.00 to 614.92 eggs/ female with highest and lowest fecundity at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp has shown significant influence whereas the interactive effect has non-significant influence on fecundity.

Third generation: At *a*CO_2, fecundity ranged between 757.40 and 656.33 eggs per female with highest and lowest fecundity at temperatures 28 and 35 ºC, respectively. At *e*CO_2, a decreased fecundity was recorded with increase in temperature with highest and lowest fecundity of 747.50 and 618.77 eggs per female at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant influence on fecundity and was higher in *a*CO_2 than *e*CO_2.

Mean of generations: The mean of three generations also indicated that in Bt cotton, the fecundity has decreased significantly (762.48, 723.54, 700.29, 665.12 and 647.99 eggs per female, respectively) with increase in temperature (28-35 °C) corresponding to decrease of 5.11, 8.16, 12.77 and 15.02 %. Similarly, under *e*CO_2 also the fecundity decreased significantly (748.00, 697.92, 664.00, 644.33 and 628.05 eggs per female) with increase in temperatures (28- 35 ºC, respectively) which has corresponded to a decrease of 6.70, 11.23, 12.52 and 14.70 %. A significant interactive effect of *e*CO_2 and *e*Temp was observed on fecundity and was higher in *a*CO_2 than *e*CO_2. Further, the fecundity under *e*CO_2 was lower (1.90, 3.54, 5.18, 1.62 and 1.53 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred that, mean fecundity of *S. litura* on Bt cotton decreased with increase in both CO_2 (by 2 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 18 %. In the study, fecundity was found to increase along the three generations as follows: 762, 768 and 757 eggs (*a*CO_2+ *a*Temp); 726, 771 and 748 eggs (*e*CO_2); and 650, 615 and 619 eggs per female *S. litura* (*e*CO_2 + *e*Temp). Fecundity appears to be high in the second generation in *a*CO_2 and *e*CO_2, but with extreme climate stress, fecundity decreased with every new generation.

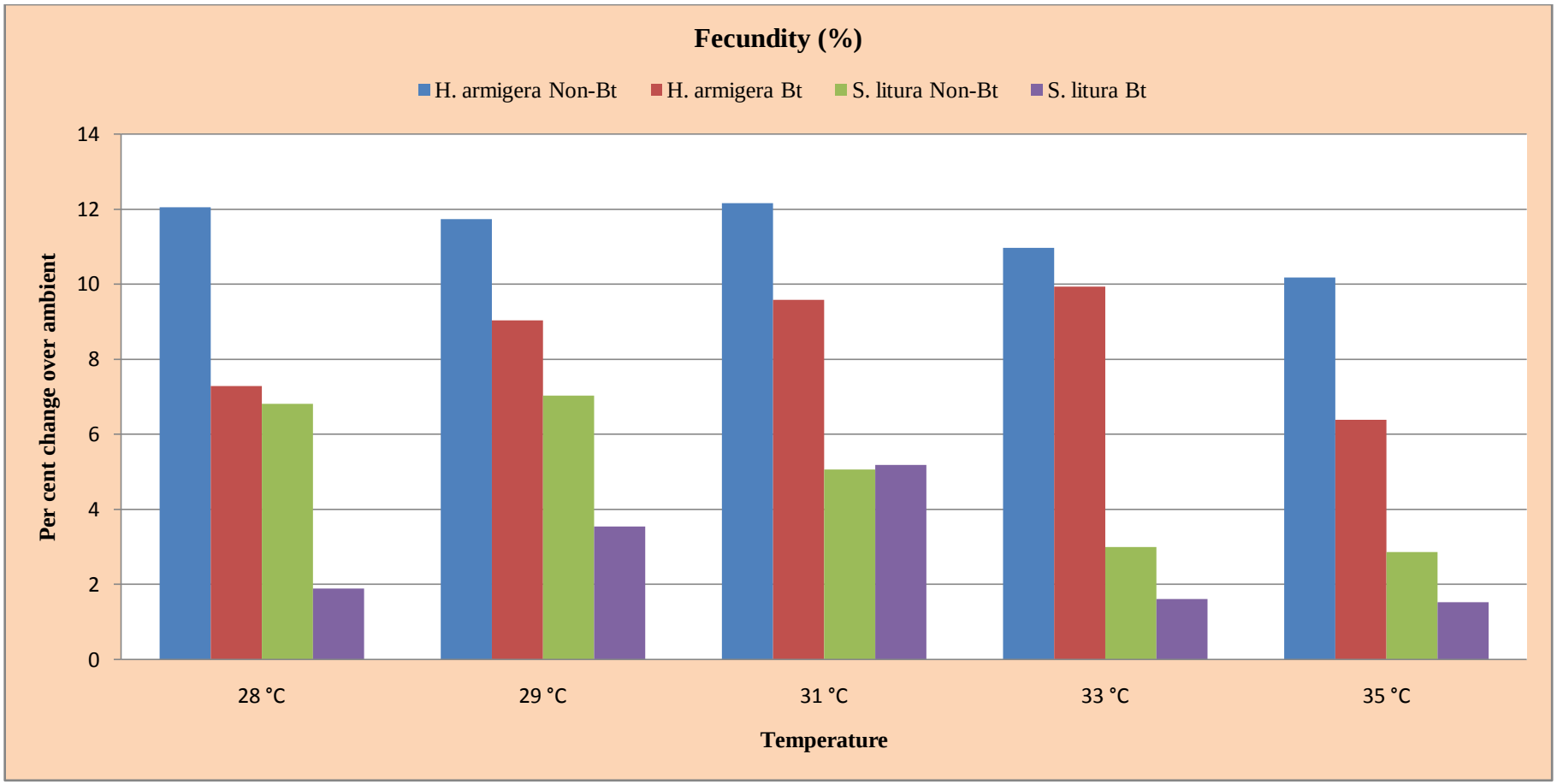


Fig. 4.33. Effect of *e*CO_2 and *e*Temp on mean fecundity of *H. armigera* and *S. litura* on non-Bt and Bt cotton

Wu *et al.* (2009) also observed loss of fecundity of *S. exigua* on Bt cotton with *e*CO_2, but the fecundity was higher in the third generation over the first (G1- 458; G2- 468; G3- 485 eggs per female moth). In the study, with interaction of CO_2 and temperature, pupal weight decreased and also fecundity decreased. Pupal weight was known to have significant correlation with fecundity (Karowe, 2007). Saethre and Hofsvang (2002) reported that pre-ovipositional period of apple codling moth, *Cydia pomonella* (L.) (Tortricidae: Lepidoptera) was strongly affected by temperature, but this duration necessarily do not affect the total number of eggs deposited per female. Calvo and Molina (2005) that temperature and food ingestion influences fecundity through their effects on larval duration, pupal weight and adult body size. In Lappet moth *Streblote panda* Hubner (Lasiocampidae: Lepidoptera), larvae were able to compensate for the low nutritional status of some host plants by protracted larval development and it may be viewed as a way to minimize adverse effects on fecundity. Zhang *et al.* (2016) reported that in predatory mite, *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) with high temperature spermatophores and oocytes were most likely to be damaged, which might cause a significantly reduced fecundity.

4.2 EFFECT OF *e*CO_2 AND *e*TEMP ON THE FEEDING INDICES OF TEST INSECTS *H. armigera and S. litura*.

The effect of *e*CO_2 and *e*Temp on the feeding indices of test insects *viz.*, Approximate Digestibility (AD), Relative Consumption Rate (RCR), Efficiency of Conversion of Ingested food (ECI), Efficiency of Conversion of Digested food (ECD) and Relative Growth Rate (RGR) on non-Bt and Bt cotton plants were discussed below.

4.2.1 Approximate Digestibility (AD)

The proportion of ingested food that is actually digested is denoted by approximate digestibility (AD) or the assimilation efficiency. With increase in test temperature (28-35 ºC), and CO_2 (380 to 550 ppm ± 25 ppm), AD of test insects increased (Tables 4.45 - 4.48 and Fig. 4.34 - 4.35). Comparative performance of both the test insects was presented in Fig. 4.36.

4.2.1.1 Effect on AD of *H. armigera* in non-Bt cotton

The data pertaining to AD of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.45 and Fig. 4.34.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp have shown significant impact on AD. At *a*CO_2, it increased with increase in temperature with lowest AD of 63.86 % at 28 ºC and highest 66.64 % at 35 ºC. At *e*CO_2, increase in AD was recorded from 64.67 to 68.17 % with corresponding increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on AD.

Second generation: At *a*CO_2, AD varied from 65.37 to 68.42 % with lowest and highest AD recorded at temperatures 28 and 35 ºC, respectively. At *e*CO_2, increase in AD was recorded with increase in temperature with lowest AD at 28 ºC with 65.77 % and highest at 35 ºC with 69.25 %. The individual effect of *e*CO_2 and *e*Temp and the interaction effect have shown significant influence on AD.

Third generation: At *a*CO_2, AD ranged from 67.03 to 69.97 % with lowest and highest AD at temperatures 28 and 35 ºC, respectively. At *e*CO_2, increase in AD was recorded with increase in temperature from 28 to 35 ºC with lowest and highest AD as 68.17 and 70.25 %, respectively. The individual effect of *e*CO_2 and *e*Temp and the interaction effect have shown significant influence on AD.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that at *a*CO_2 with increase in temperature (28-35 °C) the AD has increased significantly (65.42, 66.03, 66.41, 66.97 and 68.34 %, respectively) corresponding to 0.92, 1.52, 2.37 and 4.47 %. Similarly, under *e*CO_2 also the AD increased significantly (66.20, 66.57, 67.35, 68.71 and 69.22 %) with increase in temperatures (28-35 °C, respectively) which has corresponded to a increase of 0.55, 1.73, 3.78 and 4.56 %. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on AD of non-Bt cotton. Among *a*CO_2 and *e*CO_2, AD was lower in *a*CO_2. Further, the AD under *e*CO_2 was higher (1.20, 0.83, 1.41, 2.59 and 1.29 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred from the data that, approximate digestibility (AD) of *H. armigera* increased with increase in CO_2 (by 1 %) and increased with temperature and ultimately increased with *e*CO_2 + *e*Temp by 6 %. Abdul *et al*. (2014) reported that

Table 4.45. Effect of *e*CO_2 and *e*Temp on larval approximate digestibility (AD) of *H. armigera* in non-Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	63.86	64.67	**64.27**	65.37	65.77	**65.57**	67.03	68.17	**67.60**	65.42	66.20	**65.81**
29 ± 1°C	64.02	65.02	**64.52**	66.24	66.25	**66.25**	67.81	68.44	**68.13**	66.02	66.57	**66.30**
31 ± 1°C	64.65	66.13	**65.39**	66.36	66.76	**66.56**	68.23	69.16	**68.70**	66.41	67.35	**66.88**
33 ± 1°C	65.11	67.72	**66.42**	67.25	68.35	**67.80**	68.55	70.05	**69.30**	66.97	68.71	**67.84**
35 ± 1°C	66.64	68.17	**67.41**	68.42	69.25	**68.84**	69.97	70.25	**70.11**	68.34	69.22	**68.78**
Mean	**64.86**	**66.34**		**66.73**	**67.28**		**68.32**	**69.21**		**66.63**	**67.61**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	6.62*	0.10	0.29	4.63*	0.09	0.26	17.28*	0.09	0.26	15.39*	0.06	0.15
Temperature (°C)	25.28*	0.16	0.45	12.92*	0.15	0.41	13.19*	0.15	0.41	40.02*	0.09	0.24
Interaction (CO_2 + Temp(°C))	29.75*	0.23	0.64	28.81*	0.21	0.58	18.51*	0.21	0.58	72.42*	0.12	0.34
CV	1.73 %			1.58 %			1.56 %			0.92 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

chickpea foliage grown under *e*CO_2 condition stimulated an increase in approximate digestibility in *H. armigera*. With advancement of generations, there is an increase in AD of *H. armigera* (63.86, 65.37 and 67.03 %; 64.67, 65.77 and 68.17 %; and 68.17, 69.25 and 70.25 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively). Srinivasa Rao *et al*. (2013) quantified the effects of *e*CO_2 on the feeding indices of *A. janata* larvae on castor foliage and inferred significant increase in AD (0.32 – 12.26 %) in all four generations over *a*CO_2 conditions.

4.2.1.2 Effect on AD of *H. armigera* in Bt cotton

The data pertaining to AD of *H. armigera* in Bt cotton for all three generations was presented in Table 4.46 and Fig. 4.34.

First generation: In Bt cotton, *e*CO_2 and *e*Temp have shown significant influence on AD. Under *a*CO_2 it increased with increase in temperature with lowest AD recorded as 64.27 % at 28 ºC and highest AD 66.71 % at 35 ºC. At *e*CO_2, increase in AD was recorded from 65.35 to 68.79 % with corresponding increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on AD.

Second generation: In Bt cotton, AD varied from 65.95 to 68.08 % with lowest and highest AD at temperatures 28 ºC and 35 ºC, respectively. At *e*CO_2, increased AD was recorded with increase in temperature with lowest AD at 28 ºC with 66.52 % and highest at 35 ºC with 69.51 %. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on AD.

Third generation: At *a*CO_2, lowest AD was recorded at 28 ºC with 66.41 % and highest at 35 ºC with 69.85 %. At *e*CO_2, AD increased with increase in temperature with 67.22 % AD as lowest at 28 ºC and 70.87 % as highest AD at temperature 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on AD.

Mean of generations: The mean of three generations on Bt cotton also indicated that at *a*CO_2 with increase in temperature (28-35 °C) the AD has increased significantly (65.54, 65.97, 67.03, 67.60 and 68.21 %, respectively) corresponding to 0.66, 2.26, 3.13 and 4.07 %. Similarly, under *e*CO_2 also the AD increased significantly (66.36, 67.05, 67.98, 68.42 and 69.72 %) with increase in temperatures (28-35 ºC, respectively) which has corresponded to an increase of 1.04, 2.44, 3.10 and 5.06 %.

Table 4.46. Effect of *e*CO_2 and *e*Temp on larval approximate digestibility (AD) of *H. armigera* in Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	64.27	65.35	**64.81**	65.95	66.52	**66.24**	66.41	67.22	**66.82**	65.54	66.36	**65.95**
29 ± 1°C	64.71	66.17	**65.44**	66.65	67.43	**67.04**	66.56	67.56	**67.06**	65.97	67.05	**66.51**
31 ± 1°C	65.33	67.24	**66.29**	66.82	67.83	**67.33**	68.93	68.87	**68.90**	67.03	67.98	**67.50**
33 ± 1°C	66.07	67.40	**66.74**	67.48	68.25	**67.87**	69.24	69.62	**69.43**	67.60	68.42	**68.01**
35 ± 1°C	66.71	68.79	**67.75**	68.08	69.51	**68.80**	69.85	70.87	**70.36**	68.21	69.72	**68.97**
Mean	**65.42**	**66.99**		**67.00**	**67.91**		**68.20**	**68.83**		**66.87**	**67.91**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	156.64*	0.08	0.22	30.44*	0.08	0.22	26.46*	0.08	0.21	187.36*	0.04	0.12
Temperature (°C)	18.53*	0.12	0.34	10.52*	0.13	0.35	21.91*	0.12	0.33	47.56*	0.07	0.19
Interaction (CO_2 + Temp(°C))	17.47*	0.17	0.48	6.98*	0.18	0.50	15.78*	0.17	0.47	26.94*	0.10	0.27
CV	1.29 %			1.34 %			1.26 %			0.73 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

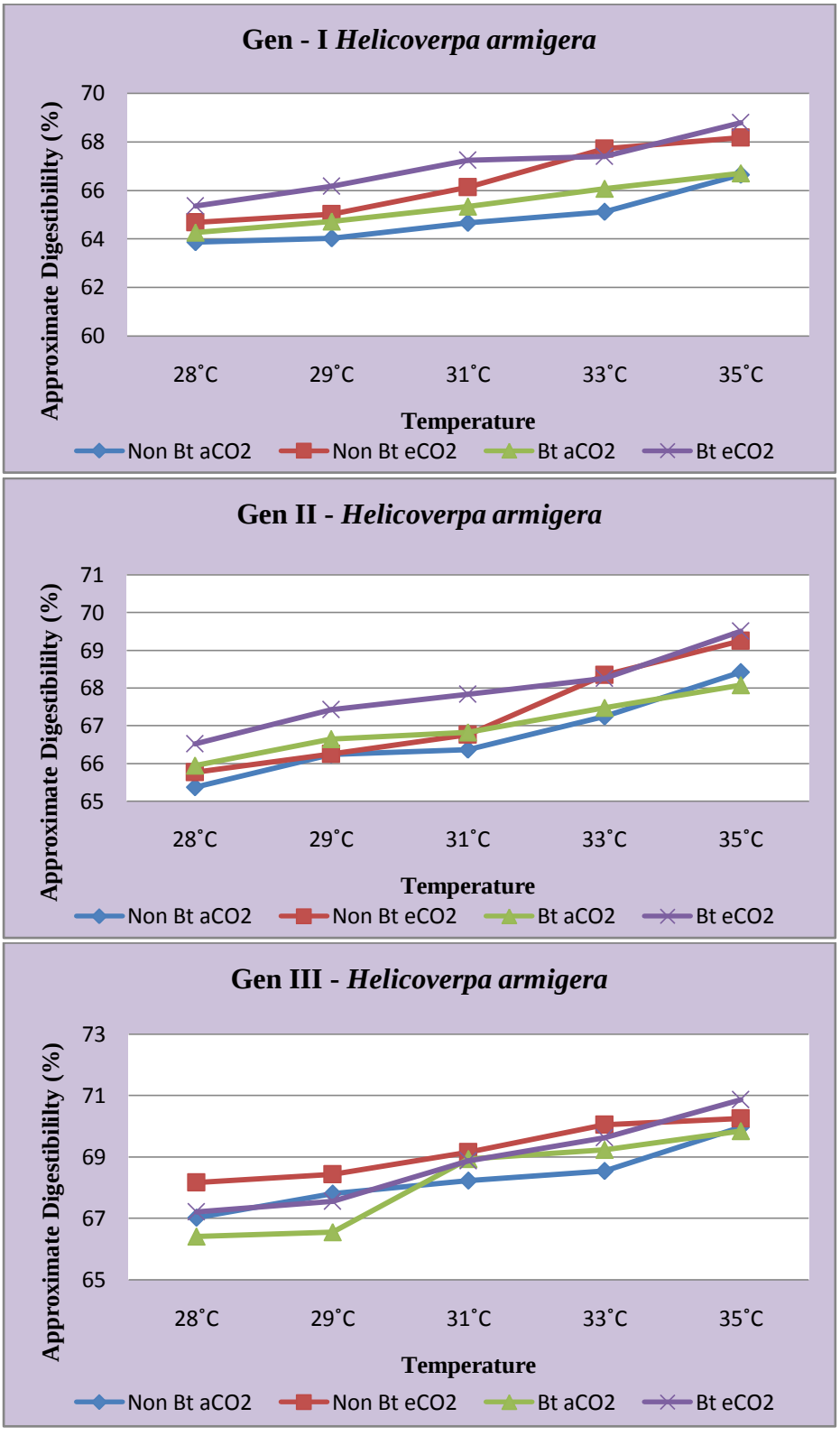


Fig. 4.34. Effect of eCO_2 and *e*Temp on larval approximate digestibility (AD) of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

The interaction effect of *e*CO_2 and *e*Temp showed significant influence on AD. And among *a*CO_2 and *e*CO_2, AD was lower in *a*CO_2. Further, the AD under *e*CO_2 was higher (1.25, 1.64, 1.42, 1.22 and 2.21 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred from the data that, approximate digestibility (AD) of *H. armigera* increased with increase in CO_2 (by 1 %) and temperature and ultimately increased with *e*CO_2 + *e*Temp by 6 %. The results are in close conformity with Chen *et al*. (2005b) who reported that approximate digestibility of *H. armigera* reared on Bt cotton under *e*CO_2 was higher compared to those under non-Bt cotton. On the contrary, Somashekara *et al*. (2012) reported that the AD of bollworm decreased after feeding on Bt cotton. In the study, with every next generation, there is a little increase in AD of *H. armigera* on Bt cotton (64.27, 65.95 and 66.41 %; 65.35, 66.52 and 67.22 %; and 68.79, 69.51 and 70.87 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively).

4.2.1.3 Effect on AD of *S. litura* in non-Bt cotton

The data pertaining to AD of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.47 and Fig. 4.35.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on AD. Under *a*CO_2, it increased with increase in temperature with lowest and highest AD of 66.02 and 69.11 % at temperatures 28 and 35 ºC. At *e*CO_2, increase in AD was recorded ranging from 68.93 to 71.82 % with corresponding increase in temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp has significant effect on AD. And AD was higher at *e*CO_2 than *a*CO_2.

Second generation: At *a*CO_2, AD increased from 68.31 to 71.69 % with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, increase in AD was recorded with increase in temperature with lowest AD at 28 ºC with 69.03 % and highest at 35 ºC with 72.21 %. And AD was higher at *e*CO_2 than *a*CO_2.The individual effect of *e*CO_2 and *e*Temp and the interaction effect has showed significant influence on AD.

Table 4.47. Effect of *e*CO_2 and *e*Temp on larval approximate digestibility (AD) of *S. litura* in non-Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	66.02	68.93	**67.48**	68.31	69.03	**68.67**	69.21	69.96	**69.59**	67.85	69.31	**68.58**
29 ± 1°C	67.96	69.40	**68.68**	68.62	70.82	**69.72**	69.60	70.63	**70.12**	68.73	70.28	**69.51**
31 ± 1°C	68.24	70.39	**69.32**	69.50	70.84	**70.17**	70.27	71.75	**71.01**	69.34	70.99	**70.17**
33 ± 1°C	69.01	70.98	**70.00**	70.17	71.05	**70.61**	71.88	72.34	**72.11**	70.35	71.46	**70.91**
35 ± 1°C	69.11	71.82	**70.47**	71.69	72.21	**71.95**	72.85	73.56	**73.21**	71.22	72.53	**71.87**
Mean	**68.07**	**70.30**		**69.66**	**70.79**		**70.76**	**71.65**		**69.50**	**70.91**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	4.63*	0.12	0.33	5.32*	0.13	0.35	6.29*	0.12	0.34	14.38*	0.07	0.19
Temperature (°C)	6.33*	0.19	0.53	11.00*	0.20	0.55	13.13*	0.19	0.54	3.11*	0.11	0.30
Interaction (CO_2 + Temp(°C))	13.43*	0.27	0.74	8.03*	0.28	0.78	2.84*	0.27	0.76	19.01*	0.15	0.42
CV	1.98 %			2.08 %			2.04 %			1.12 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

Third generation: At *a*CO_2, increase in AD was recorded from 69.21 to 72.85 % with increase in temperature from 28 to 35 ºC. At *e*CO_2, AD ranged between 69.96 to 73.56 % with lowest AD at temperature 28 ºC and highest at 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interaction effect has showed significant influence on AD. And AD was higher at *e*CO_2 than *a*CO_2.

Mean of generations: The mean of three generations on non-Bt cotton also indicated that the AD has increased significantly (67.85, 68.73, 69.34, 70.35 and 71.22 % respectively) with increase in temperature (28-35 ºC) corresponding to a increase of 1.30, 2.20, 3.69 and 4.97 %. Similarly, under *e*CO_2, the AD increased significantly (69.31, 70.28, 70.99, 71.46 and 72.53 %) with increase in temperatures (28-35 °C, respectively) which has corresponded to a increase of 1.41, 2.43, 3.10 and 4.65 %. The interaction effect of *e*CO_2 and *e*Temp has significant effect on AD. And AD was higher at *e*CO_2 than *a*CO_2. Further, the AD under *e*CO_2 was higher (2.15, 2.27, 2.39, 1.57 and 1.84 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.The data obtained suggests that, AD of *S. litura* increased with increase in CO_2 (by 2 %) and temperature and ultimately increased with *e*CO_2 + *e*Temp by 7 %. Srinivasa Rao *et al.* (2009) while examining the host mediated effects of *e*CO_2 on growth performance of *S. litura* inferred that AD increased (from 58.61 @ aCO_2 to 74.51 % @ *e*CO_2).

Srinivasa Rao *et al.* (2014b) reported that AD increased by about 9 % in *S. litura* on groundnuts under *e*CO_2. Investigations of Zhang *et al*. (2017) on the effect of *e*CO_2 on the food utilization of *S. litura* on soybean cultivars revealed that AD increased by 12.9 %, Karmakar and Pal (2017) reported that with 5 °C increase in temperature, *S. litura* AD decreased from 46.89 to 28.98 % in castor and from 47.54 to 32.84 % in tomato. Sharma and Brar (2018) also reported that *S. litura* showed significant increase in AD by 4.7 % with *e*CO_2 (500 ppm) and by 22.0 % with increase in temperature by 5 ºC. With advancement of every generation, *S. litura* AD on non-Bt cotton increased by little extent (66.02, 68.31 and 69.21 %; 68.93, 69.03 and 69.96 %; and 71.82, 72.21 and 73.56 % under the test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively).

4.2.1.4 Effect on AD of *S. litura* in Bt cotton

The data pertaining to AD of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.48 and Fig. 4.35.

First generation: In Bt cotton, CO_2 and *e*Temp has shown significant impact on AD. Under *a*CO_2, AD increased from 63.85 to 66.45 % with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, lowest AD of 65.12 % was recorded at temperature 28 ºC and highest AD 67.85 % at 35 ºC. The interaction effect of *e*CO_2 and *e*Temp has significant effect on AD.

Second generation: At *a*CO_2, increase in AD with increase in temperature was recorded with lowest and highest AD of 65.12 and 68.04 % at temperatures 28 ºC and 35 ºC, respectively. At *e*CO_2, AD increased from 66.42 to 69.85 % with corresponding increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interaction effect has showed significant influence on AD and was higher at *e*CO_2 than *a*CO_2.

Third generation: At *a*CO_2, AD ranged between 66.49 to 69.60 % with lowest and highest AD at temperatures 28 and 35 ºC, respectively. At *e*CO_2, an increased AD was recorded with increase in temperature with 67.96 % as lowest AD at 28 ºC and 70.47 % as highest AD at temperature 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interaction effect has showed significant influence on AD.

Mean of generations: The mean of three generations in Bt cotton also indicated that the AD has increased significantly (65.15, 66.08, 66.63, 67.04 and 68.03 %, respectively) with increase in temperature (28-35 ºC) corresponding to increase 1.42, 2.27, 2.90 and 4.42 %. Similarly, under *e*CO_2 also the AD increased significantly (66.50, 67.01, 67.37, 68.43 and 69.39 %) with increase in temperatures (28-35 ºC, respectively) which has corresponded to a increase of 0.77, 1.30, 2.91 and 4.35 %. The interaction effect of *e*CO_2 and *e*Temp has significant effect on AD of Bt cotton. And AD was higher at *e*CO_2 than *a*CO_2. Further, the AD under *e*CO_2 was higher (2.07, 1.41, 1.10, 2.08 and 2.00 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2. The data obtained suggests that, AD of *S. litura* on Bt cotton increased withincrease in CO_2 (by 2 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 7 %. With advancement of every generation, AD

Table 4.48. Effect of *e*CO_2 and *e*Temp on larval approximate digestibility (AD) of *S. litura* in Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	63.85	65.12	**64.49**	65.12	66.42	**65.77**	66.49	67.96	**67.23**	65.15	66.50	**65.83**
29 ± 1°C	64.44	65.51	**64.98**	65.93	67.06	**66.50**	67.86	68.46	**68.16**	66.08	67.01	**66.54**
31 ± 1°C	65.11	65.94	**65.53**	66.78	67.29	**67.04**	68.01	68.87	**68.44**	66.63	67.37	**67.00**
33 ± 1°C	65.67	66.86	**66.27**	67.20	68.84	**68.02**	68.25	69.60	**68.93**	67.04	68.43	**67.74**
35 ± 1°C	66.45	67.85	**67.15**	68.04	69.85	**68.95**	69.60	70.47	**70.04**	68.03	69.39	**68.71**
Mean	**65.10**	**66.26**		**66.61**	**67.89**		**68.04**	**69.07**		**66.59**	**67.74**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	89.35*	0.09	0.26	15.36*	0.10	0.26	19.76*	0.09	0.25	116.50*	0.05	0.14
Temperature (°C)	7.40*	0.15	0.41	4.65*	0.15	0.42	32.43*	0.14	0.40	116.50*	0.08	0.23
Interaction (CO_2 + Temp(°C))	10.54*	0.21	0.58	2.61*	0.21	0.59	11.98*	0.20	0.57	17.83*	0.11	0.32
CV	1.54 %			1.58 %			1.51 %			0.85 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

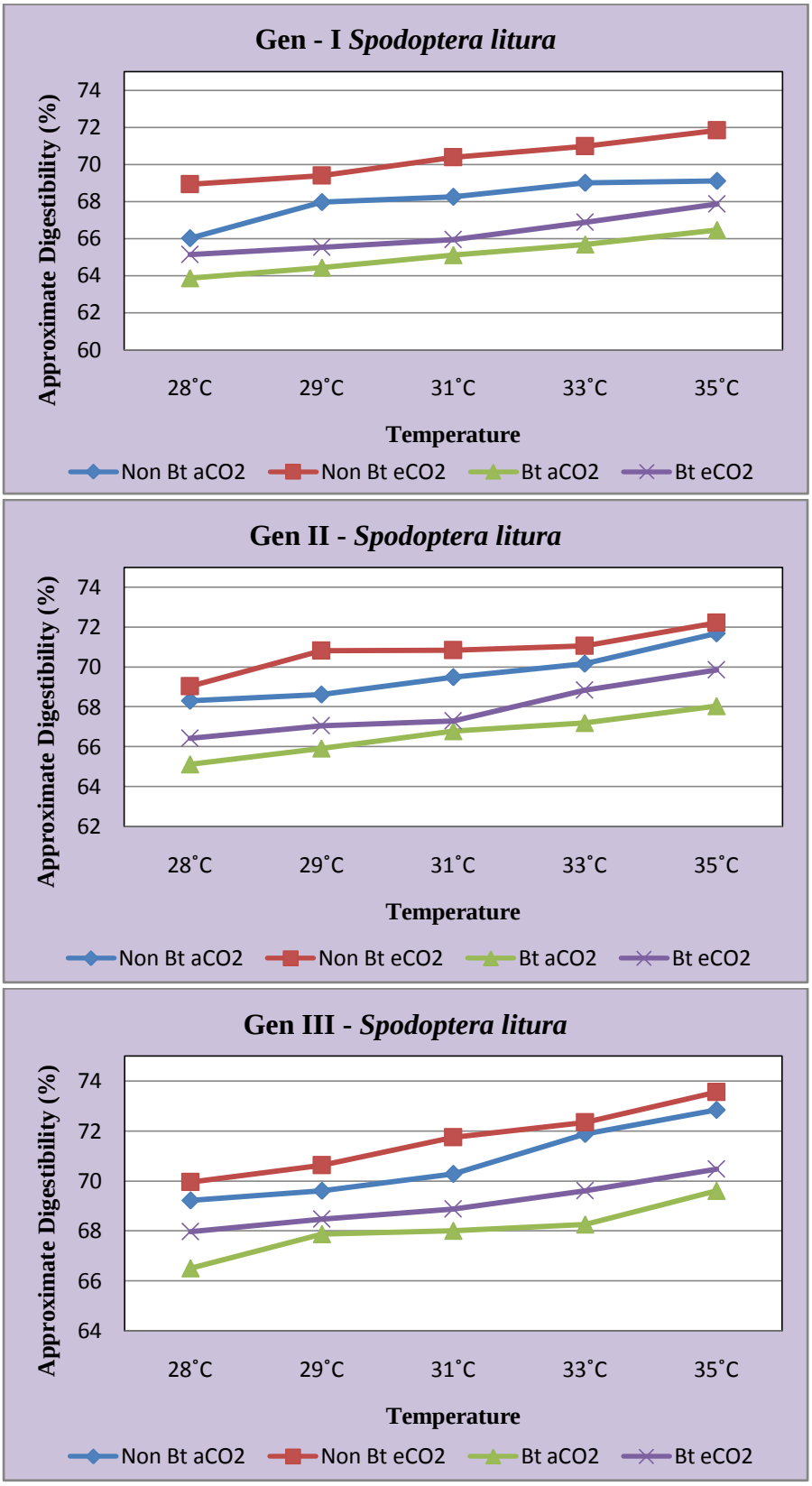


Fig. 4.35. Effect of *e*CO_2 and *e*Temp on larval approximate digestibility (AD) of *S. litura* on non-Bt and Bt cotton in first, second and third generation

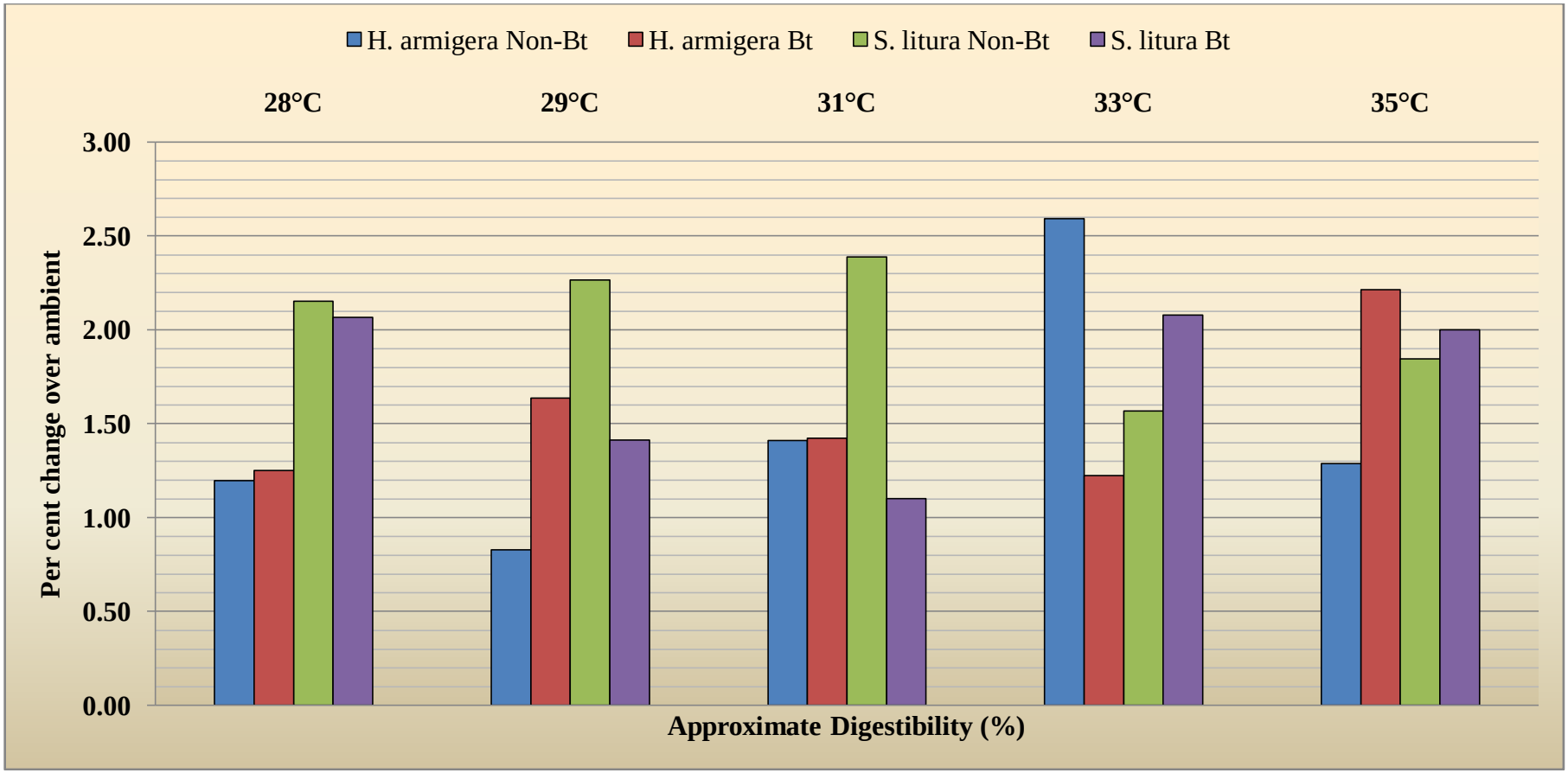


Fig. 4.36. Effect of *e*CO_2 and *e*Temp on mean larval approximate digestibility (AD) of *H. armigera* and *S. litura* on non-Bt and Bt cotton

increased by some extent (63.85, 65.12 and 66.49 %; 65.12, 66.42 and 67.96 %; and 67.85, 69.85 and 70.47 %), under the test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively.

4.2.2 Relative Consumption Rate (RCR)

Relative consumption rate (RCR) indicates the per capita consumption of food by larva per day. RCR under *e*CO_2 was higher than that of *a*CO_2.With increase in test temperatures (28-35 °C) and CO_2 (550± 25 ppm), RCR of test insects gradually increased (Tables 4.49 - 4.52 and Fig 4.37 - 4.38). Comparative performance of both the test insects was presented in Fig. 4.39.

4.2.2.1 Effect on RCR of *H. armigera* in non-Bt cotton

The data pertaining to RCR of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.49 and Fig. 4.37.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on RCR. At *a*CO_2, it increased with increase in temperature with lowest RCR of 800.52 mg g^{-1} day^{-1} at 28 ºC and highest 948.77 mg g^{-1} day^{-1}at 35 ºC. At *e*CO_2, increase in RCR was recorded with increase in temperature with lowest and highest at 28 ºC and 35 ºC with 940.35 and 1028.14 mg g^{-1} day^{-1}, respectively. The combined effect of *e*CO_2 and *e*Temp showed significant impact on RCR.

Second generation: At *a*CO_2, RCR varied from 813.03 to 904.02 mg g^{-1} $day^{-1}$with lowest and highest RCR at temperatures 28 and 35 ºC, respectively. At *e*CO_2, increased RCR was recorded with increase in temperature with lowest 953.90 mg g^{-1} day^{-1} at 28 ºC and highest 1025.43 mg g^{-1} day^{-1} at 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interaction effect have shown significant influence on RCR.

Third generation: In non-Bt cotton, increase in RCR from 849.51 to 935.88 mg g^{-1} day^{-1} was recorded with increase in temperatures from 28 to 35 ºC, respectively. At *e*CO_2, lowest RCR was recorded at 28 ºC with 987.32 mg g^{-1} $day^{-1}$and highest at 35 ºC with 1080.31 mg g^{-1} day^{-1}. The individual effect of *e*CO_2 and *e*Temp and the interaction effect have shown significant influence on RCR.

Mean of generations: The mean of three generations also indicated that at *a*CO_2 with increase in temperature (28-35 °C) the RCR has increased significantly (821.02, 854.12, 863.48, 863.70 and 929.56 mg g^{-1} day^{-1}, respectively) corresponding to 4.03, 5.17, 5.20 and 13.22 %. Similarly, under *e*CO_2 also the RCR increased significantly (960.52, 982.13, 999.02, 1019.15 and 1044.63 mg g^{-1} day^{-1}) with increase in temperatures (28-35 ºC, respectively) which has corresponded to a increase of 2.25, 4.01, 6.10 and 8.76 %. The combined effect of *e*CO_2 and *e*Temp showed significant impact on RCR. And among *a*CO_2 and *e*CO_2, RCR was lower in *a*CO_2. Further, the RCR of non-Bt cotton under *e*CO_2 was higher (16.99, 14.99, 15.70, 18.00 and 12.38 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It was observed that, RCR of *H. armigera* increased with increase in CO_2 (by 17 %) and temperature and ultimately increased with *e*CO_2 + *e*Temp by 27 %. Abdul *et al*. (2014) also reported that chickpea foliage grown under *e*CO_2 condition stimulated significant increase in RCR by 58.8 % compared with ambient conditions. In the study, with advancement of every generation, RCR increased by 801, 813 and 850 mg g^{-1} day^{-1}; 940, 954 and 987 mg g^{-1} day^{-1}; and 1028, 1025 and 1080 mg g^{-1} day^{-1}, under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively. Similarly Wu *et al*. (2006) observed that RCR of *H. armigera* on spring wheat increased from *a*CO_2 (459.9, 485, 488.5 mg mg^{-1} day^{-1} at F1, F2 and F3 generations) to *e*CO_2 (504.2, 567.1, 594.3 mg mg^{-1} day^{-1} at respective generations).

4.2.2.2 Effect on RCR of *H. armigera* in Bt cotton

The data pertaining to RCR of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.50 and Fig. 4.37.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on RCR. At *a*CO_2, it varied from 1025.81 to 1131.48 mg g^{-1} day^{-1} with lowest and highest RCR at temperatures 28 and 35 ºC, respectively. At *e*CO_2, increase in RCR was recorded with increase in temperature with lowest and highest RCR of 1077.92 and 1328.89 mg g^{-1} day^{-1} at temperatures 28 and 35 ºC, respectively. The combined effect of *e*CO_2 and *e*Temp showed significant impact on RCR.

Table 4.49. Effect of *e*CO_2 and *e*Temp on larval relative consumption rate (RCR) of *H. armigera* in non-Bt cotton

Temperature	First generation (mg g^{-1} day^{-1})			Second generation (mg g^{-1} day^{-1})			Third generation (mg g^{-1} day^{-1})			Mean (mg g^{-1} day^{-1})		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	800.52	940.35	**870.44**	813.03	953.90	**883.47**	849.51	987.32	**918.42**	821.02	960.52	**890.77**
29 ± 1°C	848.57	974.79	**911.68**	848.21	989.11	**918.66**	865.59	982.49	**924.04**	854.12	982.13	**918.13**
31 ± 1°C	845.26	983.71	**914.49**	850.65	993.57	**922.11**	894.52	1019.77	**957.15**	863.48	999.02	**931.25**
33 ± 1°C	856.72	1004.85	**930.79**	851.56	1002.43	**927.00**	882.81	1050.18	**966.50**	863.70	1019.15	**941.43**
35 ± 1°C	948.77	1028.14	**988.46**	904.02	1025.43	**964.73**	935.88	1080.31	**1008.10**	929.56	1044.63	**987.09**
Mean	**859.97**	**986.37**		**853.49**	**992.89**		**885.66**	**1024.01**		**866.37**	**1001.09**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	696.98*	3.71	10.32	632.80*	3.55	9.90	581.71*	3.97	11.06	1869.52*	2.18	6.08
Temperature (°C)	38.25*	5.86	16.32	57.89*	5.62	15.65	21.83*	6.28	17.49	104.29*	3.45	9.61
Interaction (CO_2 + Temp(°C))	2.75*	8.29	23.08	5.95*	7.95	22.13	1.03	8.88	NS	2.92*	4.88	13.59
CV	4.34 %			4.30 %			4.82 %			2.61 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

Table 4.50. Effect of *e*CO_2 and *e*Temp on larval relative consumption rate (RCR) of *H. armigera* in Bt cotton

Temperature	First generation (mg g^{-1} day^{-1})			Second generation (mg g^{-1} day^{-1})			Third generation (mg g^{-1} day^{-1})			Mean (mg g^{-1} day^{-1})		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	1025.81	1077.92	**1051.87**	1074.77	1096.08	**1085.43**	1085.98	1111.38	**1098.68**	1062.19	1095.13	**1078.66**
29 ± 1°C	1091.20	1104.07	**1097.64**	1100.28	1117.48	**1108.88**	1138.69	1232.28	**1185.49**	1110.06	1151.28	**1130.67**
31 ± 1°C	1087.47	1190.94	**1139.21**	1112.50	1199.09	**1155.80**	1193.63	1251.14	**1222.39**	1131.20	1213.72	**1172.46**
33 ± 1°C	1112.67	1240.58	**1176.63**	1132.96	1251.46	**1192.21**	1203.72	1306.80	**1255.26**	1149.78	1266.28	**1208.03**
35 ± 1°C	1131.48	1328.89	**1230.19**	1135.71	1390.38	**1263.05**	1218.22	1488.90	**1353.56**	1161.80	1402.72	**1282.26**
Mean	**1089.73**	**1188.48**		**1111.24**	**1210.90**		**1168.05**	**1278.10**		**1123.01**	**1225.83**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	41.29*	4.85	13.50	124.47*	5.37	14.97	79.37*	5.95	16.57	272.61*	2.91	8.10
Temperature (°C)	71.65*	7.66	21.35	34.85*	8.49	23.66	30.33*	9.40	26.20	145.25*	4.60	12.81
Interaction (CO_2 + Temp(°C))	47.67*	10.84	30.20	39.26*	12.01	33.46	27.76*	13.30	37.05	125.88*	6.50	18.12
CV	4.86 %			5.24 %			5.77 %			2.86 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

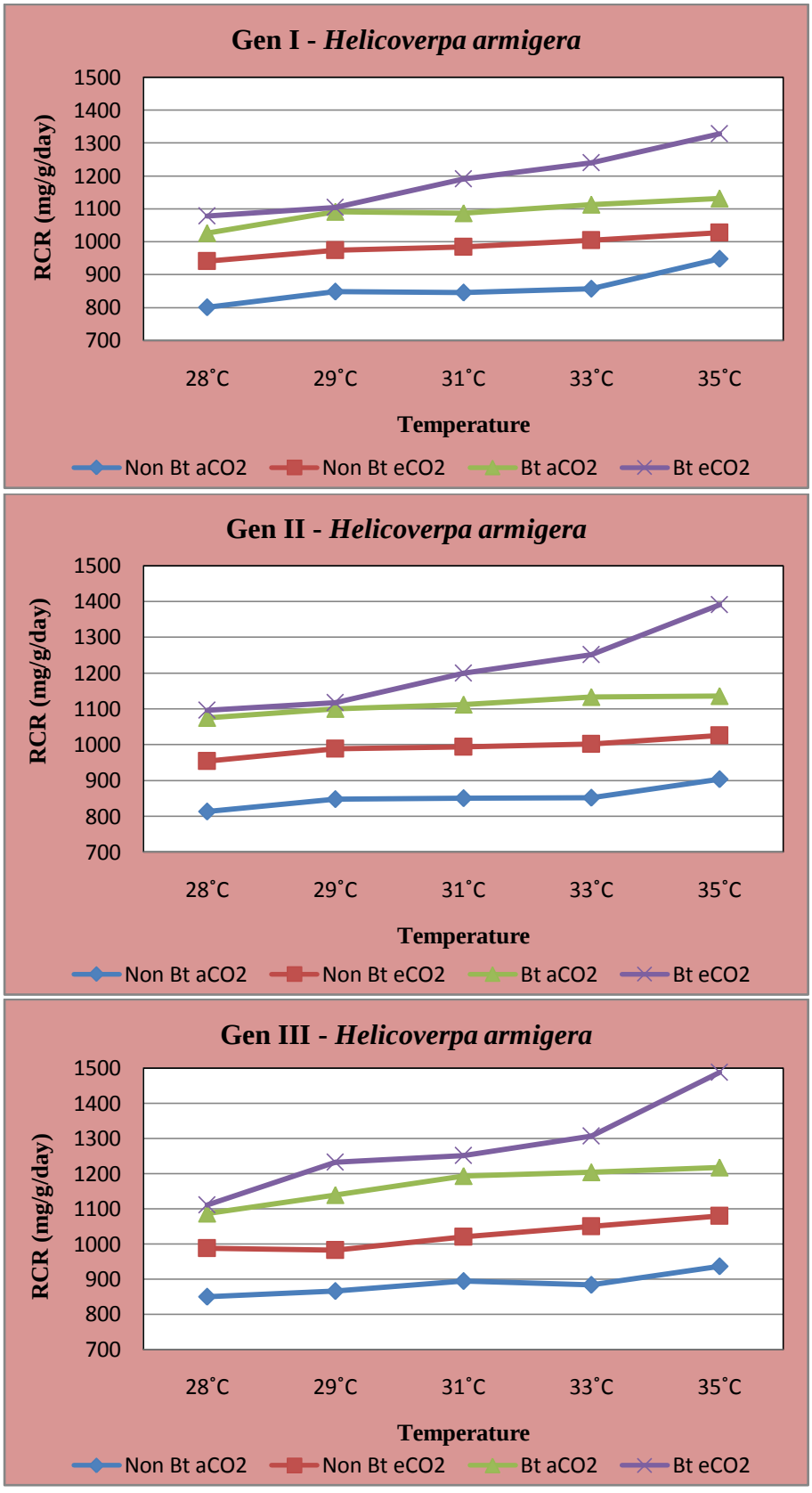


Fig. 4.37. Effect of *e*CO_2 and *e*Temp on larval relative consumption rate (RCR) of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

Second generation: At *a*CO_2, RCR varied from 1074.77 and 1135.71 mg g^{-1} day^{-1} with lowest and highest at temperatures 28 and 35 ºC, respectively. At *e*CO_2, increase in RCR was recorded with increase in temperature with lowest and highest RCR of 1096.08 and 1390.38 mg g^{-1} day^{-1} at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interaction effect has shown significant influence on RCR.

Third generation: At *a*CO_2, RCR varied from 1085.98 to 1218.22 mg g^{-1} day^{-1} with lowest and highest RCR at temperatures 28 and 35 ºC, respectively. At *e*CO_2, increase in RCR was recorded from 1111.38 to 1488.90 mg g^{-1} day^{-1} with corresponding increase in temperature from 28 and 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interaction effect has shown significant influence on RCR.

Mean of generations: The mean of three generations on Bt cotton also indicated that with increase in temperature (28-35 ºC) the RCR has increased significantly (1062.19, 1110.06, 1131.20, 1149.78 and 1161.80 mg g^{-1} day^{-1}, respectively) corresponding to 4.51, 6.50, 8.25 and 9.38 %. Similarly, under *e*CO_2 also the RCR increased significantly (1095.13, 1151.28, 1213.72, 1266.28 and 1402.72 mg g^{-1} day^{-1}) with increase in temperatures (28-35 ºC, respectively) which has corresponded to a increase of 5.13, 10.83, 15.63 and 28.09 %. The combined effect of *e*CO_2 and *e*Temp showed significant impact on RCR. And among *a*CO_2 and *e*CO_2, RCR was lower in *a*CO_2. Further, the RCR under *e*CO_2 was higher (3.10, 3.71, 7.30, 10.13 and 20.74 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It was observed that, RCR of *H. armigera* on Bt cotton increased with increase in CO_2 (by 3 %) and temperature and ultimately increased with *e*CO_2 + *e*Temp by 32 %. Chen *et al*. (2007) showed that *e*CO_2 and Bt cotton can cause an increase in RCR of *H. armigera*. With advancement of every generation, RCR increased by 1026, 1075 and 1086 mg g^{-1} day^{-1}; 1078, 1096 and 1111 mg g^{-1} day^{-1}; and 1329, 1390 and 1489 mg g^{-1} day^{-1}, under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively.

4.2.2.3 Effect on RCR of *S. litura* in non-Bt cotton

The data pertaining to RCR of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.51 and Fig. 4.38.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant impact on RCR. Under *a*CO_2, RCR increased from 776.66 to 892.17 mg g^{-1} day^{-1} with corresponding increase in temperature from 28 to 35 ºC, respectively. At *e*CO_2, an increase in RCR was recorded in the range of 871.69 to 970.66 mg g^{-1} $day^{-1}$with increase in corresponding temperature from 28 to 35 ºC, respectively. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on RCR.

Second generation: In non-Bt cotton, increase in RCR from 875.38 to 986.62 mg g^{-1} day^{-1} was recorded with corresponding increase in temperatures from 28 and 35 ºC. At *e*CO_2, lowest RCR was recorded with 881.26 mg g^{-1} day^{-1} at 28 ºC and highest RCR of 1009.87 mg g^{-1} day^{-1} at 35 ºC. The individual effect of *e*CO_2 and *e*Temp was significant and whereas a non-significant interactive effect recorded on RCR.

Third generation: At *a*CO_2, RCR varied from 877.02 to 1078.36 mg g^{-1} day^{-1} with lowest and highest RCR at temperatures 28 and 35 ºC, respectively. At *e*CO_2, an increase in RCR was recorded ranging from 914.83 to 1080.89 mg g^{-1} $day^{-1}$with corresponding increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp was significant and whereas a non-significant interactive effect recorded on RCR.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the RCR has increased significantly (843.02, 863.41, 892.78, 929.39 and 985.72 mg g^{-1} day^{-1}, respectively) with increase in temperature (28-35 ºC) corresponding to increase of 2.42, 5.90, 10.24 and 16.93 %. Similarly, under *e*CO_2 also the RCR increased significantly (889.26, 927.27, 973.07, 996.17 and 1020.47 mg g^{-1} day^{-1}) with increase in temperatures (28-35 ºC, respectively) which has corresponded to an increase of 4.27, 9.42, 12.02 and 14.76 %. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on RCR. And RCR was significantly lower in *a*CO_2 than *e*CO_2. Further, the RCR of non-Bt cotton under *e*CO_2 was higher (5.49, 7.40, 8.99, 7.19 and 3.53 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It can be inferred that, mean RCR of *S. litura* increased with increase in CO_2 (by 5 %) and temperature and ultimately increased with *e*CO_2 + *e*Temp by 21 %. The results of the study are similar to Wu *et al*. (2009) who recorded that RCR of non-Bt cotton was higher (470.7, 437.4 and 458.2 mg mg^{-1} day^{-1} in three successive

Table 4.51. Effect of *e*CO_2 and *e*Temp on larval relative consumption rate (RCR) of *S. litura* in non-Bt cotton

Temperature	First generation (mg g^{-1} day^{-1})			Second generation (mg g^{-1} day^{-1})			Third generation (mg g^{-1} day^{-1})			Mean (mg g^{-1} day^{-1})		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	776.66	871.69	**824.18**	875.38	881.26	**878.32**	877.02	914.83	**895.93**	843.02	889.26	**866.14**
29 ± 1°C	808.60	934.00	**871.30**	887.01	925.00	**906.01**	894.63	922.81	**908.72**	863.41	927.27	**895.34**
31 ± 1°C	840.67	974.54	**907.61**	921.50	959.04	**940.27**	916.17	985.62	**950.90**	892.78	973.07	**932.92**
33 ± 1°C	864.59	940.87	**902.73**	950.58	973.55	**962.07**	972.99	1074.09	**1023.54**	929.39	996.17	**962.78**
35 ± 1°C	892.17	970.66	**931.42**	986.62	1009.87	**998.25**	1078.36	1080.89	**1079.63**	985.72	1020.47	**1003.10**
Mean	**836.54**	**938.35**		**924.22**	**949.74**		**947.83**	**995.65**		**902.86**	**961.25**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	207.39*	4.99	13.92	346.52*	4.77	13.28	345.86*	4.63	12.90	910.95*	2.73	7.60
Temperature (°C)	27.35*	7.90	22.02	38.68*	7.54	21.00	28.60*	7.32	20.40	94.86*	4.31	12.01
Interaction (CO_2 + Temp(°C))	2.82*	11.17	31.14	0.76	10.66	NS	1.17	10.36	NS	3.59*	6.10	16.99
CV	6.30 %			6.01 %			5.87 %			3.44 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

generations) over Bt cotton. Srinivasa Rao *et al*. (2009) reported increase in RCR of *S. litura* from 37.12 (aCO_2) to 48.27 mg mg^{-1} day^{-1} (eCO_2) on castor. RCR increased by about 7 % in *S. litura* on groundnuts under eCO_2 (Srinivasa Rao *et al*. 2014b).

Divya (2017) revealed increased RCR in *S. exigua* larvae under eCO_2. Karmakar and Pal (2017) reported an increase in RCR for *S. litura* when reared on castor (1.27 – 1.41) and tomato (1.07-1.09). Zhang *et al*. (2017) revealed that RCR of *S. litura* on soybean cultivars increased by 12.9 % under eCO_2. Sharma and Brar (2018) reported decrease in RCR by 26.6 % at 500 ppm of CO_2 and by 35.89 % with increase in temperature of 5 ºC. In the present study, with advancement of every generation, RCR increased by some extent (777, 875 and 877 mg g^{-1} day^{-1}; 872, 881 and 915 mg g^{-1} day^{-1}; and 971, 1010 and 1081 mg g^{-1} day^{-1}, under the test conditions, aCO_2+*a*Temp, eCO_2 and eCO_2+*e*Temp respectively). Srinivasa Rao *et al*. (2013) reported increase in RCR (6-22 %) with eCO_2 in all four generations over aCO_2 conditions in *A. janata* larvae on castor foliage.

4.2.2.4 Effect on RCR of *S. litura* in Bt cotton

The data pertaining to RCR of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.52 and Fig. 4.38.

First generation: In Bt cotton, eCO_2 and *e*Temp has shown significant impact on RCR. Under aCO_2, it ranged from 928.80 to 1065.72 mg g^{-1} day^{-1} with lowest RCR at temperature 28 ºC and highest at 35 ºC. At eCO_2, increase in RCR was recorded ranging from 1113.63 to 1348.09 mg g^{-1} day^{-1} with corresponding increase in temperature from 28 ºC to 35 ºC. A significant interactive effect of eCO_2 and *e*Temp was recorded on RCR.

Second generation: At aCO_2, lowest RCR of 939.19 mg g^{-1} day^{-1} was recorded at 28 ºC and highest RCR of 1158.19 mg g^{-1} day^{-1} at 35 ºC temperature. At eCO_2, increase in RCR was recorded with increase in temperature with 1116.18 mg g^{-1} day^{-1} as lowest RCR at 28 ºC and highest RCR of 1388.26 mg g^{-1} day^{-1} at 35 ºC. RCR was significantly lower in aCO_2 than eCO_2. The individual effect of eCO_2 and *e*Temp and the interactive effect were significant on RCR.

Table 4.52. Effect of *e*CO_2 and *e*Temp on larval relative consumption rate (RCR) of *S. litura* in Bt cotton

Temperature	First generation (mg g^{-1} day^{-1})			Second generation (mg g^{-1} day^{-1})			Third generation (mg g^{-1} day^{-1})			Mean (mg g^{-1} day^{-1})		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	928.80	1113.63	**1021.22**	939.19	1116.18	**1027.69**	948.12	1157.13	**1052.63**	938.70	1128.98	**1033.84**
29 ± 1°C	963.18	1136.55	**1049.87**	956.05	1165.35	**1060.70**	971.41	1284.13	**1127.77**	963.55	1195.34	**1079.45**
31 ± 1°C	966.28	1153.52	**1059.90**	965.73	1267.46	**1116.60**	985.68	1287.09	**1136.39**	972.56	1236.02	**1104.29**
33 ± 1°C	1008.44	1213.75	**1111.10**	1014.12	1372.25	**1193.19**	1091.50	1369.17	**1230.34**	1038.02	1318.39	**1178.21**
35 ± 1°C	1065.72	1348.09	**1206.91**	1158.19	1388.26	**1273.23**	1176.78	1402.89	**1289.84**	1133.56	1379.75	**1256.66**
Mean	**986.48**	**1193.11**		**1006.66**	**1261.90**		**1034.70**	**1300.08**		**1009.28**	**1251.70**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	534.54*	5.61	15.63	341.45*	6.55	18.26	506.34*	5.24	14.59	1425.87*	3.25	9.06
Temperature (°C)	6.41*	8.87	24.71	9.94*	10.36	28.86	21.66*	8.28	23.06	35.33*	5.14	14.33
Interaction (CO_2 + Temp(°C))	3.76*	12.54	34.94	3.68*	14.65	40.82	8.57*	11.71	32.62	13.08*	7.28	20.27
CV	5.78 %			6.83 %			5.47 %			3.38 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

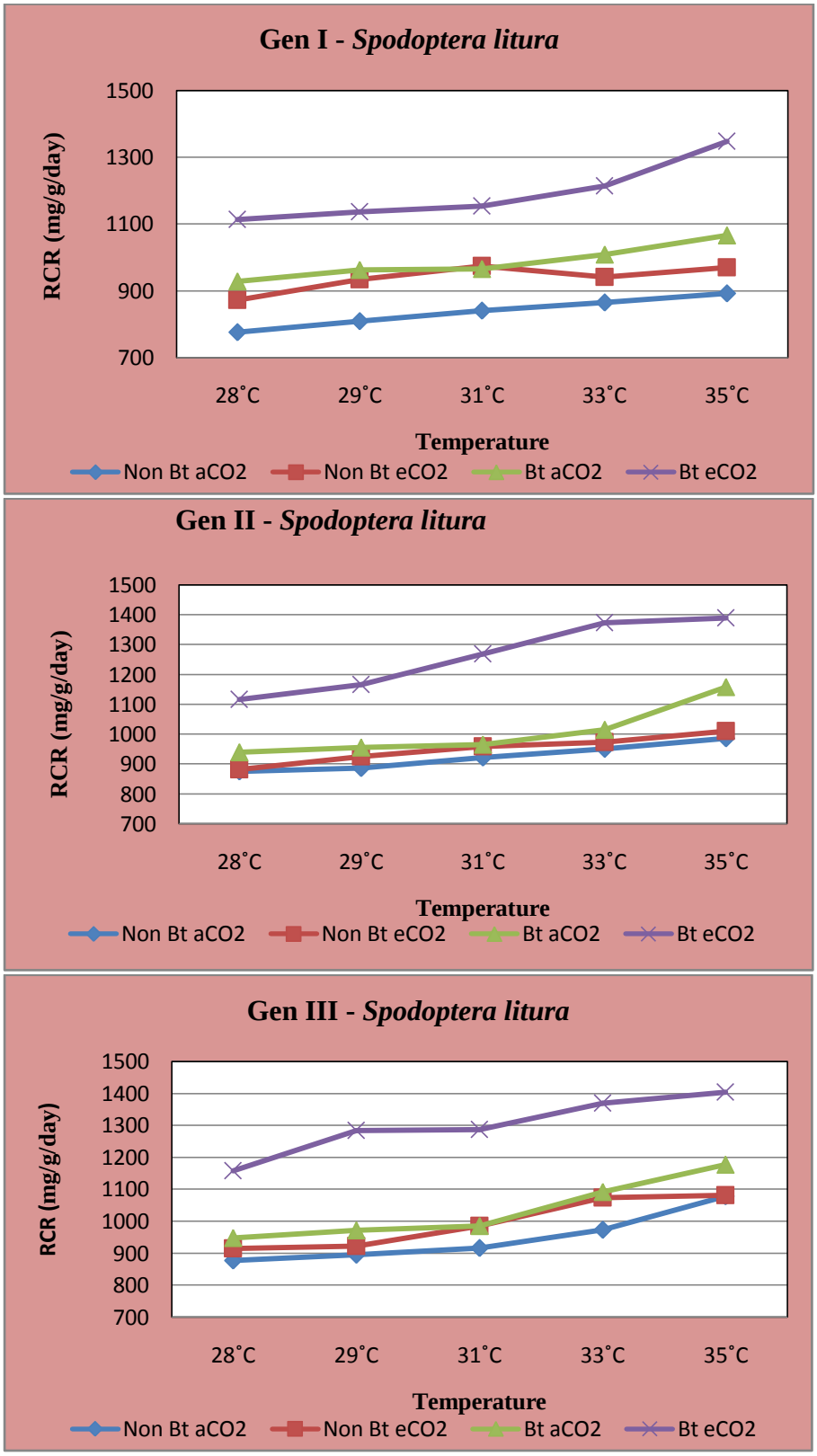


Fig. 4.38. Effect of eCO_2 and *e*Temp on larval relative consumption rate (RCR) of *S. litura* on non-Bt and Bt cotton in first, second and third generation

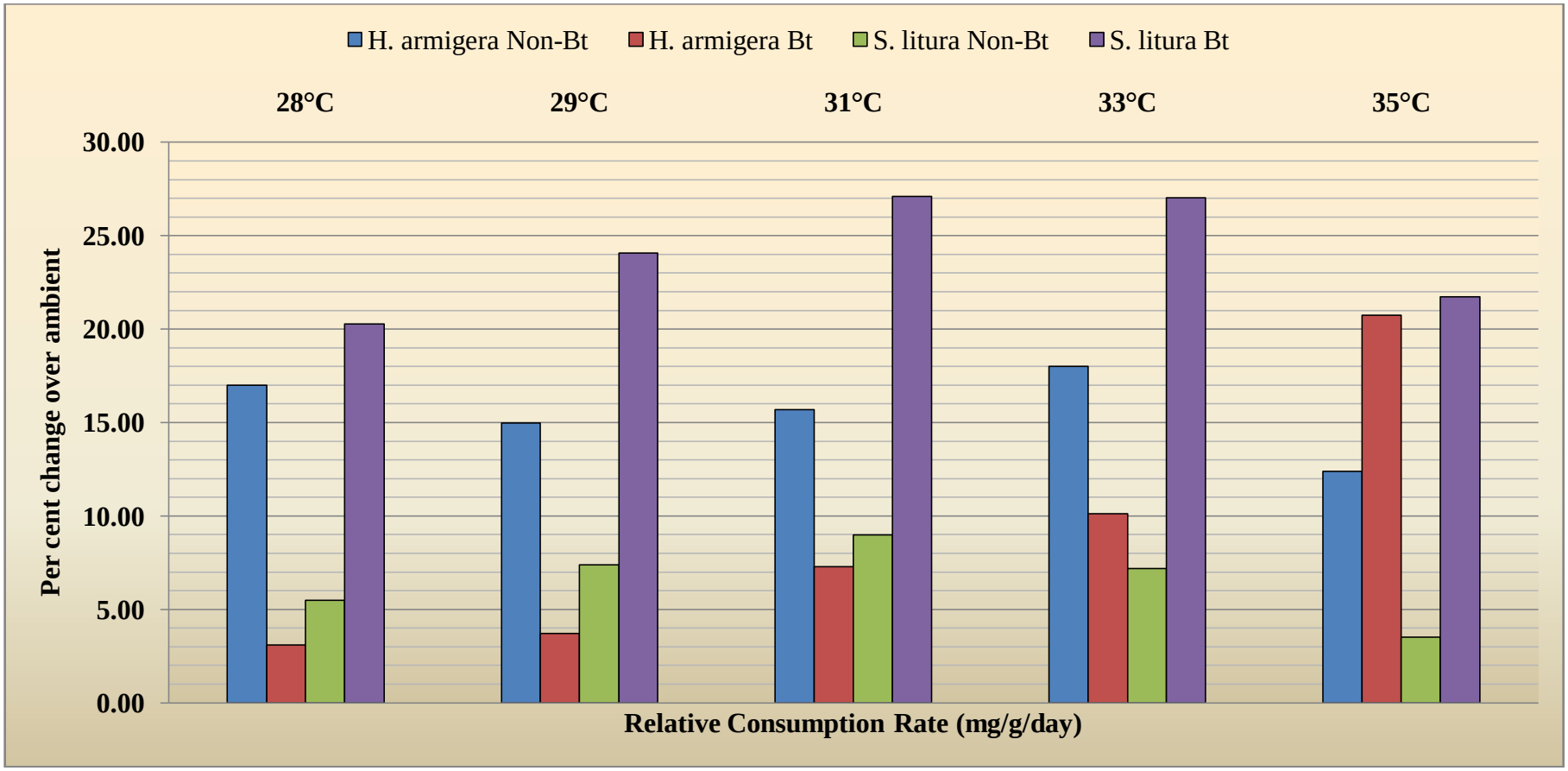


Fig. 4.39. Effect of *e*CO_2 and *e*Temp on mean larval relative consumption rate (RCR) of *H. armigera* and *S. litura* on non-Bt and Bt cotton

Third generation: In Bt cotton, increase in RCR was recorded with increase in temperature with 948.12 mg g^{-1} day^{-1} as lowest at 28 ºC and highest 1176.78 mg g^{-1} day^{-1} at temperature 35 ºC. At *e*CO_2, RCR ranged between 1157.13 and 1402.89 mg g^{-1} day^{-1} with lowest and highest RCR at temperatures 28 ºC and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect were significant on RCR.

Mean of generations: The mean of three generations also indicated that the RCR by larvae has increased significantly (938.70, 963.55, 972.56, 1038.02 and 1133.56 mg g^{-1} day^{-1}, respectively) with increase in temperature (28-35 ºC) corresponding to a increase of 2.65, 3.61, 10.58 and 20.76 %. Similarly, under *e*CO_2, the RCR increased significantly (1128.98, 1195.34, 1236.02, 1318.39 and 1379.75 mg g^{-1} day^{-1}) with increase in temperatures (28-35 ºC, respectively) which has corresponded to a increase of 5.88, 9.48, 16.78 and 22.21 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on RCR. And RCR was significantly lower in *a*CO_2 than *e*CO_2. Further, in Bt cotton the RCR under *e*CO_2 was higher (20.27, 24.06, 27.09, 27.01 and 21.72 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The data obtained suggests that, RCR of *S. litura* in Bt cotton increased with increase in CO_2 (by 20 %) and temperature and ultimately increased with *e*CO_2 + *e*Temp by 47 %. Srinivasa Rao *et al.* (2009) reported that RCR of larvae increased (37.12 @ aCO2 to 48.27 mg mg^{-1} day^{-1} @ *e*CO2) in *S. litura*. Similar increase of RCR (19 to 24 %) of *S. litura* was reported on peanut foliage at *e*CO_2 over ambient by Srinivasa Rao *et al*. (2012).In the study, with advancement of every generation, RCR increased by some extent (929, 939 and 948 mg g^{-1} day^{-1}; 1114, 1116 and 1157 mg g^{-1} day^{-1}; and 1348, 1388 and 1403 mg g^{-1} day^{-1}, under the test conditions, *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively). The study has a close proximity with that of Wu *et al*. (2009) who reported that RCR was significantly lower in the first generation with 421.3 mg mg^{-1} $day^{-1}$of *S. exigua* on Bt cotton compared with non-Bt cotton. RCR did not significantly vary in the second (430.7) and third (433.1 mg mg^{-1} day^{-1}) generations on Bt over non-Bt cotton.

4.2.3 Efficiency of Conversion of Ingested Food (ECI)

Efficiency of conversion of ingested food (ECI) indicates efficiency of larva in converting the ingested food into biomass. The results were presented in the Tables 4.53 - 4.56 and Fig. 4.40 - 4.41. With increase in CO_2 and test temperatures, ECI increased over the ambient. Comparative performance of both the test insects was presented in Fig. 4.42.

4.2.3.1 Effect on ECI of *H. armigera* in non-Bt cotton

The data pertaining to ECI of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.53 and Fig. 4.40.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on ECI. At *a*CO_2, it ranged between 17.67 and 12.91 % with highest and lowest ECI at temperatures 28 ºC and 35 ºC, respectively. At *e*CO_2, decrease in ECI was recorded with increase in temperature with highest and lowest ECI *viz.*, 17.43 and 12.65 % at temperatures 28 ºC and 35 ºC, respectively. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on ECI.

Second generation: At *a*CO_2, ECI varied from 18.22 to 13.27 % with highest ECI at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, decrease in ECI was recorded from 18.17 to 13.19 % with corresponding increase in temperature from 28 ºC to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect were significant on ECI.

Third generation: At *a*CO_2, ECI varied from 19.83 to 13.74 % with highest and lowest ECI at temperatures 28 ºC and 35 ºC, respectively. At *e*CO_2, decrease in ECI was recorded with increase in temperature with highest ECI of 18.73 at temperature 28 ºC and lowest ECI of 14.30 % at 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect were significant on ECI.

Mean of generations: The mean of three generations on non-Bt cotton also indicated that at *a*CO_2 with increase in temperature (28-35 ºC) the ECI has decreased significantly (18.57, 16.82, 15.31, 14.23 and 13.31 %, respectively) corresponding to 9.44, 17.57, 23.38 and 28.36 %. Similarly, under *e*CO_2 also the ECI decreased significantly (18.11, 16.69, 14.96, 14.24 and 13.38 %) with increase in temperatures (28-35 °C) which has corresponded to a decrease of 7.82, 17.38, 21.37 and 26.12 %. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on ECI. And among *a*CO_2 and *e*CO_2, ECI was higher in *a*CO_2. Further, the ECI under *e*CO_2 was lower (2.49, 0.75, 2.26, 0.07 and 0.55 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It was observed that, ECI of *H. armigera* decreased with increase in CO_2 (by 2 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 29 %. With the advancement of every generation, ECI increased by 17.67, 18.22 and 19.83 %;

17.43, 18.17 and 18.73 %; and 12.65, 13.19 and 14.30 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively. Similarly, Wu *et al*. (2006) noticed that ECI of *H. armigera* on spring wheat reduced from *a*CO_2 (19.23, 17.87, 17.42 % at F1, F2, F3 generations) to *e*CO_2 (16.32, 14.48, 13.41 % respectively).

4.2.3.2 Effect on ECI of *H. armigera* in Bt cotton

The data pertaining to ECI of *H. armigera* in Bt cotton for all three generations was presented in Table 4.54 and Fig. 4.40.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on ECI. At *a*CO_2, ECI decreased with increase in temperature with 17.08 % and 12.34 % as highest and lowest ECI at corresponding temperatures 28 and 35 ºC. At *e*CO_2, decrease in ECI was recorded from 14.00 to 11.51 % with increase in corresponding temperature from 28 to 35 ºC. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on ECI. And among *a*CO_2 and *e*CO_2, ECI was higher in *a*CO_2.

Second generation: In Bt cotton, ECI varied *viz.*, 17.47 to 13.01 % with highest and lowest ECI at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in ECI was recorded from 15.43 to 12.26 % with corresponding increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect were significant on ECI.

Third generation: In Bt cotton, decrease in ECI ranging from 18.30 to 13.01 % was recorded with increase in corresponding temperature from 28 and 35 ºC. At *e*CO_2, ECI decreased with increase in temperature with 16.23 and 13.83% as highest and lowest ECI at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect were significant on ECI.

Mean of generations: The mean of three generations in Bt cotton also indicated that at *a*CO_2 with increase in temperature (28-35ºC) the ECI by larvae has decreased significantly (17.62, 16.58, 14.43, 13.29 and 12.79 %, respectively) corresponding to 5.90, 18.07, 24.58 and 27.42 %. Similarly, under *e*CO_2 also the ECI decreased significantly (15.22, 14.70, 14.44, 13.77 and 12.53 %) with increase in temperatures

Table 4.53. Effect of *e*CO_2 and *e*Temp on larval efficiency of the conversion of ingested food (ECI) of *H. armigera* in non-Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	17.67	17.43	**17.55**	18.22	18.17	**18.20**	19.83	18.73	**19.28**	18.57	18.11	**18.34**
29 ± 1°C	16.27	16.04	**16.16**	16.20	16.18	**16.19**	17.99	17.86	**17.93**	16.82	16.69	**16.76**
31 ± 1°C	14.71	14.63	**14.67**	15.12	14.63	**14.88**	16.10	15.63	**15.87**	15.31	14.96	**15.14**
33 ± 1°C	13.98	13.36	**13.67**	14.44	14.16	**14.30**	14.27	15.20	**14.74**	14.23	14.24	**14.24**
35 ± 1°C	12.91	12.65	**12.78**	13.27	13.19	**13.23**	13.74	14.30	**14.02**	13.31	13.18	**13.34**
Mean	**15.11**	**14.82**		**15.45**	**15.27**		**16.39**	**16.34**		**15.65**	**15.48**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	9.39*	0.04	0.11	7.24*	0.04	0.11	4.37*	0.04	0.12	13.55*	0.02	0.06
Temperature (°C)	1005.28*	0.06	0.17	1050.41*	0.06	0.17	688.54*	0.07	0.19	3066.45*	0.03	0.09
Interaction (CO_2 + Temp(°C))	9.41*	0.09	0.24	4.00*	0.09	0.24	2.94*	0.09	0.26	7.25*	0.05	0.13
CV	2.85 %			2.76 %			3.09 %			1.57 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

Table 4.54. Effect of *e*CO_2 and *e*Temp on larval efficiency of the conversion of ingested food (ECI) of *H. armigera* in Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	17.08	14.00	**15.54**	17.47	15.43	**16.45**	18.30	16.23	**17.27**	17.62	15.22	**16.42**
29 ± 1°C	15.77	13.59	**14.68**	16.62	14.38	**15.50**	17.34	16.13	**16.74**	16.58	14.70	**15.64**
31 ± 1°C	14.01	13.43	**13.72**	14.28	14.02	**14.15**	15.01	15.86	**15.44**	14.43	14.44	**14.44**
33 ± 1°C	12.75	12.50	**12.63**	13.12	13.46	**13.29**	13.99	15.35	**14.67**	13.89	13.77	**13.83**
35 ± 1°C	12.34	11.51	**11.93**	13.01	12.26	**12.64**	13.01	13.83	**13.42**	12.79	12.53	**12.66**
Mean	**14.39**	**13.01**		**14.90**	**13.91**		**15.53**	**15.48**		**14.94**	**14.13**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	376.72*	0.04	0.11	110.12*	0.04	0.11	102.59*	0.05	0.13	563.11*	0.02	0.06
Temperature (°C)	642.81*	0.06	0.17	443.04*	0.06	0.18	332.80*	0.07	0.20	1483.18*	0.04	0.10
Interaction (CO_2 + Temp(°C))	222.39*	0.09	0.24	170.63*	0.09	0.25	117.02*	0.10	0.28	530.62*	0.05	0.14
CV	3.18 %			3.36 %			3.89 %			1.93 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

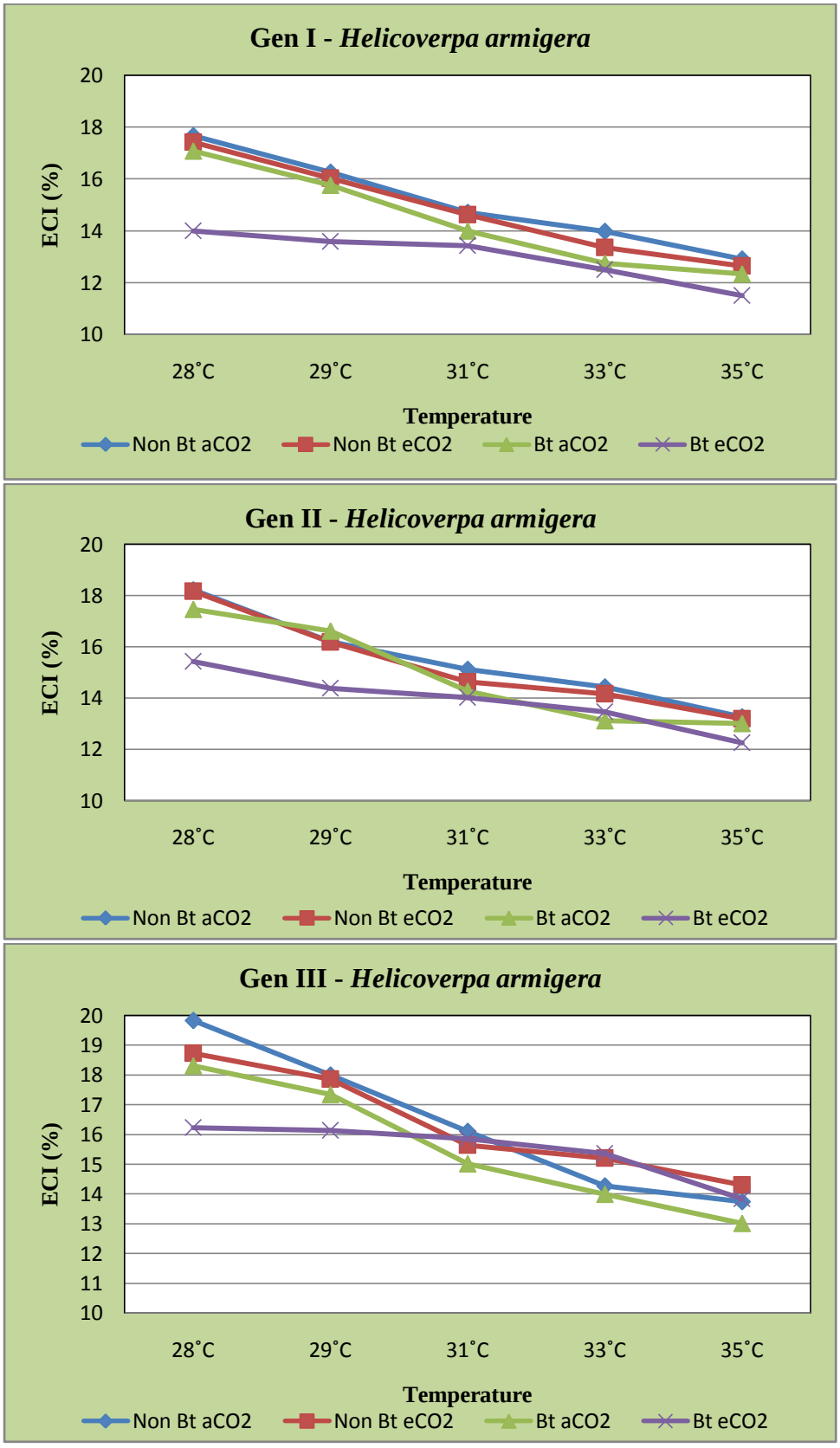


Fig. 4.40. Effect of eCO_2 and *e*Temp on larval efficiency of conversion of ingested food (ECI) of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

(28-35 °C) which has corresponded to a decrease of 3.42, 5.15, 9.53 and 17.65 %. The interaction effect of *e*CO_2 and *e*Temp showed significant influence on ECI. And among *a*CO_2 and *e*CO_2, ECI was higher in *a*CO_2. Further, the ECI under *e*CO_2 was lower (13.60, 11.32, 0.02, 3.64 and 1.98 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It was observed that, ECI of *H. armigera* in Bt cotton decreased with increase in CO_2 (by 14 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 29 %. Chen *et al*. (2004) reported that ECI of *H. armigera* significantly reduced under transgenic Bt cotton, but there was no significant CO_2 or CO_2 × cultivar interaction.Luo *et al*. (2018) observed that NaCl stress (150 mmol L^{-1}) resulted in significant decreases in ECI of *H. armigera* in Bt cotton. In the present investigation, with the advancement of every generation, ECI increased by 17.08, 17.47 and 18.30 %; 14.00, 15.43 and 16.23 %; and 11.51, 12.26 and 13.83 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively.

4.2.3.3 Effect on ECI of *S. litura* in non-Bt cotton

The data pertaining to ECI of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.55 and Fig. 4.41.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on ECI. Under *a*CO_2, ECI decreased from 16.70 to 14.96 % with increase in temperatures from 28 to 35 ºC. At *e*CO_2, decrease in ECI was recorded with increase in temperature with highest ECI at 28 ºC with 15.60 % and lowest at 35 ºC with 14.73 % ECI. A significant interactive impact of *e*CO_2 and *e*Temp was recorded on ECI and was higher in *a*CO_2 than *e*CO_2.

Second generation: At *a*CO_2, ECI ranged between 17.59 and 14.75 % with highest and lowest ECI at temperatures 28 ºC and 35 ºC. At *e*CO_2, decrease in ECI was recorded from 16.21 to 15.61 % with corresponding increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp was significant on ECI whereas the interactive effect has non-significant impact on ECI and was higher in *a*CO_2 than *e*CO_2.

Third generation: At *a*CO_2, highest ECI was recorded at temperature 28 ºC with 19.59 % and lowest ECI at 35 ºC with 15.58 %. At *e*CO_2, ECI decreased with increase in temperature with 17.03 % as highest ECI at 28 ºC and 15.12 % as lowest at 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect were significant on ECI. and was higher in *a*CO_2 than *e*CO_2.

Mean of generations: The mean of three generations in non-Bt cotton also indicated that the ECI has decreased significantly (17.96, 16.40, 16.27, 15.54 and 15.10 % respectively) with increase in temperature (28-35 °C) corresponding to a decrease of 8.70, 9.39, 13.46 and 15.94 %. Similarly, under *e*CO_2, the ECI decreased significantly (16.28, 15.75, 15.43, 15.40 and 15.15 %) with increase in temperatures (28-35 °C) which has corresponded to a decrease of 3.26, 5.24, 5.41 and 6.92 %. A significant interactive impact of *e*CO_2 and *e*Temp was recorded on ECI and was higher in *a*CO_2 than *e*CO_2. Further in non-Bt cotton, the ECI under *e*CO_2 was lower (9.35, 3.94, 5.20, 0.92 and 0.38 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It was observed that, ECI of *S. litura* decreased with increase in CO_2 (by 9 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 16 %. The ECI was also observed to be higher on non-Bt cotton over Bt cotton. Srinivasa Rao *et al.* (2009) observed that ECI significantly decreased from 380 ppm *a*CO_2 (19.83 %) in 700 ppm CO_2 (15.93 %). ECI was observed to decrease by 13-25 % in *S. litura* under *e*CO_2 over *a*CO_2 on peanut foliage by Srinivasa Rao *et al*. (2012, 2014b). Karmakar and Pal (2017) tested the effect of 5 °C rise in temperature on sixth instar larvae of *S. litura* and found that ECI tends to increase from 13.21-22.19 % in castor and 12.71-19.32 % in tomato. From the present study it was noticed that with every new generation on non-Bt cotton, ECI increased by 16.70, 17.59 and 19.59 %; 15.60, 16.21 and 17.96 %; and 14.73, 15.61 and 15.12 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively. Wu *et al*. (2009) observed that ECI of *S. exigua* is higher (19.23, 20.28 and 19.32 % in three successive generations) in non-Bt over Bt cotton.

4.2.3.4 Effect on ECI of *S. litura* in Bt cotton

The data pertaining to ECI of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.56 and Fig. 4.41.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant effect on ECI. Under *a*CO_2, it decreased from 14.32 to 12.48 % with increase in corresponding

Table 4.55. Effect of *e*CO_2 and *e*Temp on larval efficiency of the conversion of ingested food (ECI) of *S. litura* in non-Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	16.70	15.60	**16.15**	17.59	16.21	**16.90**	19.59	17.03	**18.31**	17.96	16.28	**17.12**
29 ± 1°C	15.85	15.18	**15.52**	16.12	16.10	**16.11**	17.22	15.97	**16.60**	16.40	15.75	**16.07**
31 ± 1°C	15.83	15.11	**15.47**	15.97	15.57	**15.77**	17.02	15.60	**16.31**	16.27	15.43	**15.85**
33 ± 1°C	15.36	15.05	**15.21**	15.23	15.76	**15.50**	16.04	15.39	**15.72**	15.54	15.40	**15.47**
35 ± 1°C	14.96	14.73	**14.85**	14.75	15.61	**15.18**	15.58	15.12	**15.35**	15.10	15.15	**15.13**
Mean	**15.74**	**15.13**		**15.93**	**15.85**		**17.09**	**15.83**		**16.25**	**15.60**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	8.24*	0.07	0.18	4.61*	0.06	0.17	8.69*	0.06	0.18	9.09*	0.04	0.10
Temperature (°C)	26.81*	0.10	0.28	17.50*	0.10	0.27	24.44*	0.10	0.28	67.67*	0.06	0.16
Interaction (CO_2 + Temp(°C))	8.52*	0.14	0.40	2.14	0.14	NS	3.01*	0.14	0.39	11.94*	0.08	224.00
CV	4.72 %			4.50 %			4.68 %			2.66 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

Table 4.56. Effect of *e*CO_2 and *e*Temp on larval efficiency of the conversion of ingested food (ECI) of *S. litura* in Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	14.32	14.23	**14.28**	14.90	14.20	**14.55**	15.58	15.25	**15.42**	14.93	14.56	**14.75**
29 ± 1°C	13.90	13.70	**13.80**	13.71	13.63	**13.67**	15.65	15.02	**15.34**	14.42	14.12	**14.27**
31 ± 1°C	13.26	13.07	**13.17**	13.56	13.22	**13.39**	15.42	14.10	**14.76**	14.08	13.46	**13.77**
33 ± 1°C	12.70	12.27	**12.49**	13.27	12.63	**12.95**	13.15	13.86	**13.51**	13.04	12.92	**12.98**
35 ± 1°C	12.48	12.00	**12.24**	12.93	12.53	**12.73**	13.07	13.59	**13.33**	12.83	12.71	**12.77**
Mean	**13.34**	**13.05**		**13.67**	**13.24**		**14.57**	**14.37**		**13.86**	**13.55**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	17.02*	0.05	0.13	6.19*	0.06	0.15	10.56*	0.05	0.13	27.48*	0.03	0.09
Temperature (°C)	69.07*	0.08	0.21	33.72*	0.09	0.24	32.27*	0.07	0.20	110.52*	0.05	0.14
Interaction (CO_2 + Temp(°C))	4.06*	0.11	0.30	3.89*	0.12	0.35	12.42*	0.10	0.29	12.09*	0.07	0.19
CV	4.00 %			4.62 %			3.87 %			2.58 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

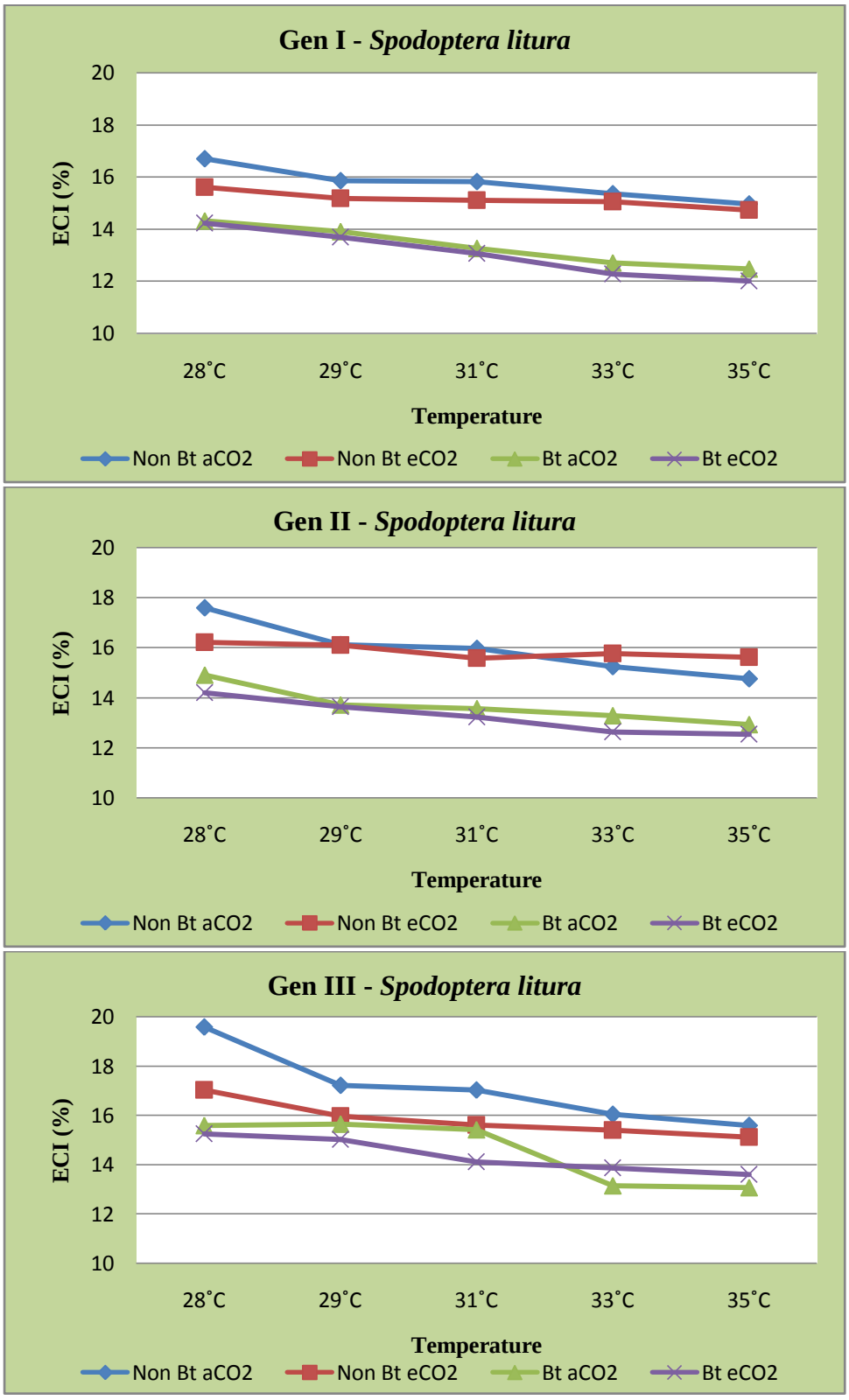


Fig. 4.41. Effect of eCO_2 and *e*Temp on larval efficiency of conversion of ingested food (ECI) of *S. litura* on non-Bt and Bt cotton in first, second and third generation

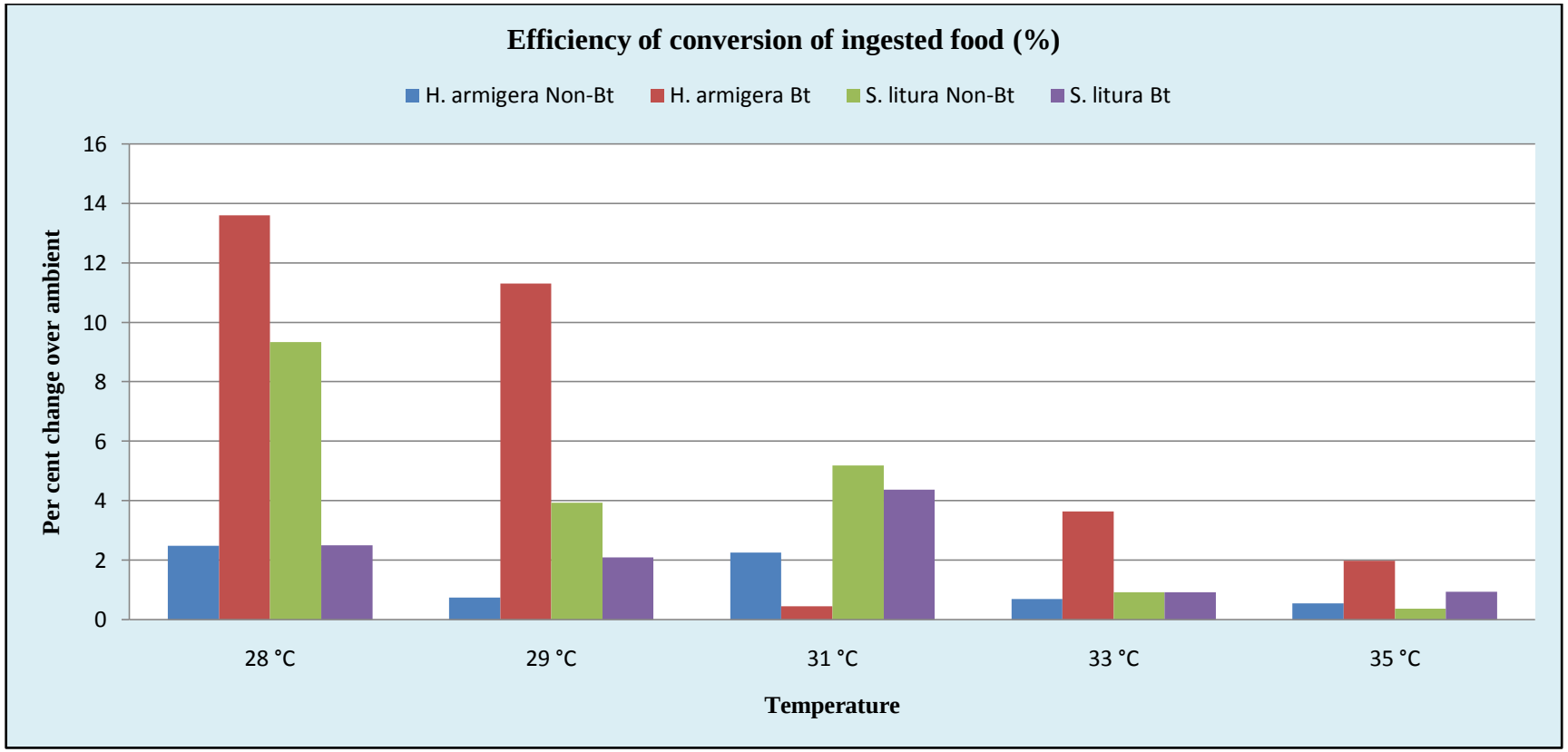


Fig. 4.42. Effect of eCO_2 and *e*Temp on mean larval efficiency of conversion of ingested food (ECI) of *H. armigera* and *S. litura* on non-Bt and Bt cotton

temperature from 28 to 35 ºC. At *e*CO_2, a decreased ECI with increase in temperature was recorded with 14.23 % as highest ECI at temperature 28 ºC and lowest ECI 12.00 % were recorded at 35 ºC. A significant interactive impact of *e*CO_2 and *e*Temp was recorded on ECI and was higher in *a*CO_2 than *e*CO_2.

Second generation: At *a*CO_2, highest and lowest ECI *viz.*, 14.90 and 12.93% was recorded respectively at temperatures 28 and 35 ºC. At *e*CO_2, ECI decreased from 14.20 to 12.53 % with highest and lowest ECI at temperatures 28 ºC and 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant influence on ECI.

Third generation: At *a*CO_2, ECI varied from 15.58 to 13.07 % with highest and lowest ECI at temperatures 28 and 35 ºC, respectively. At *e*CO_2, a decrease in ECI was recorded with increase in temperature with highest and lowest ECI of 15.25 and 13.59 % at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect has significant influence on ECI.

Mean of generations: The mean of three generations in Bt cotton, indicated that the ECI has decreased significantly (14.93, 14.42, 14.08, 13.04 and 12.83 %, respectively) with increase in temperature (28-35 ºC) corresponding to decrease 3.44, 5.71, 12.68 and 14.11 %. Similarly, under *e*CO_2 also the ECI decreased significantly (14.56, 14.12, 13.46, 12.92 and 12.71 %) with increase in temperatures (28-35ºC, respectively) which has corresponded to a decrease of 3.04, 7.53, 11.26 and 12.73 %. A significant interactive impact of *e*CO_2 and *e*Temp was recorded on ECI and was higher in *a*CO_2 than *e*CO_2. Further, the ECI of Bt cotton under *e*CO_2 was lower (2.50, 2.10, 4.38, 0.92 and 0.94 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It was observed that, ECI of *S. litura* decreased with increase in CO_2 (by 2 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 15 %. With advancement of every generation, ECI increased by 14.32, 14.90 and 15.58 %; 14.23, 14.20 and 15.25 %; and 12.00, 12.53 and 13.59 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively. Similarly, Wu *et al*. (2009) observed that ECI was significantly different among the three successive generations of *S. exigua* fed on transgenic Bt cotton ($F = 4.48$, d.f. = 2, 9, $P = 0.0447$).

4.2.4 Efficiency of Conversion of Digested Food (ECD)

A parallel parameter, ECD indicates the proportion of digested food is actually transformed into net insect biomass is denoted by efficiency of conversion of digested food (ECD). The value was lower under eCO$_2$ than that of aCO$_2$. With increase in test temperatures (upto 35 ºC) CO$_2$ (upto 550± 25 ppm) conditions, ECD of test insects gradually decreased (Tables 4.57 - 4.60 and Fig. 4.43 - 4.44). Comparative performance of both the test insects was presented in Fig. 4.45.

4.2.4.1 Effect on ECD of *H. armigera* in non-Bt cotton

The data pertaining to ECD of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.57 and Fig. 4.43.

First generation: In non-Bt cotton, eCO$_2$ and eTemp has shown significant influence on ECD. At aCO$_2$, it decreased from 23.83 to 17.66 % with increase in corresponding temperatures from 28 to 35 ºC. At eCO$_2$, decrease in ECD was recorded with increase in temperature with highest and lowest ECD as 23.35 % and 17.46 % at temperatures 28 and 35 ºC, respectively. A significant interactive effect of eCO$_2$ and eTemp was recorded on ECD.

Second generation: At aCO$_2$, ECD varied from 25.79 to 18.31 % with highest ECD at temperature 28 ºC and lowest ECD at 35 ºC. At eCO$_2$, decrease in ECD was recorded ranging from 24.51 to 17.88 % with corresponding increase in temperature from 28 to 35 ºC. The individual effect of eCO$_2$ and eTemp and the interactive effect has significant influence on ECD.

Third generation: At aCO$_2$, highest ECD was recorded at 28 ºC with 27.29 % and lowest ECD at 35 ºC with 21.59 %. At eCO$_2$, ECD decreased with increase in temperature with highest and lowest ECD at 28 and 35 ºC temperature with 26.91 % and 19.85 %, respectively. The individual effect of eCO$_2$ and eTemp and the interactive effect has significant influence on ECD.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that at aCO$_2$ with increase in temperature (28-35 ºC) the ECD has decreased significantly (25.64, 23.62, 21.52, 20.51 and 19.19 %, respectively) corresponding to 7.85, 16.07, 20.01 and 25.16 %. Similarly, under eCO$_2$ also the ECD decreased

Table 4.57. Effect of *e*CO_2 and *e*Temp on larval efficiency of the conversion of digested food (ECD) of *H. armigera* in non-Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	23.83	23.35	**23.59**	25.79	24.51	**25.15**	27.29	26.91	**27.10**	25.64	24.92	**25.28**
29 ± 1°C	21.61	21.42	**21.52**	23.43	22.43	**22.93**	25.83	24.21	**25.02**	23.62	22.69	**23.16**
31 ± 1°C	20.08	19.79	**19.94**	21.39	20.66	**21.03**	23.08	22.83	**22.96**	21.52	21.09	**21.31**
33 ± 1°C	18.94	18.18	**18.56**	20.38	18.13	**19.26**	22.20	21.81	**22.01**	20.51	19.37	**19.94**
35 ± 1°C	17.66	17.46	**17.56**	18.31	17.88	**18.10**	21.59	19.85	**20.72**	19.19	18.40	**18.79**
Mean	**20.42**	**20.04**		**21.86**	**20.72**		**24.00**	**23.12**		**22.09**	**21.30**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	28.22*	0.08	0.23	6.08*	0.07	0.21	8.45*	0.08	0.22	11.92*	0.04	0.12
Temperature (°C)	582.93*	0.13	0.37	669.37*	0.12	0.33	496.37*	0.13	0.35	1871.95*	0.07	0.19
Interaction (CO_2 + Temp(°C))	17.52*	0.19	0.52	3.15*	0.17	0.46	3.03*	0.18	0.49	19.18*	0.10	0.27
CV	4.10 %			3.58 %			3.86 %			2.13 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

significantly (24.92, 22.69, 21.09, 19.37 and 18.40 %) with increase in temperatures (28-35 ºC, respectively) which has corresponded to a decrease of 8.97, 15.37, 22.27 and 26.19 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on ECD in non-Bt cotton. And ECD was higher in *a*CO_2 than *e*CO_2. Further, the ECD under *e*CO_2 was lower (2.78, 3.97, 1.97, 5.53 and 4.12 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

Delay in development due to *e*CO_2 decreases efficiency of conversion in insect population (Masters *et al*., 1998; Williams *et al*., 1998). This study also recorded that, ECD of *H. armigera* decreased with increase in CO_2 (by 3 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 28 %. Luo *et al*. (2018) observed that NaCl stress (150 mmol L^{-1}) resulted in significant decreases in the ECD of the 5th instar *H. armigera* in Bt cotton. With advancement of every generation, ECI increased by 23.83, 25.79 and 27.29 %; 23.35, 24.51 and 26.91 %; and 17.46, 17.88 and 19.85 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively.

4.2.4.2 Effect on ECD of *H. armigera* in Bt cotton

The data pertaining to ECD of *H. armigera* in Bt cotton for all three generations was presented in Table 4.58 and Fig. 4.43.

First generation: In Bt cotton, *e*CO_2 and *e*Temp have shown significant influence on ECD. Under *a*CO_2, ECD varied from 21.55 to 16.45 % with highest and lowest ECD at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in ECD was recordedwith increase in temperature with highest ECD at 28 ºC with 20.62 % and lowest ECD at 35 ºC with 16.43 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on ECD.

Second generation: Highest ECD was recorded at temperature 28 ºC with 23.39 % and lowest ECD at 35 ºC with 18.59 %. At *e*CO_2, decrease in ECD was recorded from 21.21 to 17.87 % with increase in corresponding temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect have significant influence on ECD.

Third generation: ECD ranged between 26.52 and 18.38 % with highest and lowest ECD at temperatures 28 and 35ºC, respectively. At *e*CO_2, decrease in ECD was recorded with increase in temperature with highest and lowest ECD at temperatures 28 ºC (23.36 %) and 35 ºC (17.61 %), respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect have significant influence on ECD.

Table 4.58. Effect of *e*CO_2 and *e*Temp on larval efficiency of the conversion of digested food (ECD) of *H. armigera* in Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	21.55	20.62	**21.08**	23.39	21.21	**22.30**	26.52	23.36	**24.94**	23.82	21.73	**22.77**
29 ± 1°C	20.00	19.90	**19.95**	21.33	19.54	**20.44**	23.88	21.87	**22.88**	21.74	20.44	**21.09**
31 ± 1°C	18.88	18.33	**18.61**	19.44	19.24	**19.34**	21.13	20.68	**20.91**	19.82	19.42	**19.62**
33 ± 1°C	17.78	17.48	**17.63**	19.02	18.29	**18.66**	19.74	18.98	**19.36**	18.85	18.25	**18.55**
35 ± 1°C	16.45	16.43	**16.44**	18.59	17.87	**18.23**	18.38	17.61	**18.00**	17.81	17.30	**17.56**
Mean	**18.93**	**18.55**		**20.35**	**19.23**		**21.93**	**20.50**		**20.41**	**19.43**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	509.60*	0.06	0.18	140.37*	0.06	0.18	122.27*	0.07	0.20	770.24*	0.04	0.10
Temperature (°C)	482.08*	0.10	0.28	354.87*	0.10	0.28	254.12*	0.11	0.32	1208.34*	0.06	0.16
Interaction (CO_2 + Temp(°C))	229.59*	0.14	0.40	170.99*	0.14	0.40	123.71*	0.16	0.45	573.25*	0.08	0.22
CV	3.47 %			3.60 %			4.14 %			2.01 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

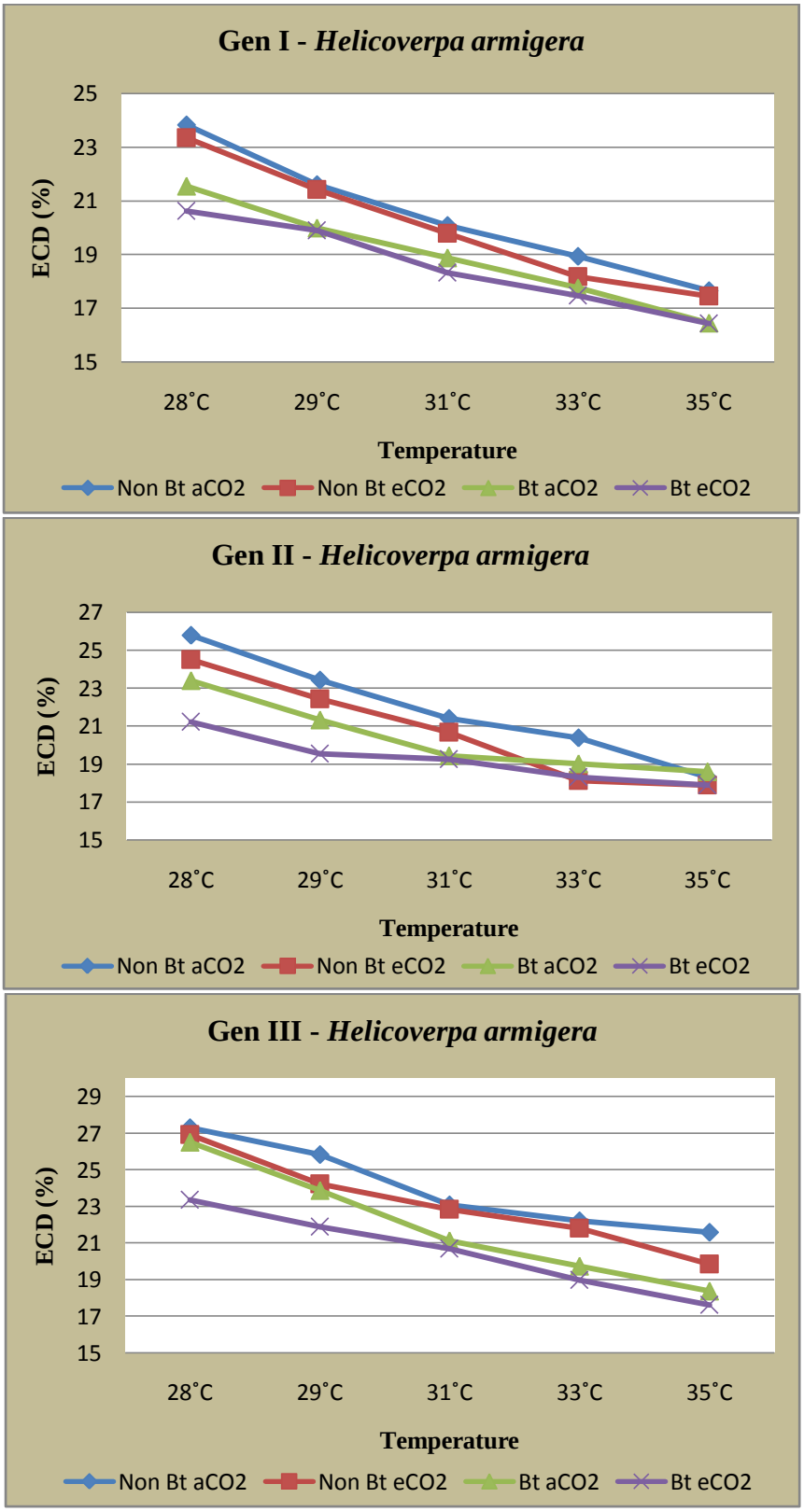


Fig. 4.43. Effect of eCO_2 and *e*Temp on larval efficiency of conversion of digested food (ECD) of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

Mean of generations: The mean of three generations in Bt cotton, indicated that with increase in temperature (28, 29, 31, 33 and 35 °C) the ECD has decreased significantly (23.82, 21.74, 19.82, 18.85 and 17.81 %, respectively) corresponding to 8.75, 16.81, 20.88 and 25.24 %. Similarly, under *e*CO_2 also the ECD decreased significantly (21.73, 20.44, 19.42, 18.25 and 17.30 %) with increase in temperatures (28-35 °C) which has corresponded to a decrease of 5.95, 10.65, 16.01 and 20.37 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on ECD. And ECD was higher in *a*CO_2 than *e*CO_2. Further, the ECD under *e*CO_2 was lower (8.77, 5.98, 2.02, 3.17 and 2.83 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

To brief this aspect, ECD of *H. armigera* in Bt cotton decreased with increase in CO_2 (by 9 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 27 %. Chen *et al*. (2004) reported that the efficiency of conversion of digested food in bollworms significantly reduced in Bt cotton, however there was no significant CO_2 or CO_2 × cotton cultivar interaction. Luo *et al*. (2018) observed that NaCl stress (150 mmol L^{-1}) resulted in significant decreases in the ECD of the 5^{th} instar *H. armigera* in Bt cotton. Further, in the present study, with advancement of every generation, ECD increased by 21.55, 23.39 and 26.52 %; 20.62, 21.21 and 23.36 %; and 16.43, 17.87 and 17.61 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively.

4.2.4.3 Effect on ECD of *S. litura* in non-Bt cotton

The data pertaining to ECD of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.59 and Fig. 4.44.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on ECD. Under *a*CO_2, ECD ranged between 24.71 to 20.40 % with highest and lowest ECD at temperatures 28 and 35 ºC, respectively. At *e*CO_2, a decrease in ECD with increase in temperature with highest and lowest ECD recorded at temperatures 28 and 35 ºC with 23.22 % and 19.35 %, respectively. A significant interactive impact of *e*CO_2 and *e*Temp was observed on ECD.

Second generation: At *a*CO_2, decrease in ECD was recorded with increase in temperature with 25.99 % ECD as highest at 28 ºC and 21.47 % ECD as lowest at 35 ºC. At *e*CO_2, ECD ranged from 24.41 to 20.10 % with highest and lowest ECD at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect have significant influence on ECD.

Third generation: In non-Bt cotton, highest ECD was recorded at temperature 28 ºC with 29.28 % and lowest ECD at 35 ºC with 23.67 %. At *e*CO_2, a decrease in ECD was recorded from 27.02 to 22.31 % with corresponding increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect have significant influence on ECD.

Mean of generations: The mean of three generations in non-Bt cotton, also indicated that the ECD has decreased significantly (26.66, 24.52, 23.46, 22.36 and 21.85 %, respectively) with increase in temperature (28, 29, 31, 33 and 35 °C) corresponding to decrease of 8.03, 11.99, 16.13 and 18.05 %. Similarly, under *e*CO_2 also the ECD decreased significantly (24.88, 23.59, 22.45, 21.90 and 20.59 %) with increase in temperatures (28-35 ºC) which has corresponded to a decrease of 5.18, 9.78, 11.99 and 17.27 %. A significant interactive impact of *e*CO_2 and *e*Temp was observed on ECD in non-Bt cotton. And among *a*CO_2 and *e*CO_2, ECD was higher at *a*CO_2. Further, the ECD under *e*CO_2 was lower (6.66, 3.78, 4.32, 2.06 and 5.77 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

It was observed that, ECD of *S. litura* decreased with increase in CO_2 (by 7 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 23 %. Srinivasa Rao *et al.* (2009) reported that ECD of *S. litura* in castor significantly decreased from 34.09 % (380 ppm *a*CO_2) to 24.49 % (700 ppm eCO_2). Elevated CO_2 caused decrease in ECD (19-35 %) of *S. litura* on peanut foliage (Srinivasa Rao *et al*., 2012, 2014b). Karmakar and Pal (2017) tested the effect of 5 °C rise in temperature on sixth instar larvae of *S. litura* and found that ECD increased from 25.11-46.56 % in castor and 20.65-38 % in tomato.

With advancement of every generation, ECD increased by 24.71, 25.99 and 29.28 %; 23.22, 24.41 and 27.02 %; and 19.35, 20.10 and 22.31 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively. Likewise, Srinivasa Rao *et al*. (2013) reported that ECD (23-34 %) decreased with *e*CO_2 in all four generations over *a*CO_2 conditions in *A. janata* larvae on castor foliage.

4.2.4.4 Effect on ECD of *S. litura* in Bt cotton

The data pertaining to ECD of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.60 and Fig. 4.44.

First generation: In Bt cotton, *e*CO_2 and *e*Temp have shown significant influence on ECD. Under *a*CO_2, ECD decreased from 21.62 to 18.37 % with increase in corresponding temperature from 28 to 35 ºC. At *e*CO_2, ECD ranged between 20.93 and 17.41 % with highest and lowest ECD at temperatures 28 and 35 ºC, respectively. A non-significant interactive impact of *e*CO_2 and *e*Temp was observed on ECD.

Second generation: ECD decreased from 24.88 to 20.95 % with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, highest and lowest ECD were recorded at temperatures 28 and 35 ºC, respectively with 21.80 and 18.48 %. The individual effect of *e*CO_2 and *e*Temp has significant influence on ECD whereas interactive effect has shown non-significant influence on ECD.

Third generation: A decrease in ECD was recorded from 26.79 to 21.94 % with corresponding increase in temperature from 28 to 35 ºC. At *e*CO_2, ECD ranged between 24.73 to 20.32 % with highest and lowest ECD at temperatures 28 and 35 ºC, respectively. A significant interactive impact of *e*CO_2 and *e*Temp was observed on ECD.

Mean of generations: The mean of three generations in Bt cotton also indicated that the ECD by larvae has decreased significantly (24.43, 23.34, 22.41, 21.69 and 20.42 %, respectively) with increase in temperature (28-35 ºC) corresponding to a decrease of 4.46, 8.25, 11.23 and 16.41 %. Similarly, under *e*CO_2, the ECD decreased significantly (22.49, 21.42, 20.94, 20.00 and 18.74 %) with increase in temperature (28-35 ºC, respectively) which has corresponded to a decrease of 4.73, 6.86, 11.04 and 16.68 %. A significant interactive impact of *e*CO_2 and *e*Temp was observed on ECD. Among *a*CO_2 and *e*CO_2, ECD was higher at *a*CO_2. Further, the ECD under *e*CO_2 was lower (7.95, 8.21, 6.56, 7.76 and 8.24 %, at 28-35 °C, respectively) compared to that of *a*CO_2.

Table 4.59. Effect of *e*CO_2 and *e*Temp on larval efficiency of the conversion of digested food (ECD) of *S. litura* in non-Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	24.71	23.22	**23.97**	25.99	24.41	**25.20**	29.28	27.02	**28.15**	26.66	24.88	**25.77**
29 ± 1°C	22.51	22.16	**22.34**	23.39	22.94	**23.17**	27.66	25.68	**26.67**	24.52	23.59	**24.06**
31 ± 1°C	21.93	20.41	**21.17**	22.73	22.51	**22.62**	25.73	24.43	**25.08**	23.46	22.45	**22.96**
33 ± 1°C	21.13	19.79	**20.46**	22.08	21.57	**21.83**	23.87	24.34	**24.11**	22.36	21.90	**22.13**
35 ± 1°C	20.40	19.35	**19.88**	21.47	20.10	**20.79**	23.67	22.31	**22.99**	21.85	20.59	**21.22**
Mean	**22.14**	**20.99**		**23.13**	**22.31**		**26.04**	**24.76**		**23.77**	**22.68**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	8.43*	0.11	0.31	6.51*	0.10	0.29	5.04*	0.10	0.28	13.25*	0.06	0.16
Temperature (°C)	29.75*	0.17	0.48	13.90*	0.16	0.45	20.75*	0.16	0.44	65.52*	0.09	0.26
Interaction (CO_2 + Temp(°C))	14.17*	0.25	0.68	3.41*	0.23	0.64	2.56*	0.22	0.62	17.63*	0.13	0.36
CV	5.39 %			5.10 %			4.93 %			2.88 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

Table 4.60. Effect of *e*CO_2 and *e*Temp on larval efficiency of the conversion of digested food (ECD) of *S. litura* in Bt cotton

Temperature	First generation (%)			Second generation (%)			Third generation (%)			Mean (%)		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	21.62	20.93	**21.28**	24.88	21.80	**23.34**	26.79	24.73	**25.76**	24.43	22.49	**23.46**
29 ± 1°C	20.81	20.25	**20.53**	23.29	20.52	**21.91**	25.92	23.50	**24.71**	23.34	21.42	**22.38**
31 ± 1°C	20.44	19.77	**20.11**	22.52	20.16	**21.34**	24.28	22.90	**23.59**	22.41	20.94	**21.68**
33 ± 1°C	19.66	18.55	**19.11**	21.88	19.79	**20.84**	23.52	21.67	**22.60**	21.69	20.00	**20.85**
35 ± 1°C	18.37	17.41	**17.89**	20.95	18.48	**19.72**	21.94	20.32	**21.13**	20.42	18.74	**19.58**
Mean	**20.18**	**19.38**		**22.70**	**20.15**		**24.49**	**22.62**		**22.46**	**20.72**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	47.67*	0.08	0.23	14.53*	0.08	0.23	20.30*	0.08	0.21	68.14*	0.05	0.14
Temperature (°C)	59.26*	0.13	0.36	27.92*	0.13	0.37	12.12*	0.12	0.33	77.93*	0.08	0.22
Interaction (CO_2 + Temp(°C))	0.79	0.18	NS	2.10	0.19	NS	10.73*	0.17	0.47	13.08*	7.28	20.27
CV	4.57 %			4.65 %			4.27 %			2.77 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

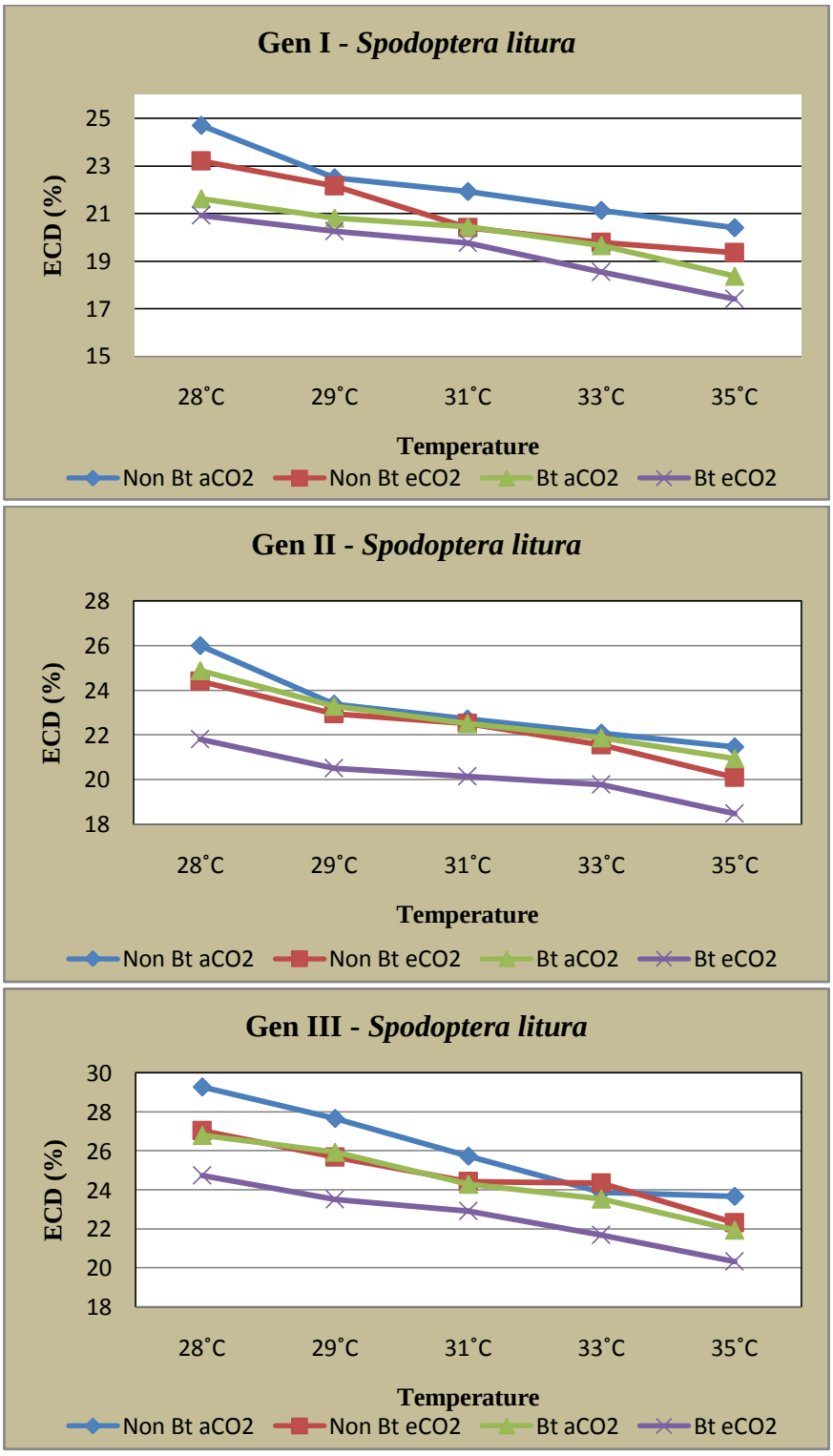


Fig. 4.44. Effect of eCO_2 and *e*Temp on larval efficiency of conversion of digested food (ECD) of *S. litura* in non-Bt and Bt cotton on first, second and third generation

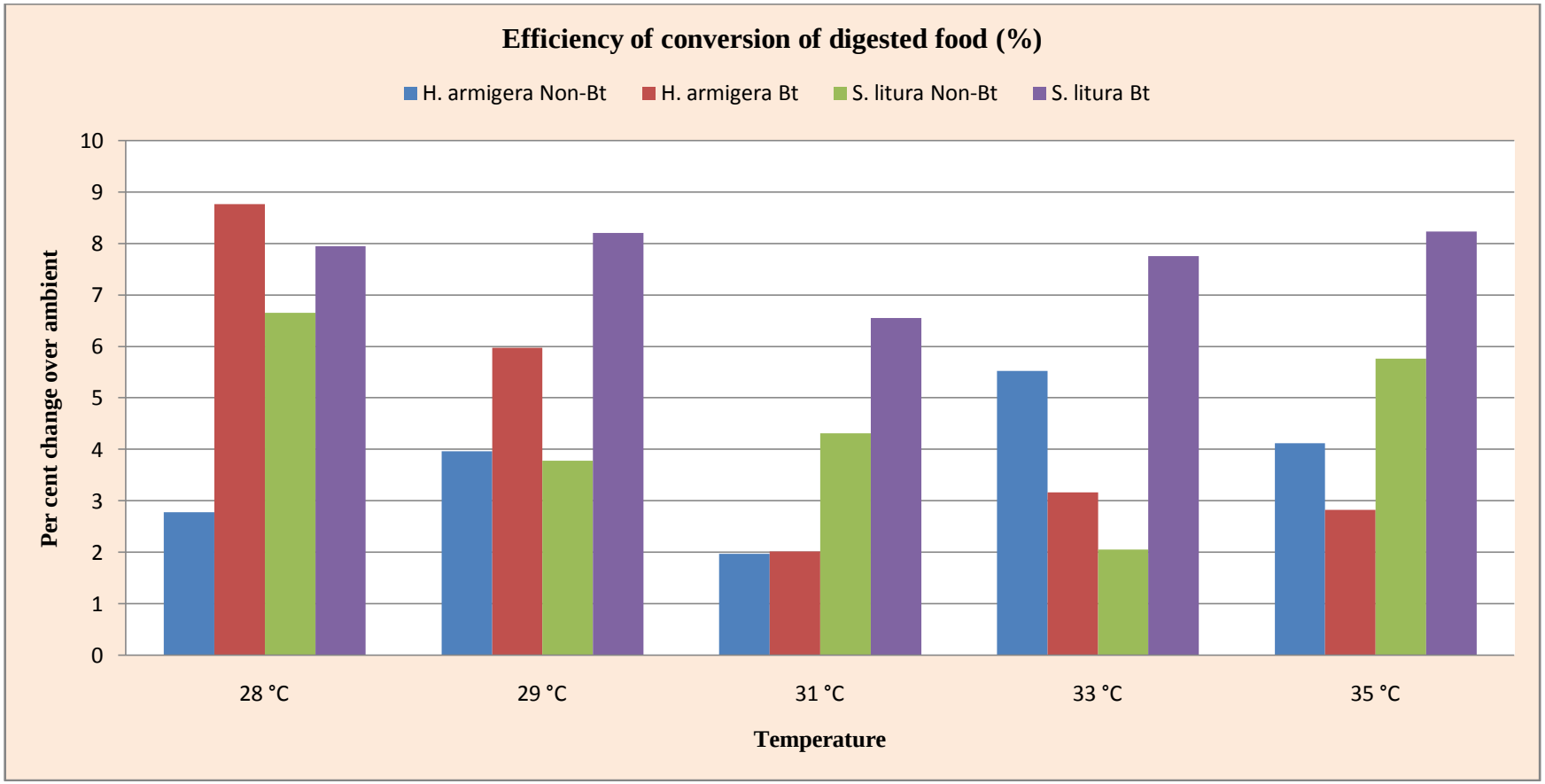


Fig. 4.45. Effect of eCO_2 and *e*Temp on mean larval efficiency of conversion of digested food (ECD) of *H. armigera* and *S. litura* on non-Bt and Bt cotton

It was observed that, ECD of *S. litura* in Bt cotton decreased with increase in CO_2 (by 8 %) and temperature and ultimately decreased with *e*CO_2 + *e*Temp by 23 %. With advancement of every generation, ECD increased by 21.62, 24.88 and 24.73 %; 20.93, 21.80 and 26.79 %; and 17.41, 18.48 and 20.32 % under *a*CO_2+*a*Temp, *e*CO_2 and *e*CO_2+*e*Temp respectively. The capacity to convert digested food to biomass may increase with every new generation, under climate change also.

4.2.5 Relative Growth Rate (RGR)

The relative growth rate (RGR) of test insect indicates the growth gained by larva per day. RGR under *e*CO_2 was lower than that of *a*CO_2.With increase in test temperatures (upto 35 ºC), and CO_2, RGR decreased (Tables 4.61 - 4.64 and Fig 4.46 - 4.47). Comparative performance of both the test insects was presented in Fig. 4.48.

4.2.5.1 Effect on RGR of *H. armigera* in non-Bt cotton

The data pertaining to RGR of *H. armigera* in non-Bt cotton for all three generations was presented in Table 4.61 and Fig. 4.46.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on RGR. Under *a*CO_2, RGR decreased in the range of 164.22 to 126.48 mg g^{-1} day^{-1} with increase in corresponding temperature from 28 to 35 ºC. At *e*CO_2, decrease in RGR was recorded from 137.92 to 110.69 mg g^{-1} day^{-1} with increase in temperature from 28 to 35 ºC. The interactive effect of *e*CO_2 and *e*Temp was significant on ECD.

Second generation: RGR ranged between 170.70 and 135.40 mg g^{-1} day^{-1} with highest and lowest RGR at temperatures 28 and 35 ºC, respectively. At *e*CO_2, highest RGR was recorded at temperature 28 ºC with 145.79 mg g^{-1} day^{-1} and lowest at 35 ºC with 123.88 mg $g^{-1}day^{-1}$. The individual effect of *e*CO_2 and *e*Temp and the interactive effect was significant on RGR.

Third generation: A decrease in RGR with increase in temperature was recorded with highest and lowest RGR at temperatures 28 ºC (178.90 mg g^{-1} day^{-1}) and 35 ºC (145.80 mg g^{-1} day^{-1}), respectively. At *e*CO_2, RGR decreased with increase in temperature with highest and lowest RGR as 154.92 and 128.68 mg g^{-1} day^{-1} respectively at temperatures 28 ºC and 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect was significant on RGR.

Mean of generations: The mean of three generations in non-Bt cotton also indicated that at *a*CO_2 with increase in temperature (28-35 ºC) the RGR has decreased significantly (171.27, 158.05, 147.88, 139.18 and 135.89 mg g^{-1} day^{-1}, respectively) corresponding to 7.72, 13.66, 18.74 and 20.66 %. Similarly, under *e*CO_2 also the RGR decreased significantly (146.21, 137.23, 129.60, 122.17 and 121.08 mg g^{-1} day^{-1}) with increase in temperatures (28-35 °C) which has corresponded to a decrease of 6.14, 1136, 16.44 and 17.19 %. The interactive effect of *e*CO_2 and *e*Temp was significant on ECD. And among *a*CO_2 and *e*CO_2, ECD was higher at *a*CO_2. Further, the RGR under *e*CO_2 was lower (14.63, 13.17, 12.36, 12.22 and 10.90 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The present findings suggest that mean RGR of *H. armigera* decreased with increase in CO_2 (by 15 %) and temperature and also decreased with *e*CO_2 + *e*Temp by 29 %. Likewise, Wu *et al*. (2006) recorded that RGR of *H. armigera* decreased with increase in CO_2 from 17.4 – 19.2 (*a*CO_2) to 13.41-16.32 mg g^{-1} day^{-1} (*e*CO_2) in spring wheat. Dalal and Arora (2016) reported that the relative growth rate (RGR) of *H. armigera* increased from 0.139 mg g^{-1} day^{-1} at 25:10 °C to 0.174 mg g^{-1} day^{-1}at 30:10 °C. From the data obtained, RGR increased with every next generation and was highest in the third generation (164, 171 and 179 mg g^{-1} day^{-1}; 138, 146 and 155 mg g^{-1} day^{-1}; and 111, 124 and 129 mg g^{-1} day^{-1} in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp respectively).

4.2.5.2 Effect on RGR of *H. armigera* in Bt cotton

The data pertaining to RGR of *H. armigera* in Bt cotton for all three generations was presented in Table 4.62 and Fig. 4.46.

First generation: In Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on RGR. Under *a*CO_2, RGR varied from 168.44 to 138.28 mg g^{-1} $day^{-1}$with highest and lowest RGR at temperatures 28 and 35 ºC. At *e*CO_2, decrease in RGR was recorded with increase in temperature with highest and lowest RGR at temperatures 28 and 35 ºC with 141.64 and 127.49 mg g^{-1} day^{-1}, respectively. The interactive effect of *e*CO_2 and *e*Temp was significant on ECD.

Table 4.61. Effect of *e*CO_2 and *e*Temp on larval relative growth rate (RGR) of *H. armigera* in non-Bt cotton

Temperature	First generation (mg g^{-1} day^{-1})			Second generation (mg g^{-1} day^{-1})			Third generation (mg g^{-1} day^{-1})			Mean (mg g^{-1} day^{-1})		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	164.22	137.92	**151.07**	170.70	145.79	**158.25**	178.90	154.92	**166.91**	171.27	146.21	**158.74**
29 ± 1°C	149.71	128.84	**139.28**	157.73	137.37	**147.55**	166.72	145.48	**156.10**	158.05	137.23	**147.64**
31 ± 1°C	140.02	120.79	**130.41**	147.03	129.50	**138.27**	156.60	138.51	**147.56**	147.88	129.6	**138.74**
33 ± 1°C	130.27	113.32	**121.80**	139.19	121.89	**130.54**	148.07	131.31	**139.69**	139.18	122.17	**130.68**
35 ± 1°C	126.48	110.69	**118.59**	135.40	123.88	**129.64**	145.80	128.68	**137.24**	135.89	121.08	**128.49**
Mean	**142.14**	**122.31**		**150.01**	**131.69**		**159.22**	**139.78**		**150.46**	**131.26**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	981.76*	0.45	1.25	821.74*	0.45	1.26	1037.33*	0.43	1.19	2544.65*	0.269	0.75
Temperature (°C)	351.02*	0.71	1.97	286.84*	0.72	1.99	327.74*	0.68	1.88	865.79*	0.425	1.185
Interaction (CO_2 + Temp(°C))	8.46*	1.00	2.79	11.68*	1.01	2.82	5.25*	0.95	2.66	21.38*	0.602	1.676
CV	3.52 %			3.59 %			3.42 %			2.82 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

Table 4.62. Effect of *e*CO_2 and *e*Temp on larval relative growth rate (RGR) of *H. armigera* in Bt cotton

Temperature	First generation (mg g^{-1} day^{-1})			Second generation (mg g^{-1} day^{-1})			Third generation (mg g^{-1} day^{-1})			Mean (mg g^{-1} day^{-1})		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	168.44	141.64	**155.04**	177.14	150.77	**163.96**	184.58	159.36	**171.97**	176.72	150.59	**163.66**
29 ± 1°C	152.48	137.06	**144.77**	161.18	145.26	**153.22**	170.04	153.96	**162.00**	161.23	145.43	**153.33**
31 ± 1°C	151.12	133.88	**142.50**	157.85	141.16	**149.51**	165.71	150.44	**158.08**	158.23	141.83	**150.03**
33 ± 1°C	140.76	130.50	**135.63**	149.99	138.29	**144.14**	157.03	147.14	**152.09**	149.26	138.64	**143.95**
35 ± 1°C	138.28	127.49	**132.89**	146.15	134.88	**140.52**	156.17	143.83	**150.00**	146.87	135.4	**141.13**
Mean	**150.22**	**134.11**		**158.46**	**142.07**		**166.71**	**150.95**		**158.46**	**142.38**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	1109.13*	0.34	0.95	936.56*	0.38	1.06	824.33*	0.39	1.08	3126.03*	0.203	0.567
Temperature (°C)	258.02*	0.54	1.51	229.67*	0.60	1.67	203.54*	0.61	1.71	753.78*	0.322	0.896
Interaction (CO_2 + Temp(°C))	38.18*	0.76	2.13	25.80*	0.85	2.36	22.57*	0.87	2.42	92.01*	0.455	1.267
CV	2.51 %			2.82 %			2.92 %			1.51 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

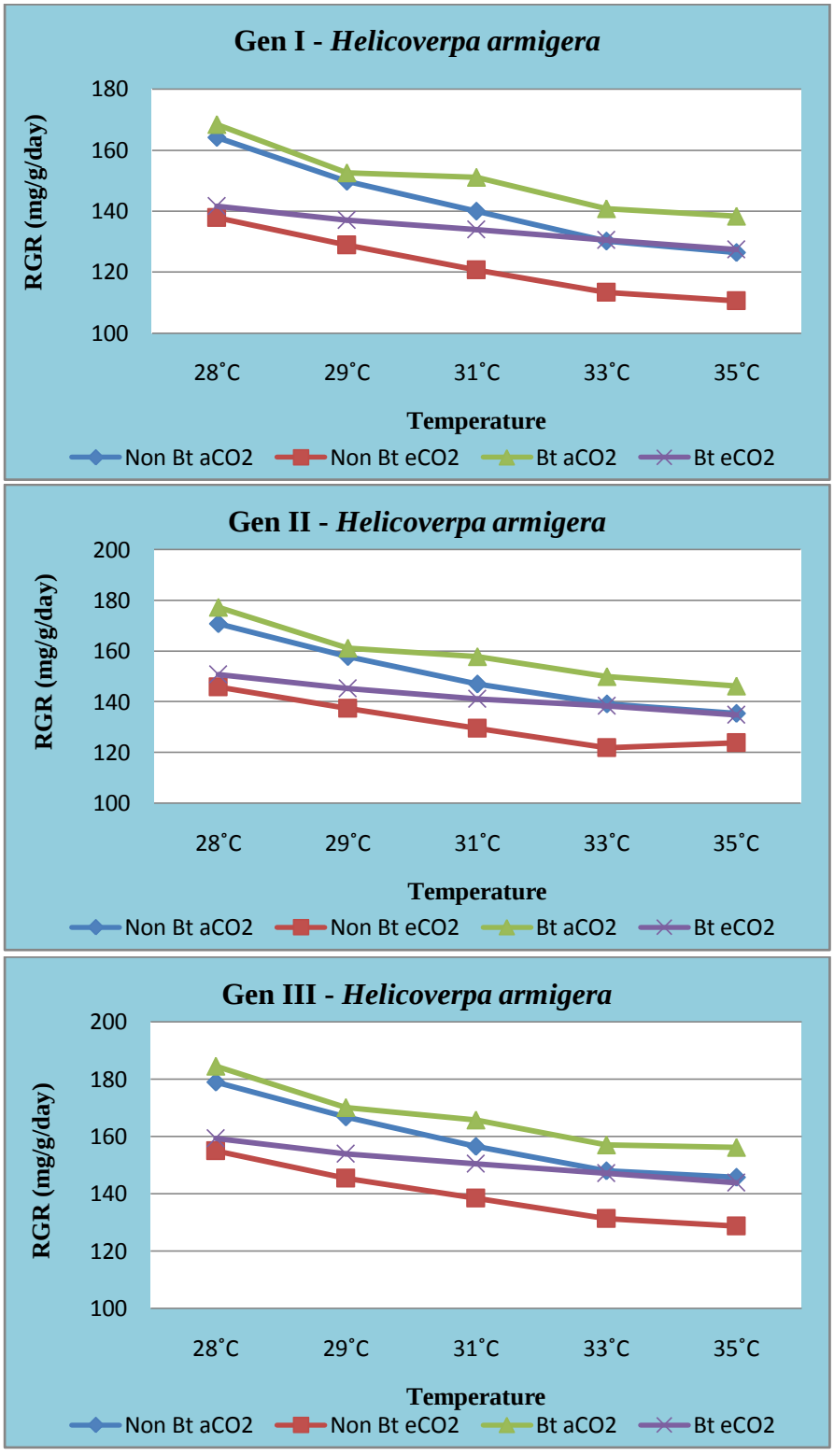


Fig. 4.46. Effect of *e*CO_2 and *e*Temp on larval relative growth rate (RGR) of *H. armigera* on non-Bt and Bt cotton in first, second and third generation

Second generation: RGR ranged from 177.14 to 146.15 mg g^{-1} $day^{-1}$with highest RGR at temperature 28 ºC and lowest at 35 ºC. At *e*CO_2, decrease in RGR was recorded from 150.77 to 134.88 mg g^{-1} day^{-1} with increase in corresponding temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect was significant on RGR.

Third generation: Highest RGR was recorded at temperature 28 ºC with 184.58 mg g^{-1} day^{-1} and lowest at 35 ºC with 156.17 mg g^{-1} day^{-1}. At *e*CO_2, decrease in RGR was recorded with increase in temperature with highest and lowest RGR at temperatures 28 and 35 ºC, respectively with 159.36 and 143.83 mg g^{-1} day^{-1}. The individual effect of *e*CO_2 and *e*Temp and the interactive effect was significant on RGR.

Mean of generations: The mean of three generations in Bt cotton also indicated that with increase in temperature (upto 35 ºC) the RGR has decreased significantly (176.72, 161.23, 158.23, 149.26 and 146.87 mg g^{-1} day^{-1}, respectively) corresponding to 8.76, 10.46, 15.54 and 16.89 %. Similarly, under *e*CO_2 also the RGR decreased significantly (150.59, 145.43, 141.83, 138.64 and 135.40 mg g^{-1} day^{-1}) with increase in temperatures (upto 35 ºC) which has corresponded to a decrease of 3.43, 5.82, 7.93 and 10.09 %. The interactive effect of *e*CO_2 and *e*Temp was significant on ECD. And among *a*CO_2 and *e*CO_2, ECD was higher at *a*CO_2. Further, the RGR under *e*CO_2 was lower (14.79, 9.80, 10.36, 7.11 and 7.81 %, at 28, 29, 31, 33 and 35 °C, respectively) compared to that of *a*CO_2.

The present findings suggest that mean RGR of *H. armigera* in Bt cotton decreased with increase in CO_2 (by 15 %) and temperature and also decreased with *e*CO_2 + *e*Temp by 23 %. Whittaker (1999) showed that Bt cotton and *e*CO_2 slows the development of bollworms and consequently reduce RGR and MRGR. Chen *et al*. (2004) also reported decreased relative growth rate (RGR), and decreased mean relative growth rate in Bt cotton under *e*CO_2 in *H. armigera*. Luo *et al*. (2018) observed that NaCl stress (150 mmol L^{-1}) resulted in significant decreases in the RGR and MRGR of the *H. armigera* in Bt cotton. RGR increased with every next generation and was highest in the third generation (168, 177 and 185 mg g^{-1} day^{-1}; 142, 151 and 159 mg g^{-1} day^{-1}; and 127, 135 and 144 mg g^{-1} day^{-1} in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp respectively).

4.2.5.3 Effect on RGR of *S. litura* in non-Bt cotton

The data pertaining to RGR of *S. litura* in non-Bt cotton for first, second and third generation was presented in Table 4.63 and Fig. 4.47.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on RGR. Under *a*CO_2, RGR ranged between 151.74 and 110.18 mg g^{-1} day^{-1} with highest and lowest RGR at temperatures 28 and 35 ºC, respectively. At *e*CO_2, a decreased RGR from 138.82 to 105.70 mg g^{-1} day^{-1} was recorded with corresponding increase in temperature from 28 and 35 ºC. A significant interactive effect of *e*CO_2 and *e*Temp on RGR was recorded. RGR was higher in *a*CO_2 than *e*CO_2.

Second generation: In non-Bt cotton, RGR varied from 159.21 to 128.53 mg g^{-1} day^{-1} with highest RGR at temperature 28 ºC and lowest RGR at 35 ºC. At *e*CO_2, RGR ranged between 137.71 and 114.15 mg g^{-1} day^{-1} with highest and lowest RGR at temperatures 28 and 35 ºC, respectively. The individual effect of *e*CO_2 and *e*Temp and the interactive effect were significant on RGR.

Third generation: RGR varied from 166.69 to 117.54 mg g^{-1} day^{-1} with highest and lowest RGR at temperatures 28 and 35 ºC, respectively. At *e*CO_2, a decreased RGR was recorded from 146.20 to 123.11 mg g^{-1} day^{-1} with increase in corresponding temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp and the interactive effect were significant on RGR.

Mean of generations: The mean of three generations in non-Bt cotton also indicated that the RGR has decreased significantly (159.21, 147.02, 141.02, 136.74 and 118.75 mg g^{-1} day^{-1}, respectively) with increase in temperature (upto 35 ºC) corresponding to decrease of 7.66, 11.43, 14.12 and 25.41 %. Similarly, under *e*CO_2 also the RGR decreased significantly (140.91, 129.15, 123.78, 120.08 and 114.32 mg g^{-1} day^{-1}) with increase in temperatures (28-35 °C) which has corresponded to a decrease of 8.35, 12.16, 14.78 and 18.87 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on RGR. And RGR was higher in *a*CO_2 than *e*CO_2. Further, the RGR under *e*CO_2 was lower (11.50, 12.16, 12.23, 12.18 and 3.73 %, at 28-35 °C, respectively) compared to that of *a*CO_2.

The present findings suggest that mean RGR of *S. litura* decreased with increase in CO_2 (by 11 %) and temperature and also decreased with *e*CO_2 + *e*Temp by 28 %. Under *e*CO_2 conditions, altered chickpea foliage quality and induced negative effect on the growth and development of *S. exigua* across the four generations (Divya, 2017). Karmakar and Pal (2017) recorded that RGR increased with 5 °C increase in temperature from 0.22 to 0.31 g g^{-1} day^{-1}in castor and 0.13 to 0.22 g g^{-1} day^{-1} in tomato. Investigations of Zhang *et al*. (2017) on the effect of *e*CO_2 on the food utilization of *S. litura* on soybean cultivars revealed that RGR decreased significantly by 16.75 % respectively. Deekshitha *et al*. (2021) reported decrease in RGR of *S. litura* with application of sublethal doses of emamectin benzoate, (0.053 as against 0.148 g $g^{-1}day^{-1}$ in control) but when coupled with *e*CO_2 it decreased further (0.043); with *e*Temp it increased further (0.074). RGR was 0.066 g $g^{-1}day^{-1}$ with *e*CO_2 + *e*Temp.

Further, RGR increased with every next generation and was highest in the third generation (152, 159 and 167 mg g^{-1} day^{-1}; 139, 138 and 146 mg g^{-1} day^{-1}; and 106, 114 and 123 mg g^{-1} day^{-1} in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp respectively). Similarly, Wu *et al*. (2009) recorded that RGR of *S. exigua* on non-Bt cotton was higher (90.24, 88.1 and 88.27 mg g^{-1} day^{-1} in three successive generations) than that in Bt. RGR was reported to decrease by 12-15 % with *e*CO_2 in all four generations over *a*CO_2 conditions in *A. janata* larvae on castor foliage (Srinivasa Rao *et al*. 2013) and by 9 % for *S. litura* on groundnut foliage (Srinivasa Rao *et al.* 2014b).

4.2.5.4 Effect on RGR of *S. litura* in Bt cotton

The data pertaining to RGR of *S. litura* in Bt cotton for first, second and third generation was presented in Table 4.64 and Fig. 4.47.

First generation: In non-Bt cotton, *e*CO_2 and *e*Temp has shown significant influence on RGR. Under *a*CO_2, RGR decreased from 158.76 to 133.66 mg g^{-1} day^{-1} with increase in temperature from 28 to 35 ºC. At *e*CO_2, highest RGR was recorded at temperature 28 ºC with 138.67 mg g^{-1} $day^{-1}$and lowest RGR at 35 ºC with 113.21 mg g^{-1} day^{-1}. A non-significant interactive effect of *e*CO_2 and *e*Temp was recorded on RGR.

Table 4.63. Effect of *e*CO_2 and *e*Temp on larval relative growth rate (RGR) of *S. litura* in non-Bt cotton

Temperature	First generation (mg g^{-1} day^{-1})			Second generation (mg g^{-1} day^{-1})			Third generation (mg g^{-1} day^{-1})			Mean (mg g^{-1} day^{-1})		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	151.74	138.82	**145.28**	159.21	137.71	**148.46**	166.69	146.20	**156.45**	159.21	140.91	**150.06**
29 ± 1°C	138.75	121.12	**129.94**	146.58	128.49	**137.54**	155.72	137.83	**146.78**	147.02	129.15	**138.08**
31 ± 1°C	134.22	116.34	**125.28**	139.60	122.78	**131.19**	149.24	132.21	**140.73**	141.02	123.78	**132.4**
33 ± 1°C	128.49	111.34	**119.92**	136.23	119.60	**127.92**	145.49	129.31	**137.40**	136.74	120.08	**128.41**
35 ± 1°C	110.18	105.70	**107.94**	128.53	114.15	**121.34**	117.54	123.11	**120.33**	118.75	114.32	**116.54**
Mean	**132.68**	**118.66**		**142.03**	**124.55**		**146.94**	**133.73**		**140.55**	**125.65**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	850.78*	0.44	1.22	943.84*	0.40	1.12	990.75*	0.39	1.08	3229.06*	0.219	0.609
Temperature (°C)	223.86*	0.69	1.92	261.88*	0.64	1.77	261.10*	0.61	1.70	863.69*	0.346	0.963
Interaction (CO_2 + Temp(°C))	4.91*	0.98	2.72	4.21*	0.90	2.51	3.35*	0.86	2.41	14.34*	0.489	1.362
CV	3.60 %			3.38 %			3.26 %			1.83 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm

NS – Non significant

* Significant @ 5 % level of significance

Table 4.64. Effect of *e*CO_2 and *e*Temp on larval relative growth rate (RGR) of *S. litura* in Bt cotton

Temperature	First generation (mg g^{-1} day^{-1})			Second generation (mg g^{-1} day^{-1})			Third generation (mg g^{-1} day^{-1})			Mean (mg g^{-1} day^{-1})		
	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**	***a*CO_2**	***e*CO_2**	**Mean**
28 ± 1°C	158.76	138.67	**148.72**	165.25	146.71	**155.98**	173.12	154.57	**163.85**	165.71	146.65	**156.18**
29 ± 1°C	151.40	130.13	**140.77**	159.32	138.24	**148.78**	167.45	147.26	**157.36**	159.39	138.54	**148.97**
31 ± 1°C	146.87	124.45	**135.66**	153.50	130.71	**142.11**	160.65	139.51	**150.08**	153.67	131.56	**142.62**
33 ± 1°C	139.53	118.64	**129.09**	146.65	126.57	**136.61**	155.77	136.42	**146.10**	147.32	127.21	**137.26**
35 ± 1°C	133.66	113.21	**123.44**	140.21	121.26	**130.74**	149.33	130.53	**139.93**	141.07	121.67	**131.37**
Mean	**146.04**	**125.02**		**152.99**	**132.70**		**161.26**	**141.66**		**153.43**	**133.13**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	1829.86*	0.35	0.97	820.99*	0.50	1.40	1719.96*	0.33	0.93	1174.37*	0.202	0.563
Temperature (°C)	322.09*	0.55	1.53	157.13*	0.79	2.21	314.73*	0.53	1.47	924.97*	0.319	0.89
Interaction (CO_2 + Temp(°C))	0.67	0.78	NS	1.17	1.12	NS	1.01	0.75	NS	3.67*	0.452	1.258
CV	2.67 %			3.92 %			2.64 %			1.58 %		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
NS – Non significant
* Significant @ 5 % level of significance

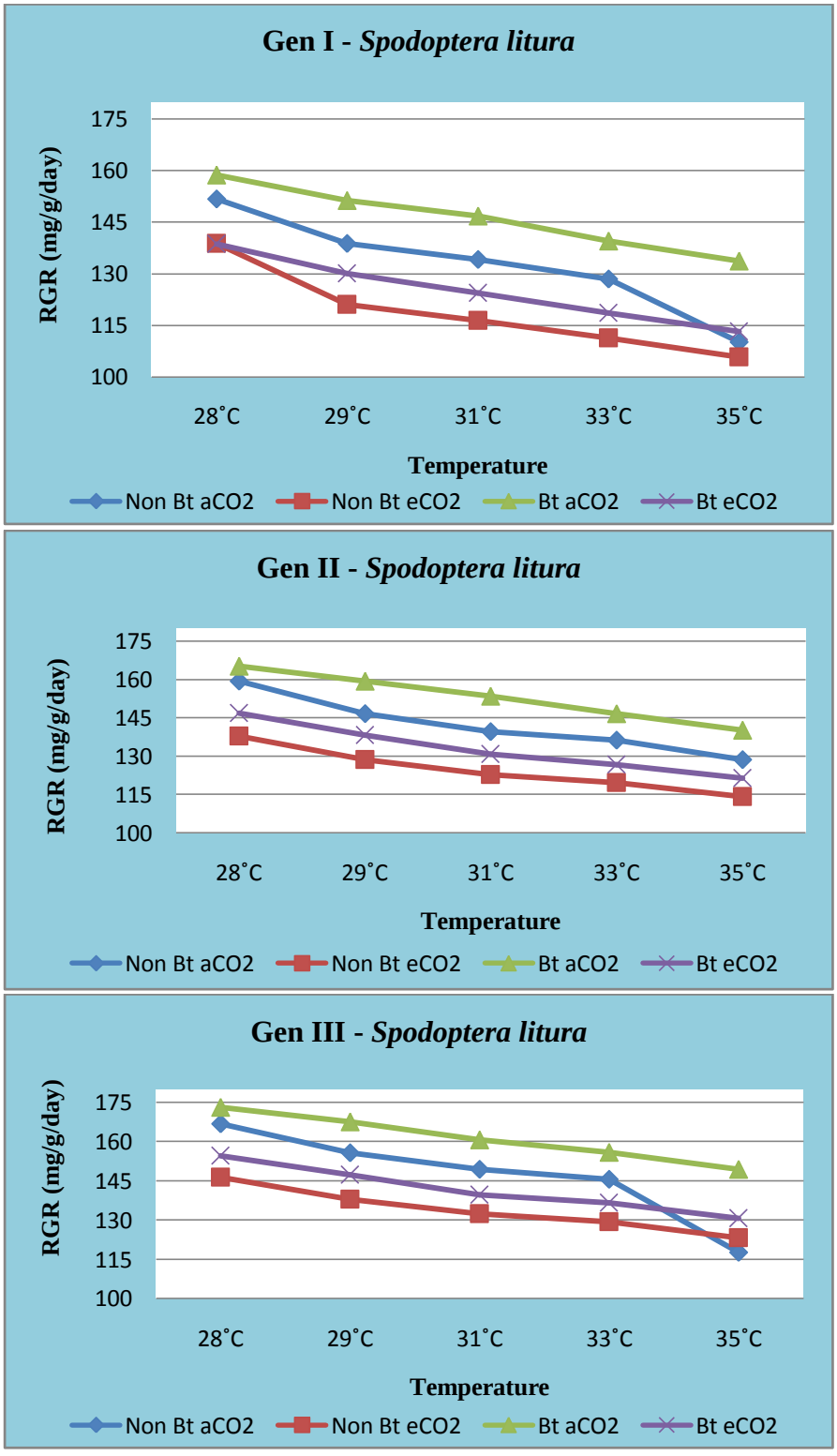


Fig. 4.47. Effect of eCO_2 and *e*Temp on larval relative growth rate (RGR) of *S. litura* on non-Bt and Bt cotton on first, second and third generation

Second generation: RGR ranged between 165.25 to 140.21 mg g^{-1} day^{-1} with highest and lowest RGR at temperatures 28 and 35 ºC, respectively. At *e*CO_2, decrease in RGR was recorded from 146.71 to 121.26 mg g^{-1} day^{-1} with increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence on RGR whereas non-significant interactive effect was recorded on RGR.

Third generation: In Bt cotton highest RGR was recorded at temperature 28 ºC with 173.12 mg g^{-1} day^{-1} and lowest at 35 ºC with 149.33 mg g^{-1} day^{-1}. At *e*CO_2, RGR decreased from 154.57 to 130.53 mg g^{-1} day^{-1} with increase in temperature from 28 to 35 ºC. The individual effect of *e*CO_2 and *e*Temp has shown significant influence on RGR whereas non-significant interactive effect was recorded on RGR.

Mean of generations: The mean of three generations also indicated that the RGR of larvae in Bt cotton has decreased significantly (165.71, 159.39, 153.67, 147.32 and 141.07 mg g^{-1} day^{-1}, respectively) with increase in temperature (28-35 ºC) corresponding to a decrease of 3.81, 7.26, 11.10 and 14.87 %. Similarly, under *e*CO_2, the RGR decreased significantly (146.65, 138.54, 131.56, 127.21 and 121.67 mg g^{-1} day^{-1}) with increase in temperatures (28, 29, 31, 33 and 35 °C, respectively) which hascorresponded to a decrease of 5.53, 10.29, 13.26 and 17.04 %. A significant interactive effect of *e*CO_2 and *e*Temp was recorded on RGR. And RGR was higher in *a*CO_2 than *e*CO_2. Further, the RGR under *e*CO_2 was lower (11.50, 13.08, 14.39, 13.65 and 13.75 %, at respective temperatures) compared to that of *a*CO_2.

The present findings suggest that mean RGR of *S. litura* in Bt cotton decreased with increase in CO_2 (by 11 %) and temperature and also decreased with *e*CO_2 + *e*Temp by 27 %. RGR increased with every next generation and was highest in the third generation (159, 165 and 167 mg g^{-1} day^{-1}; 139, 147 and 146 mg g^{-1} day^{-1}; and 113, 121 and 123 mg g^{-1} day^{-1} in three successive generations at ambient condition, *e*CO_2 and *e*CO_2 + *e*Temp respectively). The results are in agreement with Wu *et al*. (2009) recorded significantly lower RGRs in the first 82.54 ($F = 38.81$, d.f. = 1, 6, $P = 0.0008$), second 81.87 ($F = 27.19$, d.f. = 1, 6, $P = 0.002$) and third 80.68 mg g^{-1} day^{-1} ($F = 159.75$, d.f. = 1, 6, $P = 0.0001$) generations of *S. exigua* on Bt cotton compared with non-Bt cotton. Decrease in RGR with *e*CO_2 and *e*Temp appears to be advantageous for cotton crop, but the gradual increase of RGR along the generations can be a challenge to cotton crop production and protection.

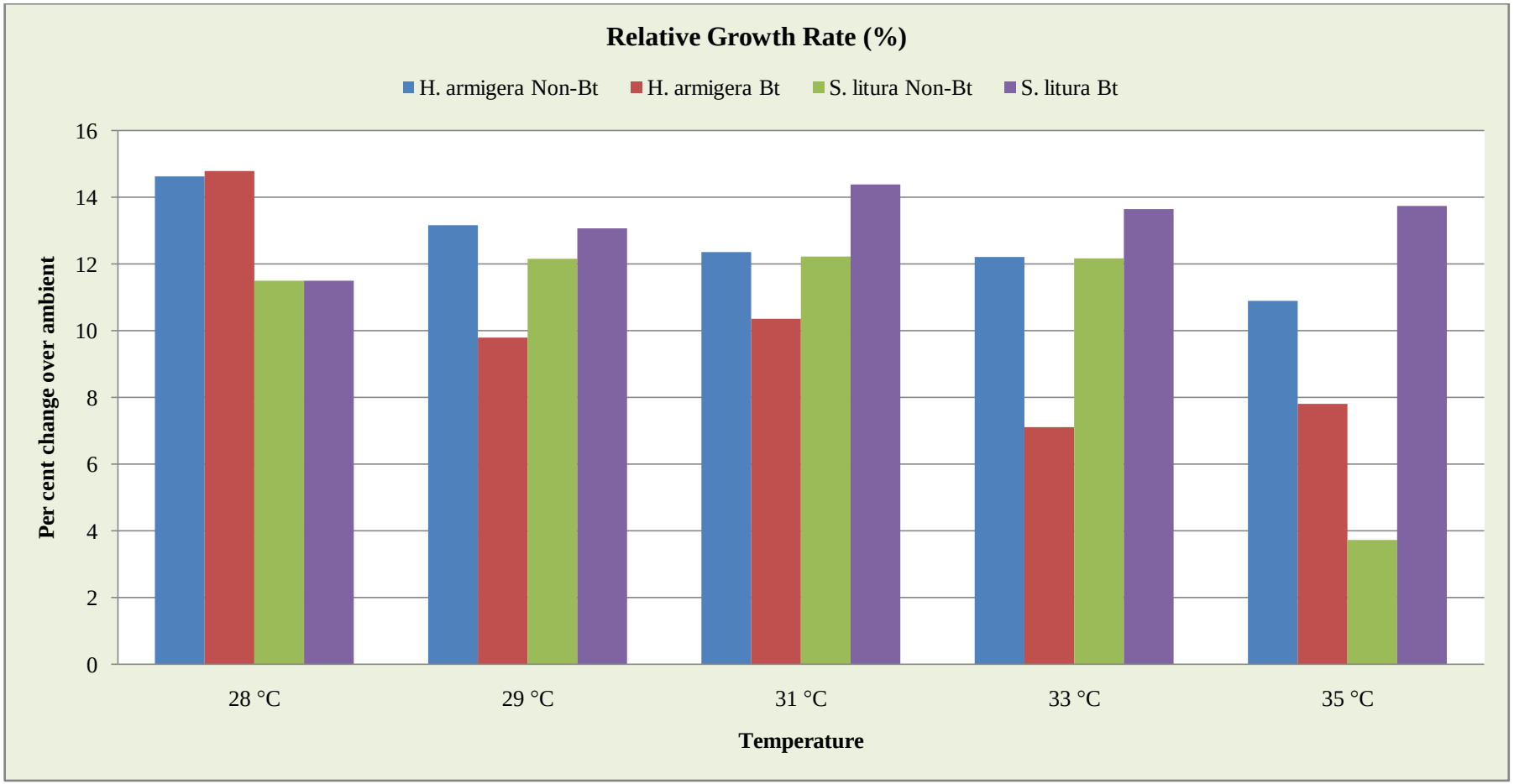


Fig. 4.48. Effect of *e*CO_2 and *e*Temp on mean larval relative growth rate (RGR) of *H. armigera* and *S. litura* on non-Bt and Bt cotton

4.3 QUANTIFICATION OF VARIATION OF BIOCHEMICAL PARAMETERS IN COTTON FOLIAGE AND TEST INSECTS AT *e*CO_2 AND *e*TEMP CONDITIONS

4.3.1 Biochemical Constituents in Cotton Foliage at *e*CO_2 and *e*Temp Conditions.

The biochemical constituents *viz.*, carbon, nitrogen, C: N ratio, polyphenol content, , proteins, carbohydrates and Bt endotoxin content of cotton foliage at 30, 45, 60, 75 and 90 days after sowing (DAS) at different test conditions *viz*., *a*CO_2 (380 ± 25 ppm), *e*CO_2 (550 ± 25 ppm), *a*Temp (28 ºC), *e*Temp (29, 31, 33, and 35 ± 1 °C), and *e*CO_2+ *e*Temp (550 ± 25 ppm with 29, 31, 33 and 35 ± 1 °C) were estimated and the results were discussed here under.

4.3.1.1 Nitrogen content: Nitrogen is the major constituent required for nutrition of plants. It has an important role in protein synthesis for various metabolic activities in plants. There is strong correlation between the nitrogen, photosynthetic capacity and the concentration of Rubisco in leaves (Evans and Seeman, 1989). Both *e*CO_2 (Stitt and Krapp, 1999) and temperature (Tjoelker *et al.*, 1999) may decrease foliar nitrogen concentration. In the cotton foliage used for the study, the per cent nitrogen content in either cotton foliage has significantly decreased with increase in temperature (28 to 35 ºC) under both *a*CO_2 and *e*CO_2 conditions, at different days after sowing *i.e.*, 30, 45, 60, 75 and 90 DAS. Higher per cent of nitrogen content was recorded at *a*CO_2 than at *e*CO_2. In both *a*CO_2 and *e*CO_2 conditions, at each level of temperature the per cent nitrogen content in foliage gradually increased from 30 DAS and reached its peak at 60 DAS and gradually reduced thereafter till 90 DAS. Under *e*CO_2 and *e*Temp conditions, the per cent nitrogen decreases with increase in temperatures.

Nitrogen content in Non-Bt cotton

The highest nitrogen content was recorded in *a*CO_2 at 28 ºC (6.03 %) at 60 DAS interval and lowest in *e*CO_2 at 35 ºC (4.36 %) at 90 DAS interval (Table 4.65 and Fig. 4.49). The effect of elevated carbon dioxide on nitrogen content was significant at every stage. The nitrogen content was lower under *e*CO_2 (5.10 to 4.61 %) and five temperatures (28 to 35 ºC) at 30 DAS compared to *a*CO_2 conditions (5.34 to 4.98 %).

Table 4.65. Effect of *e*CO_2 and *e*Temp on per cent nitrogen content in non-Bt cotton foliage

Temperature	Nitrogen (%) in non-Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	5.34	5.10	**5.22**	5.59	5.38	**5.49**	6.03	5.69	**5.86**	5.29	5.14	**5.22**	4.97	4.85	**4.91**
29 ± 1°C	5.28	5.01	**5.15**	5.47	5.24	**5.35**	5.83	5.36	**5.59**	5.25	5.01	**5.13**	4.94	4.78	**4.86**
31 ± 1°C	5.23	4.83	**5.03**	5.40	5.22	**5.33**	5.63	5.19	**5.41**	5.10	4.94	**5.02**	4.84	4.58	**4.71**
33 ± 1°C	5.07	4.64	**4.81**	5.35	5.17	**5.26**	5.60	5.11	**5.35**	5.06	4.78	**4.92**	4.68	4.54	**4.61**
35 ± 1°C	4.98	4.61	**4.80**	5.18	5.05	**5.11**	5.50	4.96	**5.23**	5.00	4.69	**4.85**	4.66	4.36	**4.51**
Mean	**5.18**	**4.81**		**5.39**	**5.21**		**5.71**	**5.26**		**5.14**	**4.91**		**4.82**	**4.62**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	153.573*	0.02	0.06	45.333*	0.02	0.06	276.992*	0.02	0.06	125.159*	0.01	0.04	105.800*	0.014	0.4
Temperature (°C)	35.229*	0.03	0.10	20.698*	0.03	0.09	63.099*	0.03	0.09	43.326*	0.02	0.07	59.253*	0.022	0.064
Interaction (CO_2 + Temp(°C))	3.149*	0.05	0.14	0.49	0.04	NS	1.46	0.04	NS	2.50	0.03	NS	3.350*	0.031	0.09
CV	1.60%			1.39%			1.37%			1.11%			1.12%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

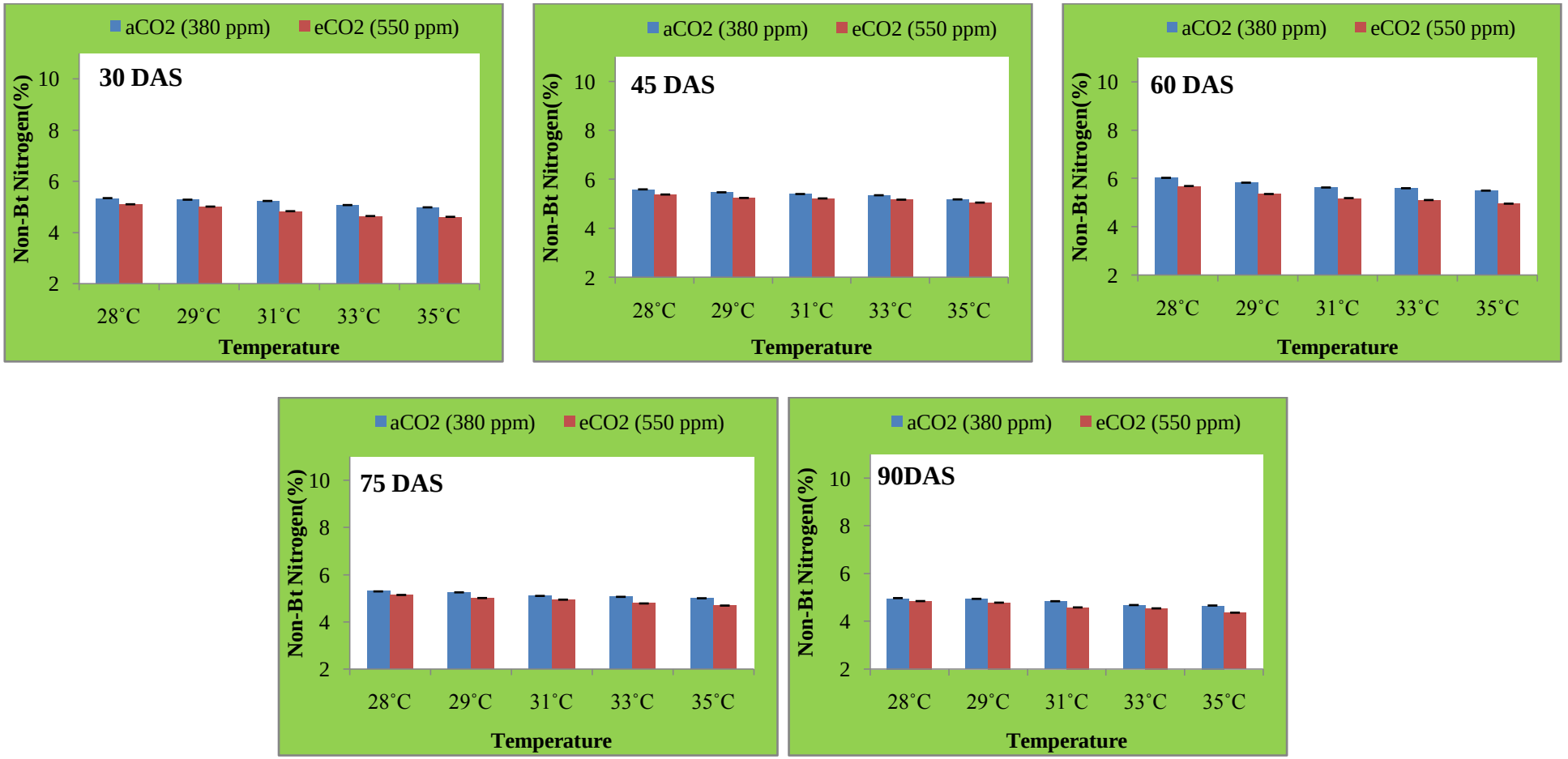


Fig. 4.49. Effect of *e*CO_2 and *e*Temp on per cent nitrogen content in non-Bt cotton foliage

Similar is the case with 45 DAS, with low nitrogen content at temperatures (28 to 35 ºC) under *e*CO_2 (5.38 to 5.05 %) than at *a*CO_2 (5.59 to 5.18 %). At 60 DAS, the nitrogen content was more under *a*CO_2 (6.03 to 5.50 %) at temperatures (28 to 35ºC) compared to *e*CO_2 (5.69 to 4.96 %). At 75 DAS, higher per cent nitrogen was found under *a*CO_2 (5.29 to 5.00 %) compared to *e*CO_2 (5.14 to 4.69 %). Similarly, at 90 DAS, the nitrogen content in the cotton foliage was higher under *a*CO_2 (4.97 to 4.66 %) than at *e*CO_2 (4.84 to 4.36 %) at corresponding temperatures. The interactive effect of *e*CO_2 and *e*Temp has shown significant influence only at 30 DAS and 90 DAS.

To sumup, in non-Bt cotton, nitrogen content decreased by 6 % (*e*CO_2) and by 18 % (*e*CO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect in drastically reducing nitrogen levels of foliage especially in non-Bt cotton, and that temperature is an indispensible factor in climate change investigations on crop pests. The present results are in conformity with Wu *et al*. (2006) who observed that nitrogen reduced from 12.71 to 11.8 mg g^{-1} with enhancement of CO_2 to 750 ppm. Srinivasa Rao *et al.* (2014b) reported that *e*CO_2 stimulates decrease in nitrogen (3.2-2.95 %) content of groundnut foliage by 7.8 %. Manimanjari *et al*., (2014) reported that foliar nitrogen content decreased under *e*CO_2 (2.65 %) compared to *a*CO_2 (2.81 %) in sunflower. Similarly, Meena *et al.* (2017) reported decreased nitrogen content in elevated CO_2 (550 ppm) + *e*Temp (4.46 %) compared to natural conditions (4.83 %) in Bell pepper foliage. Decreased nitrogen under *e*CO_2 impacts the herbivore's efficiency of conversion to body mass, larval growth and survival rates (Masters *et al*., 1998).

Nitrogen content in Bt cotton: The highest nitrogen content (Table 4.66 and Fig. 4.50) was recorded in *a*CO_2 at 28 ºC (6.05 %) at 60 DAS interval and lowest in *e*CO_2 at 35 ºC (4.81 %) at 90 DAS interval. With increase in temperature there was a decrease in nitrogen content in leaves. At 30 DAS, the nitrogen content was lower under *e*CO_2 (5.22 to 4.88 %) at five temperatures (28 to 35 ºC) as compared to *a*CO_2 conditions (5.54 to 4.95 %). At 45 DAS, also low nitrogen content was recorded at temperatures (28 to 35 ºC) under *e*CO_2 (5.50 to 5.21%) than at *a*CO_2 (5.71 to 5.33 %). At 60 DAS, the nitrogen content was more under *a*CO_2 (6.05 to 5.47%) at temperatures (28 to 35 ºC) compared to *e*CO_2 (5.80 to 5.33 %). At 75 DAS, higher per cent nitrogen was found under *a*CO_2 (5.66 to 5.18 %) compared to *e*CO_2 (5.43 to

Table 4.66. Effect of *e*CO_2 and *e*Temp on per cent nitrogen content in Bt cotton foliage

Temperature	Nitrogen (%) in Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	5.54	5.22	**5.38**	5.71	5.50	**5.60**	6.05	5.80	**5.92**	5.66	5.43	**5.55**	5.4	5.24	**5.32**
29 ± 1°C	5.28	5.13	**5.21**	5.64	5.46	**5.55**	5.87	5.66	**5.77**	5.51	5.32	**5.41**	5.24	5.15	**5.2**
31 ± 1°C	5.18	5.04	**5.11**	5.47	5.39	**5.43**	5.71	5.52	**5.62**	5.40	5.25	**5.32**	5.14	5.00	**5.07**
33 ± 1°C	5.12	4.98	**5.05**	5.45	5.28	**5.36**	5.68	5.36	**5.52**	5.29	5.15	**5.22**	5.07	4.97	**5.02**
35 ± 1°C	4.95	4.88	**4.92**	5.33	5.21	**5.27**	5.47	5.33	**5.40**	5.18	5.02	**5.10**	5.01	4.81	**4.91**
Mean	**5.21**	**5.04**		**5.51**	**5.36**		**5.75**	**5.53**		**5.40**	**5.23**		**5.17**	**5.03**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	71.348*	0.01	0.04	38.782*	0.02	0.05	64.113*	0.02	0.06	135.070*	0.01	0.03	80.219*	0.011	0.032
Temperature (°C)	62.223*	0.02	0.07	24.804*	0.03	0.08	43.161*	0.03	0.09	109.742*	0.02	0.05	85.647*	0.017	0.051
Interaction (CO_2 + Temp(°C))	4.503*	0.03	0.09	0.84	0.04	NS	1.17	0.04	NS	1.35	0.02	NS	1.991	0.024	0.072
CV	1.05%			1.22%			1.35%			0.76%			0.82%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

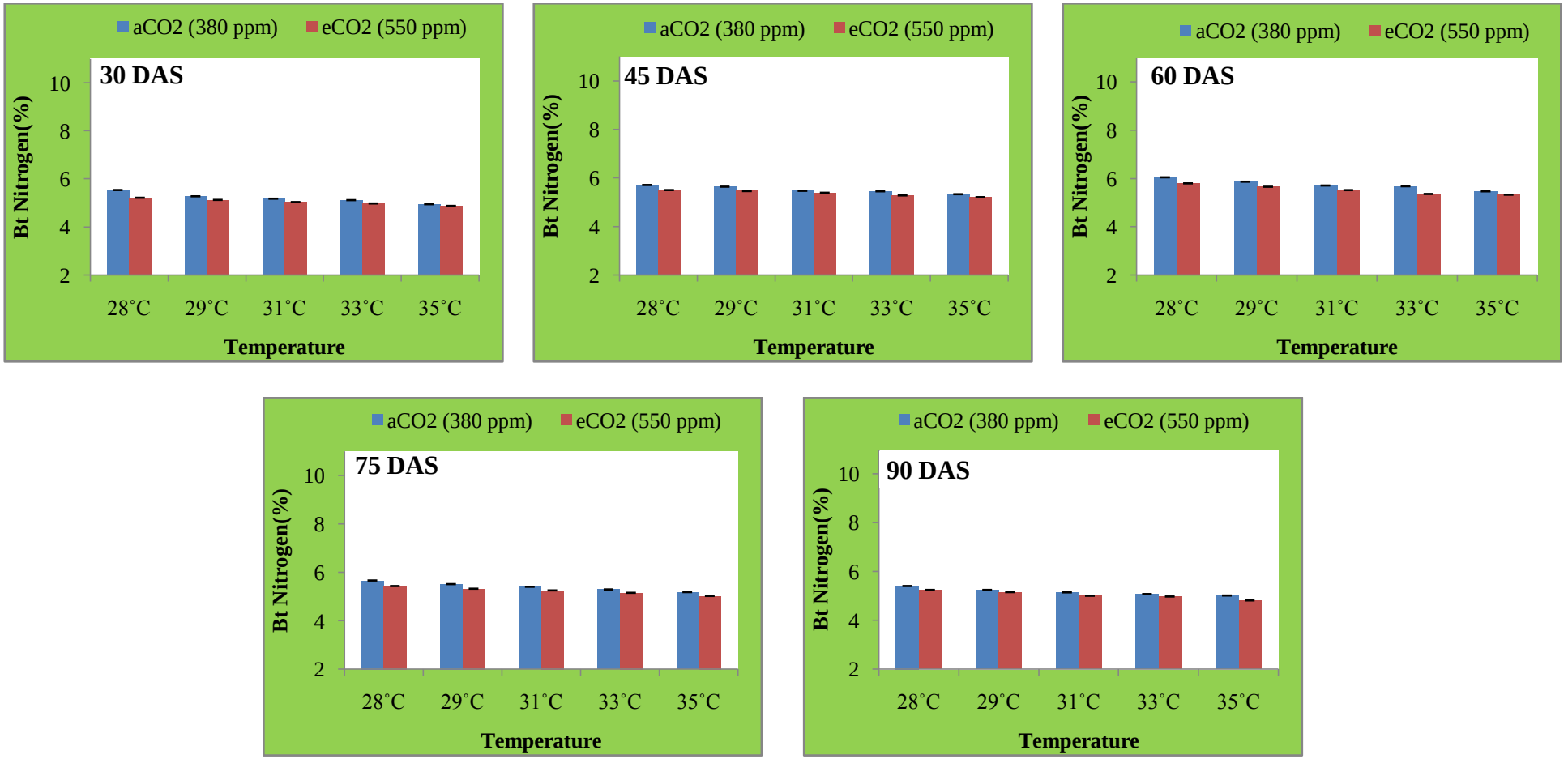


Fig. 4.50 Effect of *e*CO_2 and *e*Temp on per cent nitrogen content in Bt cotton foliage

5.02 %). Similarly, at 90 DAS, the nitrogen content in the cotton foliage was higher under *a*CO_2 (5.40 to 5.01 %) than at *e*CO_2 (5.24 to 4.81 %) at corresponding temperatures. In Bt cotton the interaction effect was significant only at 30 DAS and non significant in the later stages of development.

In a nutshell, nitrogen content in Bt cotton decreased by 4 % (*e*CO_2) and by 12 % (*e*CO_2 +*e*Temp) at 60 DAS, lower than that recorded for non-Bt cotton. It shows the impact of interactive effect on drastically reducing nitrogen levels of foliage especially in non-Bt cotton, and that temperature is an indispensible factor in climate change investigations on crop pests. The observations are in line with Chen *et al*. (2005b) who reported decrease in nitrogen content in the young bolls under *e*CO_2 over those in *a*CO_2 for both GK-12 Bt (~22-19 mg g^{-1}) and Simian -3 non-Bt (~24-22 mg g^{-1}) cotton. On the contrary, Wu *et al*. (2009) noticed significantly lower foliar nitrogen content ($p < 0.05$) in Bt cotton compared with non-transgenic cotton.

4.3.1.2 Carbon

Carbon content in Non-Bt cotton: The carbon content in the cotton foliage increased with advancement of the crop stage till 60 DAS, thereafter it decreased gradually (Table 4.67 and Fig. 4.51). As the CO_2 level increased to 550 ppm, carbon content increase was noticed at all intervals. Significant difference was observed in the carbon content of cotton foliage grown under *a*CO_2 and *e*CO_2 at five temperatures (28 to 35 ºC) at 30, 45, 60, 75 and 90 DAS. The carbon content in the cotton foliage is higher under *e*CO_2 at all temperatures (28 to 35ºC) at 30 DAS (22.28 to 28.38 %), 45 DAS (26.85 to 31.71 %), 60 DAS (30.95 to 38.00 %), 75 DAS (28.95 to 34.85 %) and at 90 DAS (22.85 to 30.19 %) than the carbon content at *a*CO_2 at corresponding temperatures with 30 DAS (14.19 to 23.42 %), 45 DAS (18.47 to 26.57 %), 60 DAS (24.76 to 33.71 %), 75 DAS (20.38 to 30.47 %) and at 90 DAS (16.95 to 23.90 %).

It can be briefed that in non-Bt cotton, carbon content increased by 25 % (*e*CO_2) and by 53 % (*e*CO_2 + *e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically increasing carbon levels of foliage, and that temperature is an indispensible factor in climate change investigations on crops. The current findings are in conformity with Shwetha *et al*. (2017) who reported increased carbon content under *e*CO_2 and *e*Temp (46 %) conditions compared to reference (42 %) in ground

Table 4.67. Effect of *e*CO_2 and *e*Temp on per cent carbon content in non-Bt cotton foliage

Temperature	Carbon (%) in non-Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	14.19	22.28	**18.23**	18.47	26.85	**22.66**	24.76	30.95	**27.85**	20.38	28.95	**24.66**	16.95	22.85	**20.9**
29 ± 1°C	16.47	22.47	**19.47**	20.85	28.38	**24.61**	26.76	31.33	**29.04**	22.57	29.23	**25.90**	18.38	23.33	**20.85**
31 ± 1°C	17.33	25.24	**21.28**	23.33	29.23	**26.28**	29.23	33.14	**31.19**	25.33	31.33	**28.33**	19.80	25.90	**22.85**
33 ± 1°C	20.95	27.14	**24.04**	24.54	30.19	**27.37**	31.71	36.19	**33.95**	29.04	33.62	**31.33**	21.99	28.76	**25.38**
35 ± 1°C	23.42	28.38	**25.90**	26.57	31.71	**29.14**	33.71	38.00	**35.85**	30.47	34.85	**32.66**	23.9	30.19	**27.05**
Mean	**18.47**	**25.10**		**22.75**	**29.27**		**29.23**	**33.92**		**25.55**	**31.59**		**20.2**	**26.6**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	658.848*	0.18	0.54	1412.601*	0.12	0.36	627.686*	0.13	0.39	914.472*	0.14	0.42	922.225*	0.149	0.44
Temperature (°C)	120.690*	0.29	0.85	165.248*	0.19	0.57	253.51	0.21	0.62	234.904*	0.22	0.66	136.066*	0.236	0.695
Interaction (CO_2 + Temp(°C))	5.383*	0.41	1.21	12.443*	0.27	0.81	4.413*	0.30	0.87	14.673*	0.32	0.93	5.178*	0.333	0.983
CV	3.25%			1.83%			1.62%			1.91%			2.47%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

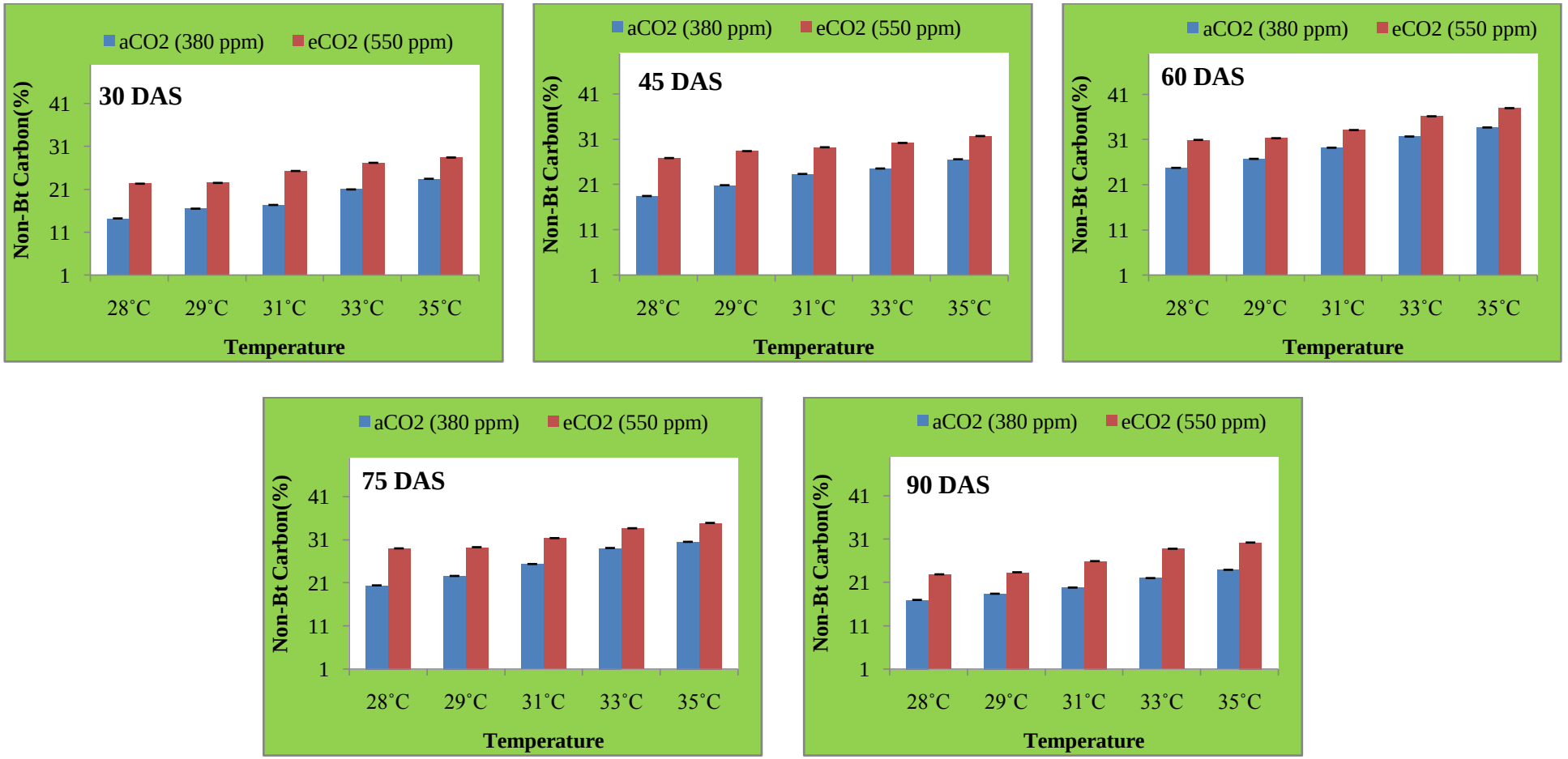


Fig. 4.51. Effect of *e*CO_2 and *e*Temp on per cent carbon content in non-Bt cotton foliage

Table 4.68. Effect of *e*CO_2 and *e*Temp on per cent carbon content in Bt cotton foliage

Temperature	Carbon (%) in Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	13.80	20.18	**18.99**	18.66	25.85	**23.76**	23.71	30.42	**28.57**	21.42	26.33	**26.38**	16.57	21.71	**21.14**
29 ± 1°C	15.42	20.66	**18.04**	21.14	25.90	**23.52**	25.90	31.00	**27.95**	22.95	27.90	**25.42**	17.80	23.04	**20.42**
31 ± 1°C	17.33	24.09	**20.71**	23.14	27.71	**25.42**	28.85	32.00	**30.43**	26.18	30.09	**28.14**	20.19	26.19	**23.19**
33 ± 1°C	19.80	25.80	**22.80**	24.00	30.57	**27.28**	30.28	34.57	**32.43**	27.90	32.85	**30.38**	21.81	28.76	**25.28**
35 ± 1°C	22.47	27.71	**25.09**	26.85	32.85	**29.85**	32.57	37.80	**35.19**	29.33	35.42	**32.38**	24.18	30.57	**27.38**
Mean	**17.76**	**24.48**		**22.75**	**29.17**		**28.26**	**33.55**		**25.55**	**31.52**		**20.1**	**26.85**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	836.23	0.16	0.49	1162.662*	0.13	0.39	871.809*	0.13	0.37	702.627*	0.16	0.47	814.05*	0.167	0.493
Temperature (°C)	121.27*	0.26	0.77	157.99*	0.21	0.62	218.119*	0.20	0.59	129.014*	0.25	0.74	119.38*	0.264	0.779
Interaction (CO_2 + Temp(°C))	16.943*	0.37	1.08	29.031*	0.30	0.88	41.384*	0.28	0.84	21.559*	0.36	1.05	7.815*	0.374	1.102
CV	3.01%			1.99%			1.59%			2.16%			2.76%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowin

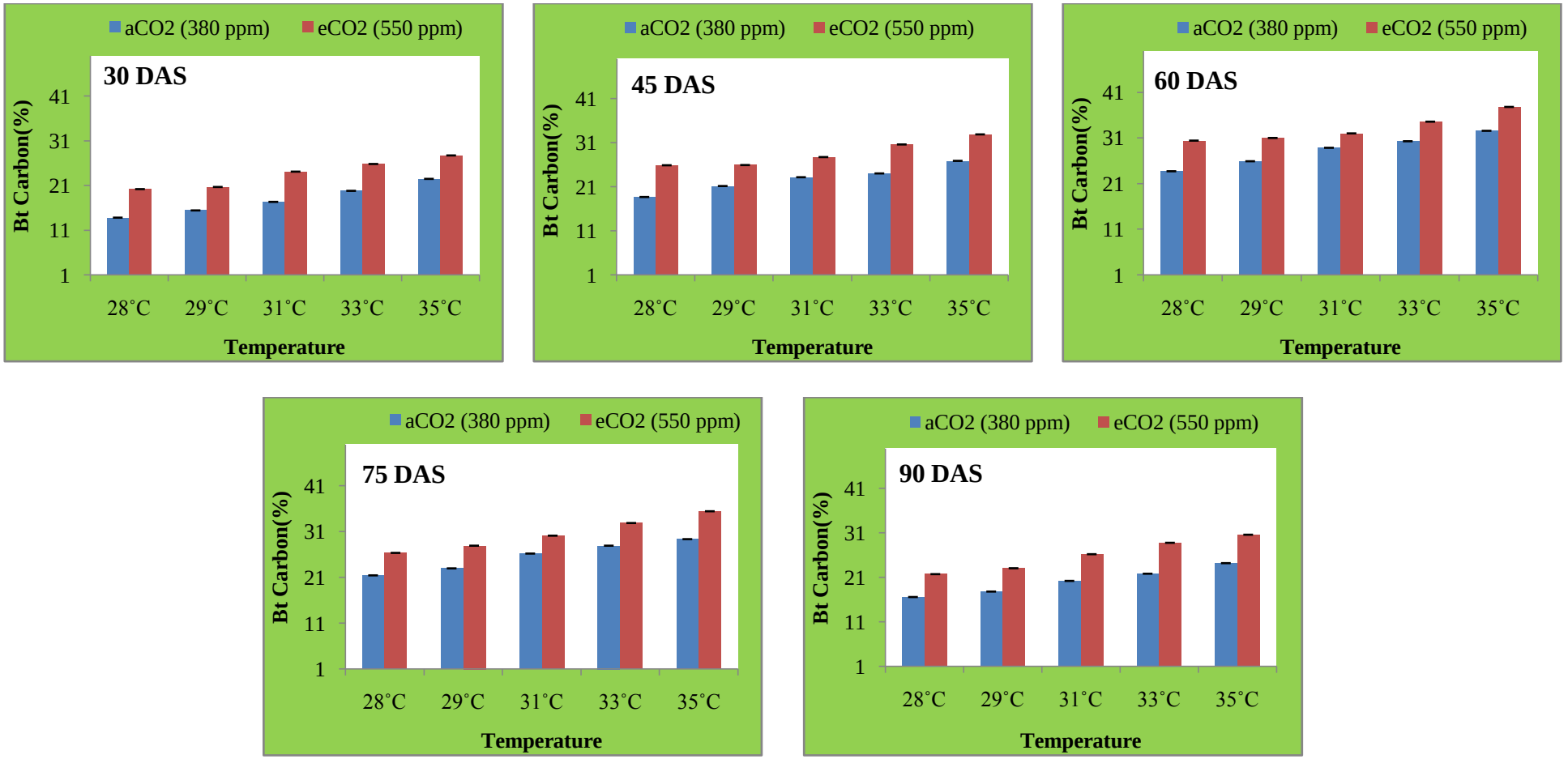


Fig. 4.52. Effect of *e*CO_2 and *e*Temp on per cent carbon content in Bt cotton foliage

nut foliage. Srinivasa Rao *et al.* (2014b) reported that *e*CO_2 stimulates increase in carbon content of groundnut foliage by 3-6 %. Increase in availability of carbon content under *e*CO_2 may enhance nitrogen limitation, leading to a reduction in plant nitrogen concentration (Manimanjari, 2017).

Carbon content in Bt cotton: At particular temperature, with increase in CO_2 level, carbon content increase was noticed at all the intervals (Table 4.68 & Fig. 4.52). Significant effect of *e*CO_2 and *e*Temp was observed in the carbon content of cotton foliage grown under. Similar to non-Bt cotton, the carbon content in the Bt cotton foliage is more under *e*CO_2 at all temperatures and at all intervals *viz.*, 30 DAS (20.18 to 27.71 %), 45 DAS (25.85 to 32.85 %), 60 DAS (30.42 to 37.80 %), 75 DAS (26.33 to 35.42 %) and at 90 DAS (21.71 to 30.57 %). It was relatively lesser under *a*CO_2 conditions where, the carbon content recorded at various intervals *viz.*, 30 DAS (13.80 to 22.47 %), 45 DAS (18.66 to 26.85 %), 60 DAS (23.71 to 32.57 %), 75 DAS (21.42 to 29.33 %) and at 90 DAS (16.57 to 24.18 %).

It can be briefed that in Bt cotton, carbon content increased by 28 % (*e*CO_2) and by 59 % (*e*CO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically increasing carbon levels of foliage especially in Bt cotton, and that temperature is an indispensible factor in climate change investigations on crop pests.

4.3.1.3 C: N Ratio

C: N Ratio in Non-Bt cotton: Significant difference was observed in C: N ratio of cotton foliage (Table 4.69 and Fig. 4.53) grown under *e*CO_2 and *a*CO_2 with five temperature conditions. It was higher under *e*CO_2 at five temperatures (28 to 35 ºC) compared to *a*CO_2 conditions. The highest C: N ratio was recorded at 60 DAS interval at 35 °C temperature (7.66) and lowest at 30 DAS at 28 °C (2.66). The C: N ratio was higher under *e*CO_2 at all temperatures (28 to 35 ºC) compared to *a*CO_2 conditions. The C: N ratio increased in foliage with increase in temperature under *e*CO_2 at 30 DAS (4.37 to 6.16), 45 DAS (4.99 to 6.28), 60 DAS (5.44 to 7.66), 75 DAS (5.63 to 7.43) and 90 DAS (4.13 to 6.93). The value was less under *a*CO_2, which increased with increase in temperatures at 30 DAS (2.66 to 4.70), 45 DAS (3.30 to 5.13), 60 DAS (4.11 to 6.13), 75 DAS (3.85 to 6.10) and at 90 DAS (3.41 to 5.13).

Table 4.69. Effect of *e*CO_2 and *e*Temp on C:N ratio in non-Bt cotton foliage

Temperature	C:N ratio in non Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	2.66	4.37	**3.51**	3.30	4.99	**4.15**	4.11	5.44	**4.78**	3.85	5.63	**4.74**	3.41	4.13	**4.27**
29 ± 1°C	3.12	4.48	**3.80**	3.81	5.42	**4.62**	4.59	5.85	**5.22**	4.30	5.84	**5.07**	3.72	4.88	**4.30**
31 ± 1°C	3.32	5.22	**4.27**	4.32	5.57	**4.94**	5.19	6.39	**5.79**	4.97	6.34	**5.65**	4.09	5.65	**4.87**
33 ± 1°C	4.13	5.98	**5.06**	4.59	5.84	**5.21**	5.67	7.08	**6.37**	5.74	7.03	**6.38**	4.7	6.34	**5.52**
35 ± 1°C	4.70	6.16	**5.43**	5.13	6.28	**5.71**	6.13	7.66	**6.90**	6.10	7.43	**6.76**	5.13	6.93	**6.03**
Mean	**3.58**	**5.24**		**4.23**	**5.62**		**5.13**	**6.48**		**4.98**	**6.45**		**4.2**	**5.78**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	772.51*	0.04	0.12	789.63*	0.04	0.10	751.67*	0.04	0.10	1224.32*	0.03	0.09	1213.021*	0.032	0.094
Temperature (°C)	149.13*	0.07	0.20	114.48*	0.06	0.16	241.50*	0.06	0.16	333.098*	0.05	0.14	230.698*	0.051	0.149
Interaction (CO_2 + Temp(°C))	3.293*	0.09	0.28	4.670*	0.08	0.23	1.46	0.08	NS	4.623*	0.07	0.20	6.094*	0.072	0.211
CV	3.70%			2.75%			2.32%			2.00%			2.48%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

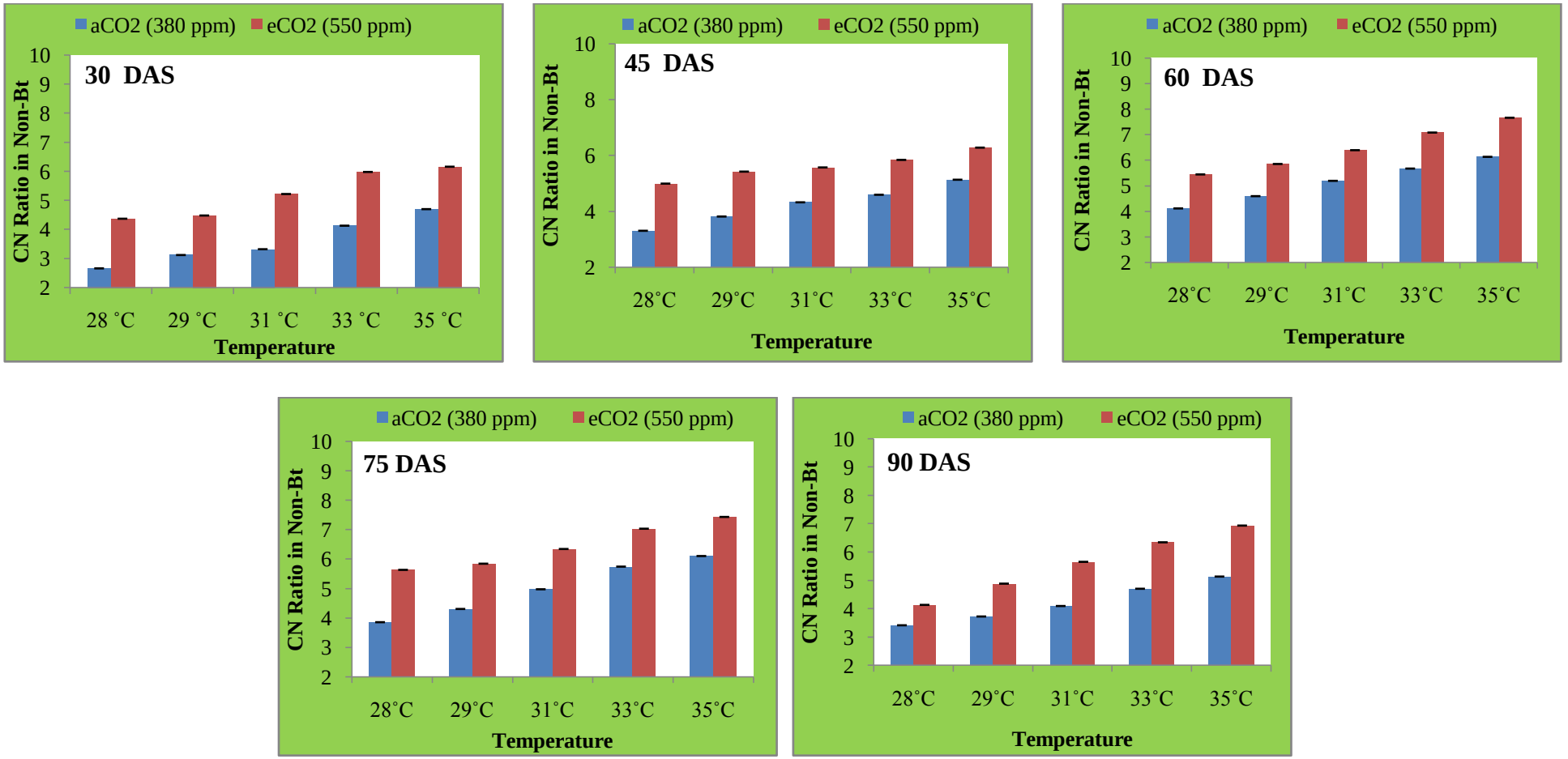


Fig. 4.53 Effect of *e*CO_2 and *e*Temp on C:N ratio in non-Bt cotton foliage

To sum up, in non-Bt cotton, C: N ratio increased by 32 % (eCO_2) and by 86 % (eCO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically increasing C: N ratio of foliage especially in non-Bt cotton, and that temperature is an indispensible factor in climate change investigations on crop pests. Norby *et al*. (1999) stated that crops belonging to both C_3 and C_4 biochemical pathway exhibit reduced nitrogen, increased carbon and C: N ratio due to increased photosynthesis and plant growth. Growth under eCO_2 results in increased photosynthesis, plant biomass, leaf area and C: N ratio which occurs through the accumulation of non structural carbohydrates in the plants grown under eCO_2 (Wu *et al*., 2007). Srinivasa Rao *et al.* (2014b) reported that eCO_2 stimulates 11-15 % increase in C: N ratio (12.13 to 13.93) of groundnut foliage. Deekshitha (2020) recorded significant increase in carbon and C:N ratio under eCO_2 conditions compared with aCO_2 condition in sunflower, which might be accrued to increased carbon intake. The increase in C:N ratio and decrease in nitrogen content in leaves under eCO_2, indicates reduction in food quality that might alter the consumption of *S. litura* larvae. Further, present findings can be supported with experimental evidences shown in other oil seed crops *viz*., soybean (Lincoln *et al*., 1984), chickpea (Abdul *et al*., 2014), cotton (Coviella *et al*., 2002) and castor (Srinivasa Rao *et al*., 2009) where the plants had significantly lower nitrogen, higher carbon and C: N ratio at elevated levels of CO_2. In non-Bt cotton, C: N increased by 32 % (eCO_2) and by 81 % (eCO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically increasing C:N value of foliage, which was brought about by involvement of temperature.

C: N ratio content in Bt cotton

Similar to carbon levels, with each temperature setting, the C: N ratio increased with plant growth (Table 4.70 and Fig. 4.54). The C: N value increased with growth and also along the CO_2 gradient. The interaction effect of CO_2 and temperature also witnessed increase in the ratio across the temperature gradient at each growth stage. Across the growth stages, the value increased linearly for aCO_2 and was curvilinear for eCO_2. The C: N ratio increased in foliage with increase in temperature under eCO_2 at 30 DAS (4.64 to 5.68), 45 DAS (4.25 to 6.31), 60 DAS (5.17 to 7.10 %), 75 DAS (5.07 to 7.06 %) and 90 DAS (4.01 to 6.36 %). Under aCO_2, the C:N ratio increased with increase in temperature at 30 DAS (2.49 to 4.54), 45 DAS (3.27 to 5.03), 60 DAS (3.92 to 5.95), 75 DAS (3.78 to 5.66) and at 90 DAS (3.07 to 4.82) but were lower than at eCO_2 and corresponding temperatures. Wu *et al*. (2006) reported that C: N ratio increased from 17.63 (aCO_2) to 20.16 (eCO_2) in spring wheat.

Table 4.70. Effect of *e*CO_2 and *e*Temp on C:N ratio in Bt cotton foliage

Temperature	C:N ratio in Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	2.49	4.64	**3.56**	3.27	4.25	**4.26**	3.92	5.17	**4.84**	3.78	5.07	**4.78**	3.07	4.01	**3. 99**
29 ± 1°C	2.92	4.72	**3.47**	3.75	4.75	**4.25**	4.42	5.30	**4.86**	4.17	5.25	**4.71**	3.40	4.47	**3.93**
31 ± 1°C	3.35	4.78	**4.06**	4.23	5.14	**4.68**	5.05	5.79	**5.42**	4.85	5.73	**5.29**	3.93	5.24	**4.58**
33 ± 1°C	3.87	5.18	**4.53**	4.41	5.79	**5.10**	5.33	6.45	**5.89**	5.28	6.38	**5.83**	4.30	5.78	**5.04**
35 ± 1°C	4.54	5.68	**5.11**	5.03	6.31	**5.67**	5.95	7.10	**6.52**	5.66	7.06	**6.36**	4.82	6.36	**5.59**
Mean	**3.43**	**4.86**		**4.13**	**5.44**		**4.93**	**6.08**		**4.74**	**6.03**		**3.9**	**5.35**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	871.91*	0.03	0.10	990.79*	0.03	0.09	907.71*	0.03	0.08	711.91*	0.03	0.10	1171.03*	0.03	0.088
Temperature (°C)	159.73*	0.05	0.16	168.93*	0.05	0.14	282.96*	0.04	0.13	170.66*	0.05	0.16	222.91*	0.047	0.139
Interaction (CO_2 + Temp(°C))	15.266*	0.08	0.23	20.608*	0.07	0.19	24.886*	0.06	0.18	15.93*	0.08	0.23	8.818*	0.067	0.197
CV	3.20%			2.37%			1.89%			2.45%			2.50%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

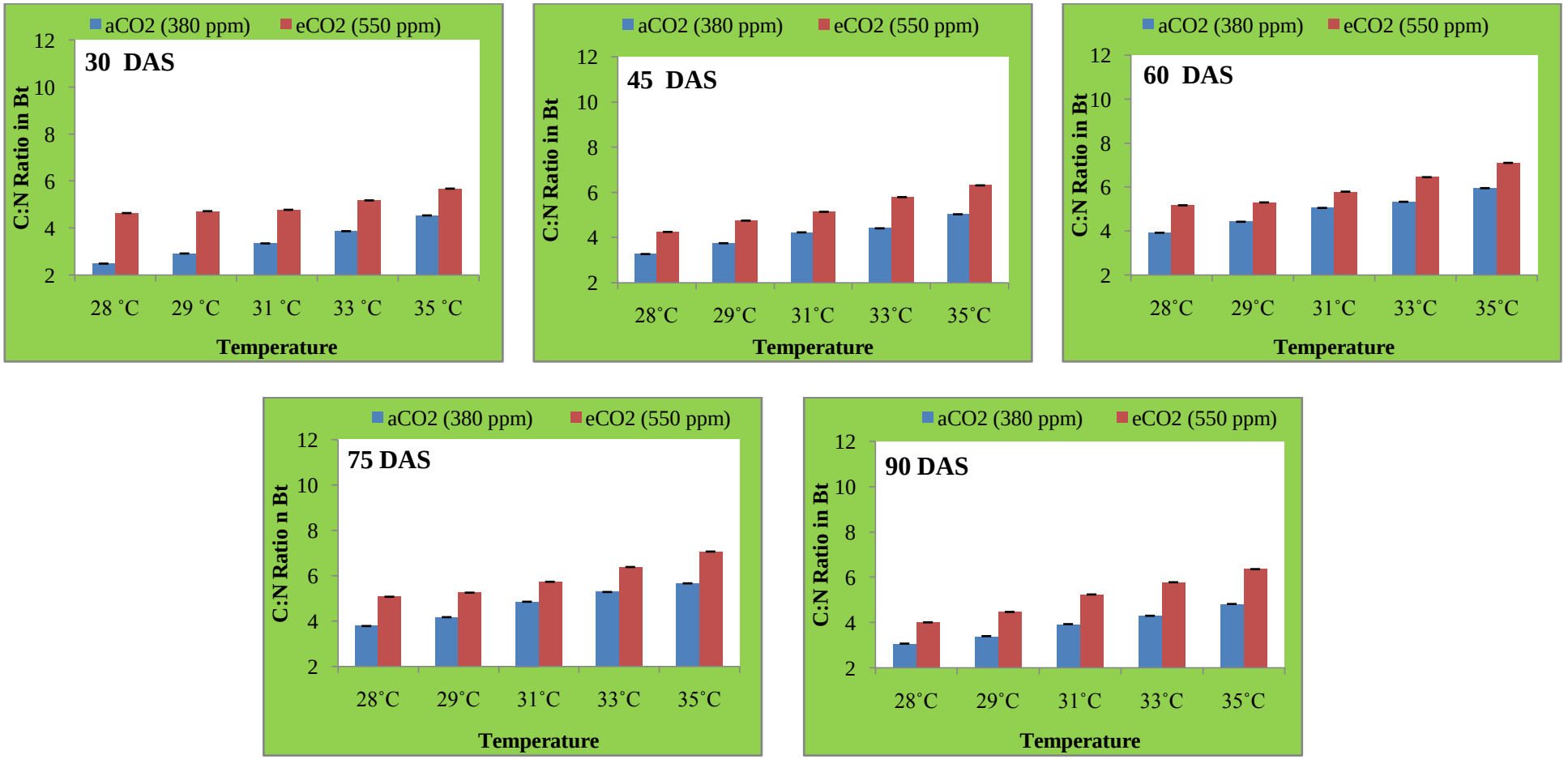


Fig. 4.54. Effect of *e*CO_2 and *e*Temp on C:N ratio in Bt cotton foliage

4.3.1.4 Carbohydrate content

Carbohydrates in Non-Bt cotton: The carbon dioxide and temperature has significant impact on carbohydrate content in cotton foliage at all intervals *viz.*, 30, 45, 60, 75 and 90 DAS (Table 4.71 and Fig. 4.55). The carbohydrate content gradually increased from 30 DAS and reached its peak at 60 DAS and thereafter gradually decreased upto 90 DAS. Carbohydrate content increased with increase in carbon dioxide concentration and temperature. Significantly higher carbohydrate content was recorded under *e*CO_2 in the cotton foliage at 30 DAS (45.53 to 52.17 mg g^{-1}), 45 DAS (47.15 to 53.20 mg g^{-1}), 60 DAS (48.20 to 54.53 mg g^{-1}), 75 DAS (47.75 to 53.65 mg g^{-1}) and at 90 DAS (46.05 to 51.22 mg g^{-1}) at five temperatures (28 to 35 ºC).

The carbohydrate content of cotton foliage was significantly lower under *a*CO_2 and five temperatures (28 to 35 ºC) at 30 DAS (43.43 to 48.75 mg g^{-1}), 45 DAS (44.55 to 50.17 mg g^{-1}), 60 DAS (45.03 to 51.47 mg g^{-1}), 75 DAS (43.38 to 50.20 mg g^{-1}) and at 90 DAS (42.80 to 48.48 mg g^{-1}) compared to *e*CO_2 at corresponding temperatures. The interactive effect of *e*CO_2 and *e*Temp has shown significant effect at 30 DAS but in later stages it is non-significant.

It can be briefed that in non-Bt cotton, carbohydrate content increased by 7 % (*e*CO_2) and by 21 % (*e*CO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically increasing carbohydrate levels of foliage, and that temperature is an indispensible factor in climate change investigations on crop pests. Similarly, Wu *et al*. (2006) reported that TNC of spring wheat increased from 223.98 mg g^{-1} to 237.75 mg g^{-1} with increase in CO_2 (370 to 750 ppm). Lavanya *et al*. (2017) also reported increase in carbohydrate content under *e*CO_2 + *e*Temp (5.18 mg g^{-1}) compared to *a*CO_2 + *e*Temp (4.84 mg g^{-1}). Deekshitha (2020) indicated that total carbohydrate content of sunflower foliage at *e*CO_2+ *e*Temp increased by 18.70, 23.10, 21.82, 25.54 and 20.17 % at 30, 45, 60, 75 and 90 DAS, respectively compared to *a*CO_2+*a*Temp.

Table 4.71. Effect of *e*CO_2 and *e*Temp on carbohydrate content in non-Bt cotton foliage

Temperature	Carbohydrate (mg g^{-1}) content in non Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	43.43	45.53	**44.48**	44.55	47.15	**45.85**	45.03	48.20	**46.62**	43.38	47.75	**45.57**	42.8	46.05	**44.43**
29 ± 1°C	44.20	46.83	**45.53**	45.95	48.45	**47.20**	46.47	48.88	**47.68**	45.72	48.73	**47.23**	44.28	47.17	**45.73**
31 ± 1°C	45.70	48.40	**47.05**	47.67	49.42	**48.54**	47.97	50.20	**49.08**	47.47	50.87	**49.17**	45.20	48.67	**46.93**
33 ± 1°C	46.98	50.45	**48.72**	48.90	51.27	**50.08**	50.17	52.82	**51.49**	48.58	52.05	**50.32**	47.50	49.82	**48.66**
35 ± 1°C	48.75	52.17	**50.46**	50.17	53.20	**51.68**	51.47	54.53	**53.00**	50.20	53.65	**51.93**	48.48	51.22	**49.85**
Mean	**45.82**	**48.67**		**47.44**	**49.89**		**48.22**	**50.92**		**47.07**	**50.61**		**45.65**	**48.58**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	639.20*	0.08	0.24	395.48*	0.09	0.26	330.66*	0.11	0.31	624.49*	0.10	0.30	459.08*	0.097	0.285
Temperature (°C)	361.65*	0.13	0.37	395.48*	0.14	0.41	252.82*	0.17	0.49	250.43*	0.16	0.47	203.88*	0.153	0.451
Interaction (CO_2 + Temp(°C))	5.282*	0.18	0.53	2.84	0.20	NS	1.47	0.24	NS	2.47	0.22	NS	2.159	0.216	NS
CV	0.65%			0.69%			0.82%			0.79%			0.79%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

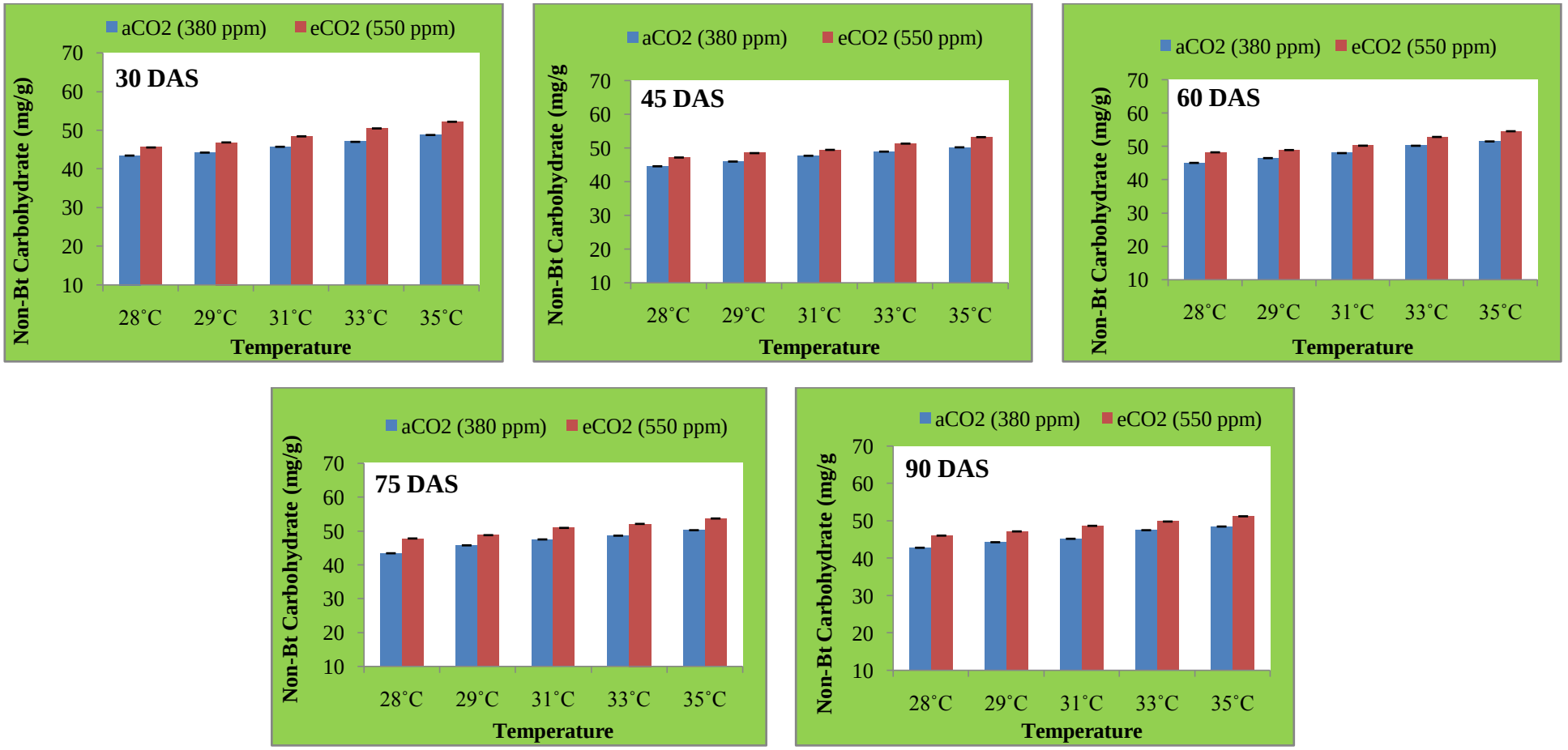


Fig. 4.55. Effect of *e*CO_2 and *e*Temp on carbohydrate content in non-Bt cotton foliage

Table 4.72. Effect of *e*CO_2 and *e*Temp on carbohydrate content in Bt cotton foliage

Temperature	Carbohydrate (mg g^{-1}) content in Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	42.48	43.78	**43.13**	43.87	46.18	**45.03**	45.30	47.27	**46.28**	43.40	44.73	**44.07**	42.42	43.3	**42.86**
29 ± 1°C	43.30	45.18	**44.24**	44.60	47.67	**46.13**	46.68	48.75	**47.72**	44.78	45.57	**45.18**	43.55	43.72	**43.63**
31 ± 1°C	44.57	47.22	**45.89**	46.62	49.23	**47.93**	48.57	50.17	**49.37**	46.23	47.20	**46.72**	44.97	44.72	**44.84**
33 ± 1°C	46.40	48.72	**47.56**	48.08	50.38	**49.23**	49.87	52.32	**51.09**	47.87	49.00	**48.43**	46.22	46.32	**46.27**
35 ± 1°C	47.67	49.90	**48.78**	49.20	51.30	**50.25**	50.52	54.38	**52.45**	49.20	50.15	**49.68**	47.08	48.03	**47.56**
Mean	**44.88**	**46.96**		**46.47**	**48.95**		**48.18**	**50.57**		**46.29**	**47.33**		**44.84**	**45.21**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	201.41*	0.10	0.31	405.22*	0.09	0.26	314.62*	0.10	0.28	51.528*	0.10	0.30	7.485*	0.096	0.282
Temperature (°C)	200.51	0.16	0.48	243.9*	0.14	0.41	272.21	0.15	0.44	203.17*	0.16	0.48	159.92*	0.151	0.446
Interaction (CO_2 + Temp(°C))	2.46	0.23	NS	1.87	0.20	NS	8.515*	0.21	0.63	0.42	0.23	NS	3.003*	0.214	0.631
CV	0.87%			0.71%			0.75%			0.84%			0.82%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

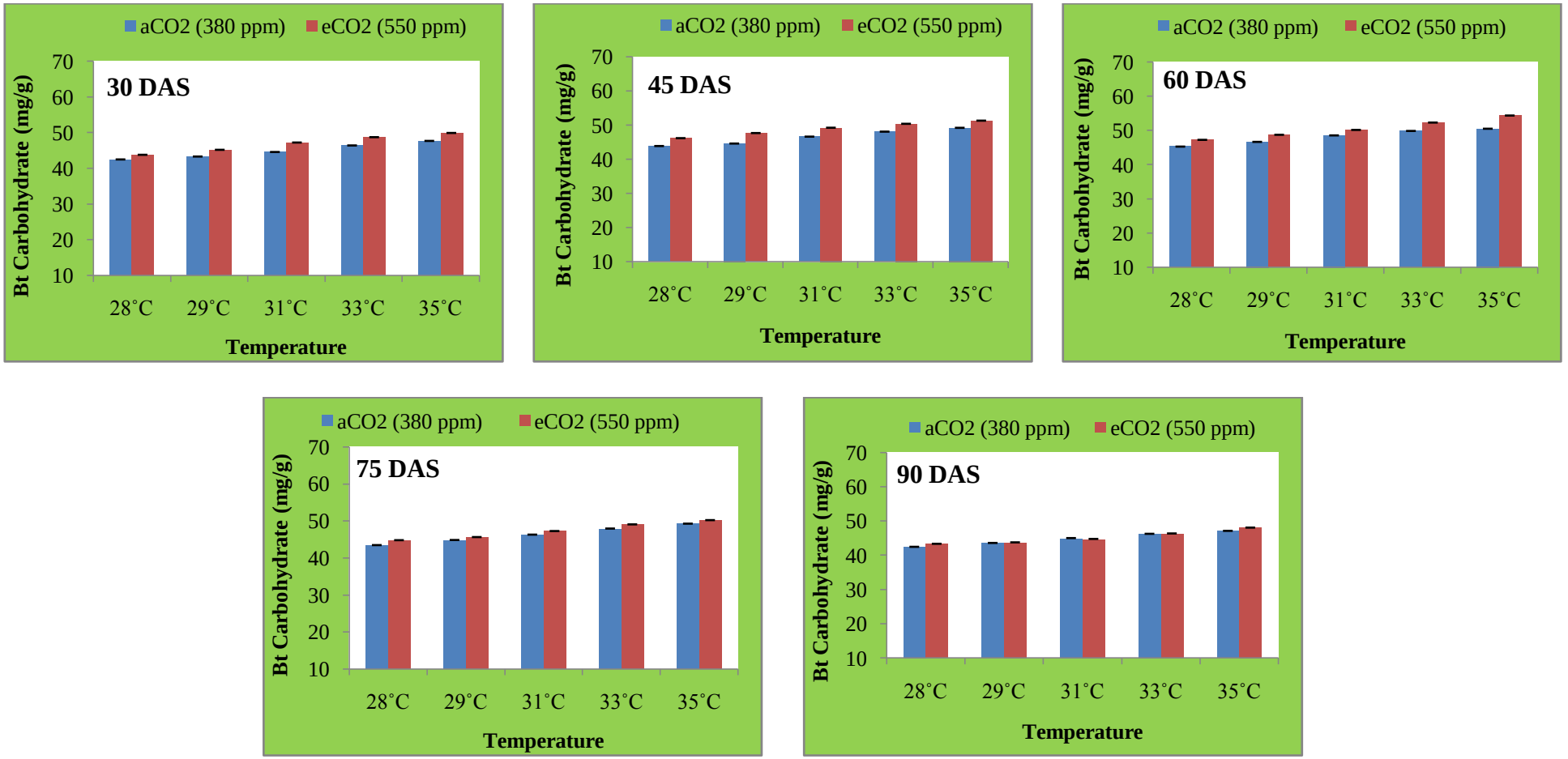


Fig. 4.56. Effect of *e*CO_2 and *e*Temp on carbohydrate content in Bt cotton foliage

Carbohydrate content in Bt cotton: Carbohydrate content in cotton leaves increased upto 60 DAS and thereafter it decreased (Table 4.72 and Fig. 4.56). The highest carbohydrate content recorded was 54.38 mg g^{-1} under *e*CO_2 at 35 ºC temperature at 60 DAS and lowest recorded was 42.42 mg g^{-1} under *a*CO_2 at 28 ºC at 90 DAS. A significant impact of carbon dioxide and temperature was observed with respect to the carbohydrate content at different intervals (30, 45, 60, 75 and 90 DAS) in cotton foliage.. Significantly higher carbohydrates content was found in the cotton foliage at 30 DAS (43.78 to 49.90 mg g^{-1}), 45 DAS (46.18 to 51.30 mg g^{-1}), 60 DAS (47.27 to 54.38 mg g^{-1}), 75 DAS (44.73 to 50.15 mg g^{-1}) and at 90 DAS (43.30 to 48.03 mg g^{-1}) under *e*CO_2 at all temperatures (28 to 35 ºC). The carbohydrate content of cotton foliage was significantly lower under *a*CO_2 and all temperatures (28 to 35 ºC) at 30 DAS (42.48 to 47.67 mg g^{-1}), 45 DAS (43.87 to 49.20 mg g^{-1}), 60 DAS (45.30 to 50.52 mg g^{-1}), 75 DAS (43.40 to 49.20 mg g^{-1}) and at 90 DAS (42.42 to 47.08 mg g^{-1}) compared to *e*CO_2 at corresponding temperatures. Interactive effect of *e*CO_2 and *e*Temp has shown significant effect only at 60 DAS and 90 DAS intervals.

It can be briefed that in Bt cotton, carbohydrate content increased by 4 % (*e*CO_2) and by 20 % (*e*CO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically increasing carbohydrate levels of foliage, and that temperature is an indispensible factor in climate change investigations on crop pests.

4.3.1.5 Polyphenol content

Polyphenol content in Non-Bt cotton: The polyphenol content was highest at 60 DAS at *e*CO_2 and temperature 35 °C with 4.22 mg g^{-1} and lowest at 30 DAS at *a*CO_2 at temperature 28 °C with 2.81 mg g^{-1}. At particular temperature the polyphenol content was higher at *e*CO_2 compared to *a*CO_2 conditions (Table 4.73 and Fig. 4.57). The polyphenol content increased in with increase in temperature under *e*CO_2 at 30 DAS (2.93 to 3.36 mg g^{-1}), 45 DAS (3.13 to 3.72 mg g^{-1}), 60 DAS (3.74 to 4.22 mg g^{-1}), 75 DAS (3.28 to 3.87 mg g^{-1}) and 90 DAS (2.98 to 3.45 mg g^{-1}). Under *a*CO_2, the polyphenol content increased with increase in temperature at 30 DAS (2.81 to 3.18 mg g^{-1}), 45 DAS (3.06 to 3.57 mg g^{-1}), 60 DAS (3.44 to 4.05 mg g^{-1}), 75 DAS (3.17 to 3.74 mg g^{-1}) and at 90 DAS (2.88 to 3.25 mg g^{-1}) and was lower than at *e*CO_2 and corresponding temperatures.

Table 4.73. Effect of *e*CO_2 and *e*Temp on polyphenol content in non-Bt cotton foliage

Temperature	Polyphenols (mg g^{-1}) content in non Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	2.81	2.93	**2.87**	3.06	3.13	**3.09**	3.44	3.74	**3.59**	3.17	3.28	**3.23**	2.88	2.98	**2.93**
29 ± 1°C	2.86	2.98	**2.82**	3.02	3.28	**3.15**	3.51	3.88	**3.64**	3.23	3.42	**3.28**	2.9	3.06	**2.88**
31 ± 1°C	2.93	3.14	**3.03**	3.25	3.44	**3.34**	3.66	3.98	**3.82**	3.38	3.55	**3.46**	2.97	3.24	**3.11**
33 ± 1°C	3.09	3.24	**3.17**	3.37	3.56	**3.47**	3.80	3.99	**3.90**	3.52	3.64	**3.58**	3.2	3.33	**3.26**
35 ± 1°C	3.18	3.36	**3.27**	3.57	3.72	**3.64**	4.05	4.22	**4.14**	3.74	3.87	**3.80**	3.25	3.45	**3.35**
Mean	**2.93**	**3.13**		**3.25**	**3.42**		**3.67**	**3.96**		**3.38**	**3.55**		**3**	**3.21**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	167.11*	0.01	0.03	126.33*	0.01	0.03	395.81*	0.01	0.03	165.92*	0.01	0.03	363.77*	0.008	0.023
Temperature (°C)	123.106	0.02	0.05	184.06*	0.02	0.05	180.09*	0.02	0.05	263.7*	0.01	0.04	262.19*	0.013	0.037
Interaction (CO_2 + Temp(°C))	5.457*	0.02	0.07	4.181*	0.02	0.07	13.754*	0.02	0.07	6.698*	0.02	0.06	17.061*	0.018	0.052
CV	1.39%			1.23%			1.05%			1.01%			0.99%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

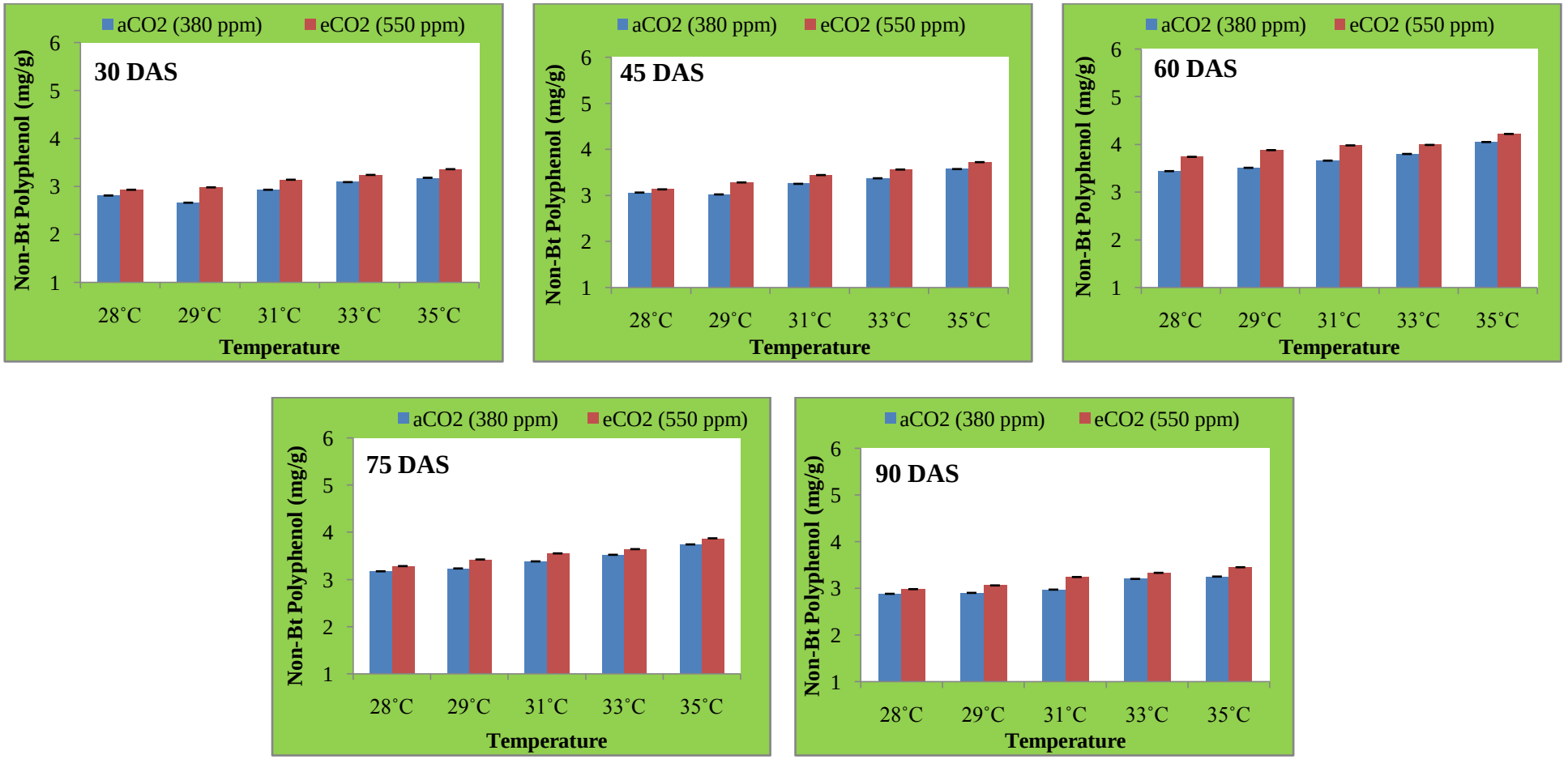


Fig. 4.57. Effect of *e*CO_2 and *e*Temp on polyphenol content in non-Bt cotton foliage

It can be briefed that in non-Bt cotton, polyphenol content increased by 9 % (eCO_2) and by 23 % (eCO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically increasing polyphenol levels of foliage, and that temperature is an indispensible factor in climate change investigations on crop pests. Results are in line with the findings of Srinivasa Rao *et al.* (2014b) who recorded 2-14 % higher polyphenol content in eCO_2 (1.90 % and 1.69 %) over ambient (1.66 %) in groundnut. Dury *et al*. (1998) reported that larval development of *Operophthera brumata* was detrimentally affected by increased phenolic content and decreased nitrogen content of oak leaves due to higher CO_2 levels. It is a general perception that secondary metabolites inhibit growth by reducing the efficiency of conversion of assimilated and ingested food. However they might stimulate growth, survival and pupation at low concentrations apparently due to *hormoligosis* (Annadurai *et al.,* 1990), the phenomenon in which harmful quantities of certain stress agents might be stimulating (Luckey, 1968). Polyphagous insects feeding on diverse diet may have certain fitness advantages that outweigh diet specialization (Muller *et al*., 2014), through adaptation to those plant defensive substances by evolving physiological detoxification (Raffa, 1987) and biochemical detoxiofication mechanisms (Gould, 1984).

Polyphenol content in Bt cotton: The influence of eCO_2 and *e*Temp was significant on polyphenol content of cotton foliage at all intervals. The polyphenol content was higher under eCO_2 at all temperatures (28-35 ºC) compared to aCO_2 conditions (Table 4.74 and Fig. 4.58). The polyphenol content increased with increase in temperature under eCO_2 at 30 DAS (2.94 to 3.38 mg g^{-1}), 45 DAS (3.30 to 3.72 mg g^{-1}), 60 DAS (3.98 to 4.30 mg g^{-1}), 75 DAS (3.43 to 3.82 mg g^{-1}) and 90 DAS (3.07 to 3.48 mg g^{-1} respectively). Under aCO_2, the polyphenol content increased with increase in temperature at 30 DAS (2.87 to 3.28 mg g^{-1}), 45 DAS (3.18 to 3.63 mg g^{-1}), 60 DAS (3.66 to 4.14 mg g^{-1}), 75 DAS (3.30 to 3.77 mg g^{-1}) and at 90 DAS (2.95 to 3.37 mg g^{-1}) but were lower than at eCO_2 and corresponding temperatures.

It can be briefed that in Bt cotton, polyphenol content increased by 9 % (eCO_2) and by 17 % (eCO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically increasing polyphenol levels of foliage, and that temperature is an indispensible factor in climate change investigations on crop pests.

Table 4.74. Effect of *e*CO_2 and *e*Temp on polyphenol content in Bt cotton foliage

Temperature	Polyphenols (mg g^{-1}) content in Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	2.87	2.94	**2.92**	3.18	3.30	**3.24**	3.66	3.98	**3.82**	3.30	3.43	**3.37**	2.95	3.07	**3.01**
29 ± 1°C	2.93	2.98	**2.79**	2.93	3.29	**3.11**	3.73	3.86	**3.65**	3.40	3.52	**3.31**	2.72	3.12	**2.92**
31 ± 1°C	2.95	3.03	**2.99**	3.30	3.44	**3.37**	3.82	4.00	**3.91**	3.49	3.63	**3.56**	3.09	3.2	**3.14**
33 ± 1°C	3.07	3.23	**3.15**	3.43	3.55	**3.49**	3.93	4.19	**4.06**	3.57	3.73	**3.65**	3.15	3.29	**3.22**
35 ± 1°C	3.28	3.38	**3.33**	3.63	3.72	**3.67**	4.14	4.30	**4.22**	3.77	3.82	**3.79**	3.37	3.48	**3.42**
Mean	**2.96**	**3.11**		**3.29**	**3.45**		**3.79**	**4.06**		**3.44**	**3.62**		**3.05**	**3.23**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	110.72*	0.01	0.03	142.57*	0.01	0.03	320.99*	0.01	0.03	217.00*	0.01	0.03	132.12*	0.01	0.032
Temperature (°C)	166.34*	0.02	0.05	203.6*	0.02	0.05	169.68*	0.02	0.05	225.30*	0.01	0.04	125.40*	0.01	0.051
Interaction (CO_2 + Temp(°C))	8.026*	0.02	0.07	12.962*	0.02	0.06	10.910*	0.02	0.07	28.022*	0.02	0.06	13.23*	0.02	0.072
CV	1.31%			1.11%			1.06%			0.92%			1.35%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

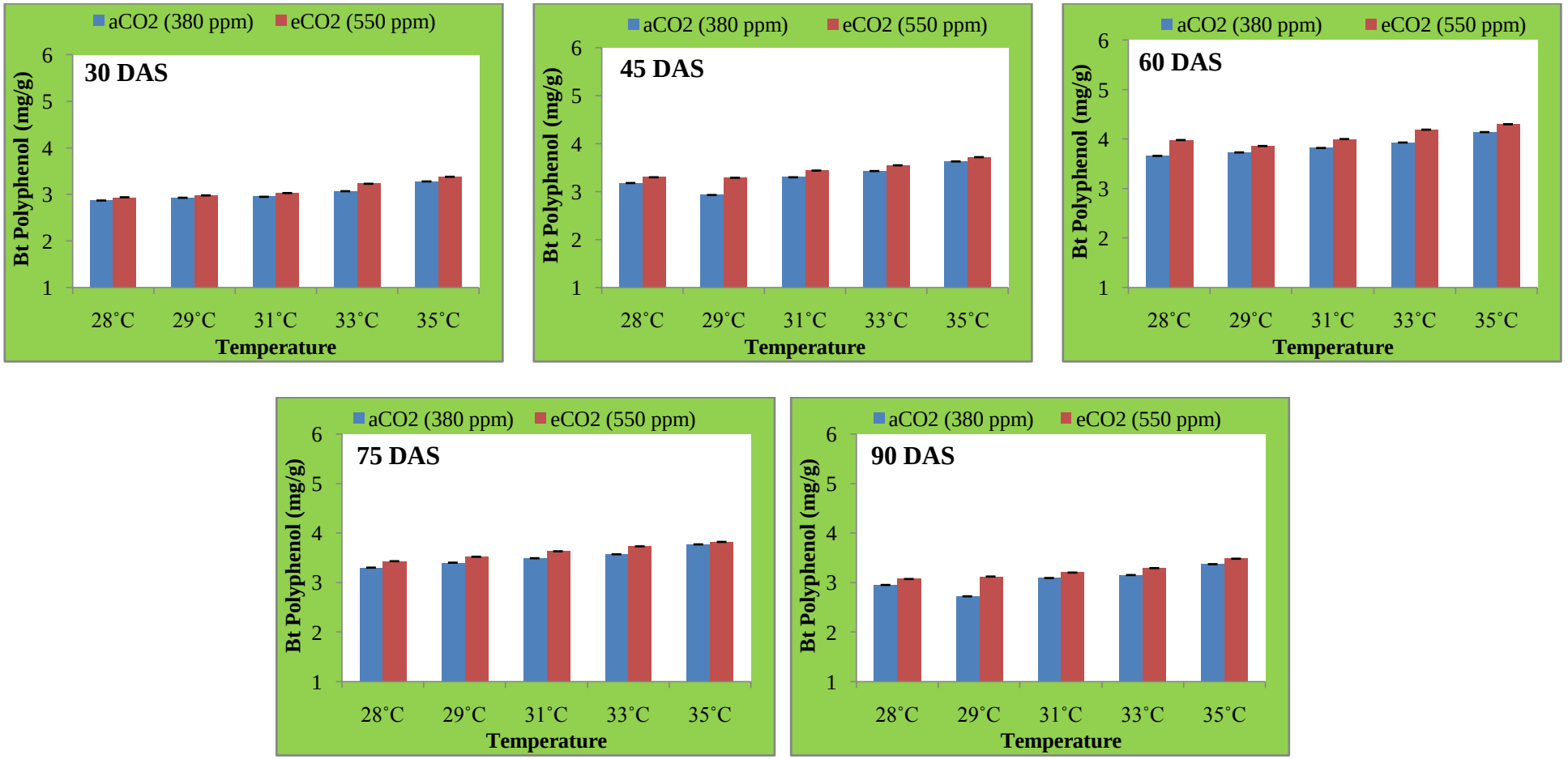


Fig. 4.58. Effect of *e*CO_2 and *e*Temp on polyphenol content in Bt cotton foliage

4.3.1.6 Protein content

Protein content in Non-Bt cotton: As the growth progresses, the protein content also increased upto 60 DAS after which it declined. The highest protein content was recorded at 60 DAS at *a*CO_2 and 28 °C with 29.71 mg g^{-1} and lowest at 30 DAS at *e*CO_2 and 35 °C with 12.52 mg g^{-1}. The data on protein content in the cotton foliage under *e*CO_2 and *e*Temp was presented in the Table 4.75 and Fig. 4.59. At 30 DAS, the protein content was lower under *e*CO_2 (20.22 to 12.52 mg g^{-1}) at five temperatures (28 to 35 ºC) as compared to *a*CO_2 conditions (25.32 to 17.37 mg g^{-1}).

At 45 DAS, with low protein content was recorded at temperatures (28 to 35 °C) under *e*CO_2 (22.23 to 14.35 mg g^{-1}) than at *a*CO_2 (27.50 to 19.21 mg g^{-1}). At 60 DAS, the protein content was more under *a*CO_2 (29.71 to 20.11 mg g^{-1}) at temperatures (28 to 35 ºC) compared to *e*CO_2 (26.67 to 17.30 mg g^{-1}). At 75 DAS, higher per cent protein was found under *a*CO_2 (26.58 to 15.28 mg g^{-1}) compared to *e*CO_2 (23.15 to 14.14 mg g^{-1}). Similarly, at 90 DAS, the protein content in the cotton foliage was higher under *a*CO_2 (22.35 to 14.97 mg g^{-1}) than at *e*CO_2 (21.94 to 13.44 mg g^{-1}) at corresponding temperatures. Interaction effect has significant influence on protein content of cotton foliage at all stages of the plant.

It can be briefed that in non-Bt cotton, protein content decreased by 10 % (*e*CO_2) and by 42 % (*e*CO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically decreasing protein levels of foliage, and that temperature is an indispensible factor in climate change investigations on crop pests. The present results were in agreement with Wu *et al*. (2006) who reported that protein content of spring wheat decreases from 1.53 to 1.34 g L^{-1} with increase in CO_2 to 750 ppm. The decrease in protein content with leaf age is due to degradation of chloroplast proteins during senescence (Martinez *et al.,* 2008). High temperature affects cell division as well as the cell expansion, which is one of the major stresses stimulating protein degradation in turn causes tissue death (Scheurwater *et al*., 2000). Haba *et al*. (2014) reported marked decrease in protein content at higher temperatures (33:19 ºC) by 70 % compared to ambient conditions *i.e.,* 23:19 ºC (40 %). Deekshita (2020) observed that protein content of sunflower foliage grown at *e*CO_2 + *e*Temp reduced by 35.90, 33.74, 28.20, 24.62 and 23.45 % at 30, 45, 60, 75 and 90 DAS, respectively compared to ambient temperature *a*CO_2 + *a*Temp.

Table 4.75. Effect of *e*CO_2 and *e*Temp on protein content in non-Bt cotton foliage

Temperature	Protein (mg g^{-1}) content in non-Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	25.32	20.22	**22.77**	27.50	22.23	**24.86**	29.71	26.67	**28.19**	26.58	23.15	**24.86**	22.35	21.94	**22.14**
29 ± 1°C	21.26	18.46	**19.86**	23.94	20.40	**22.17**	27.55	21.15	**24.35**	24.11	20.64	**22.37**	20.43	19.41	**19.92**
31 ± 1°C	19.92	16.95	**18.43**	21.24	19.86	**20.55**	23.92	19.57	**21.74**	22.34	17.73	**20.03**	19.51	15.48	**17.49**
33 ± 1°C	18.57	14.58	**16.57**	20.80	16.67	**18.73**	22.92	18.56	**20.74**	18.45	16.58	**17.51**	15.09	14.7	**14.89**
35 ± 1°C	17.37	12.52	**14.94**	19.21	14.35	**16.78**	20.11	17.30	**18.70**	15.28	14.14	**14.71**	14.97	13.44	**14.2**
Mean	**20.48**	**16.54**		**22.53**	**18.70**		**24.84**	**20.65**		**21.35**	**18.44**		**18.47**	**16.99**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	21120.19*	0.05	0.14	10656.77*	0.08	0.24	10408.39*	0.06	0.18	5736.02*	0.06	0.19	887.86*	0.092	0.271
Temperature (°C)	2682.548*	0.07	0.22	851.368*	0.13	0.38	1257.605*	0.10	0.28	953.412*	0.10	0.30	438.72*	0.145	0.429
Interaction (CO_2 + Temp(°C))	522.392*	0.10	0.31	163.257*	0.18	0.54	253.724*	0.14	0.40	86.119*	0.14	0.43	20.986*	0.206	0.607
CV	0.97%			1.41%			0.94%			1.43%			2.39%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

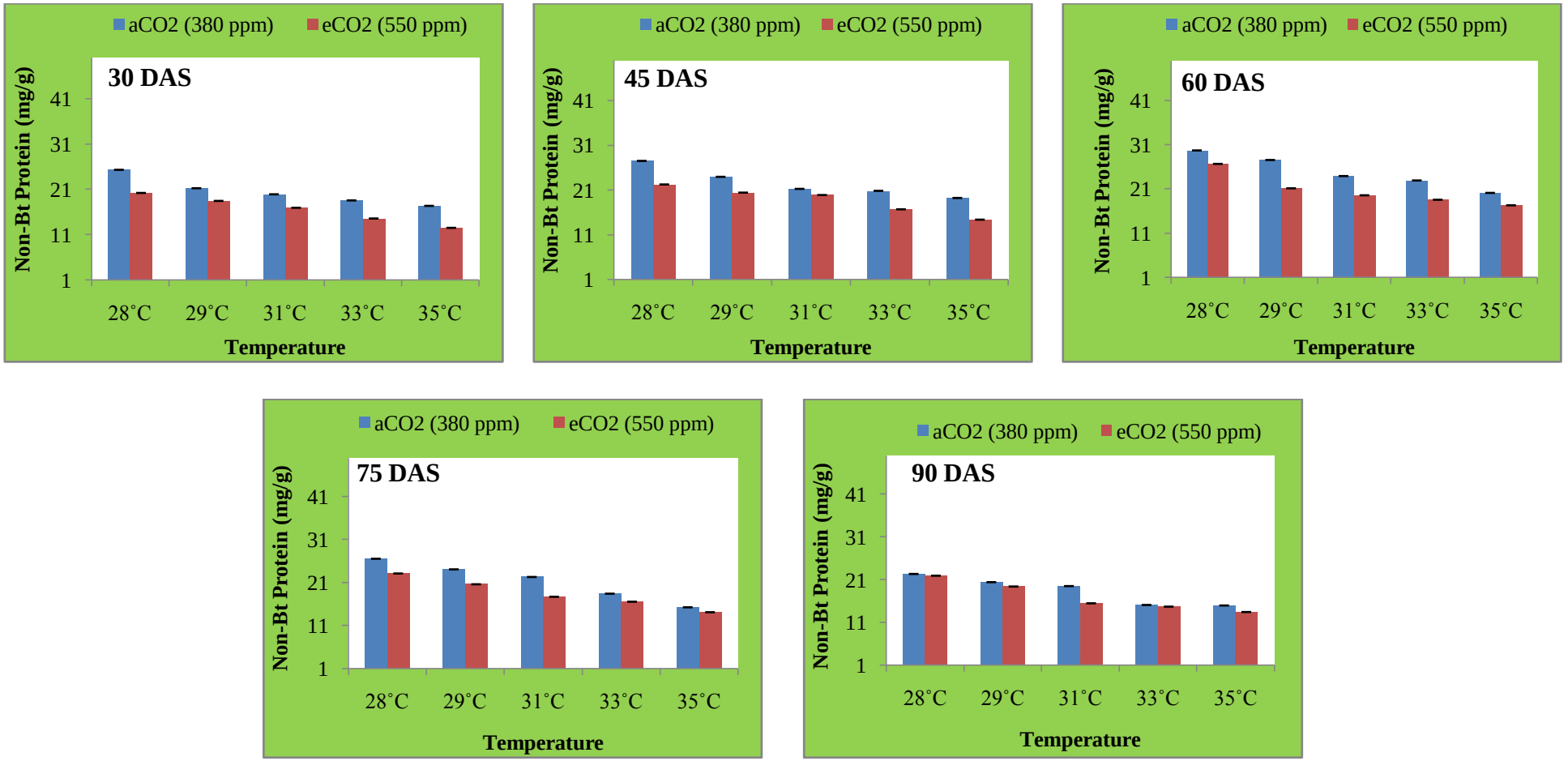


Fig. 4.59. Effect of *e*CO_2 and *e*Temp on protein content in non-Bt cotton foliage

Table 4.76. Effect of *e*CO_2 and *e*Temp on protein content in Bt cotton foliage

Temperature	Protein (mg g^{-1}) content in Bt foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	22.33	20.50	**21.41**	27.57	23.59	**25.58**	31.42	28.25	**29.83**	26.27	22.00	**24.13**	23.26	19.5	**21.38**
29 ± 1°C	19.17	17.50	**18.33**	24.71	21.24	**22.97**	29.77	24.36	**27.06**	24.72	20.05	**22.38**	22.88	17.66	**20.27**
31 ± 1°C	18.09	17.03	**17.56**	22.30	19.50	**20.90**	27.78	22.59	**25.18**	23.06	17.11	**20.08**	20.89	16.11	**18.5**
33 ± 1°C	15.43	15.57	**15.50**	19.48	17.01	**18.24**	25.16	21.87	**23.51**	20.65	16.68	**18.66**	17.55	14.49	**16.02**
35 ± 1°C	14.09	13.89	**14.04**	17.18	16.82	**17.00**	24.77	20.22	**22.49**	19.14	15.62	**17.38**	16.03	13.96	**14.99**
Mean	**17.82**	**16.91**		**22.24**	**19.63**		**27.78**	**23.45**		**22.76**	**18.29**		**20.12**	**16.34**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	4356.33*	0.13	0.40	250.82*	0.36	1.06	3669.81*	0.09	0.26	6460.44*	0.05	0.16	1357.78*	0.065	0.191
Temperature (°C)	240.516*	0.21	0.63	135.79*	0.57	1.67	358.867*	0.14	0.41	981.97*	0.09	0.25	816.506*	0.103	0.303
Interaction (CO_2 + Temp(°C))	36.581*	0.30	0.88	1.03	0.80	NS	102.386*	0.20	0.58	23.559*	0.12	0.35	37.877*	0.145	0.428
CV	2.13%			6.29%			1.25%			1.05%			1.42%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

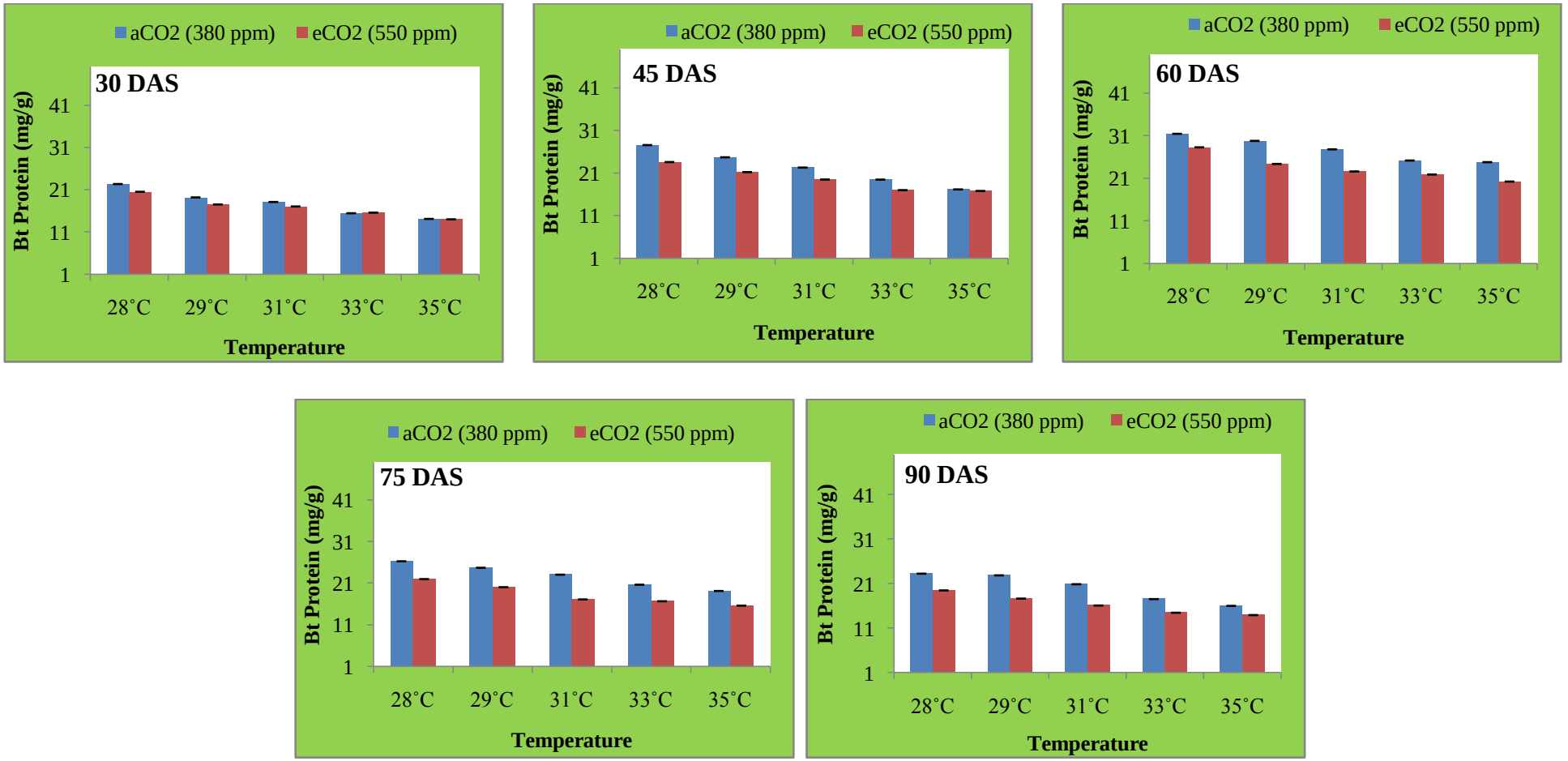


Fig. 4.60. Effect of *e*CO_2 and *e*Temp on protein content in Bt cotton foliage

Protein content in Bt cotton: With the progress of growth, the protein content also increased upto 60 DAS after which it declined below the initial level. The data on protein content in the cotton foliage under *e*CO_2 and *e*Temp was presented in the Table 4.76 and Fig. 4.60. Higher CO_2 concentration negatively affected the protein level. It also realized peak protein concentration around 60 DAS. At 30 DAS, the protein content was lower under *e*CO_2 (20.50 to 13.99 mg g^{-1}) at five temperatures (28- 35 ºC) as compared to *a*CO_2 conditions (22.33 to 14.09 mg g^{-1}). At 45 DAS, with low protein content was recorded at temperatures (28-35 ºC) under *e*CO_2 (23.59 to 16.82 mg g^{-1}) than at *a*CO_2 (27.57 to 17.18 mg g^{-1}). At 60 DAS, the protein content was more under *a*CO_2 (31.42 to 24.77 mg g^{-1}) at temperatures (28 to 35ºC) compared to *e*CO_2 (28.25 to 20.22 mg g^{-1}). At 75 DAS, higher per cent protein was found under *a*CO_2 (26.27 to 19.14 mg g^{-1}) compared to *e*CO_2 (22.00 to 15.62 mg g^{-1}). Similarly, at 90 DAS, the protein content in the cotton foliage was higher under *a*CO_2 (23.26 to 16.03 mg g^{-1}) than at *e*CO_2 (19.50 to 13.96 mg g^{-1}) at corresponding temperatures.

It can be briefed that in Bt cotton, protein content decreased by 10 % (*e*CO_2) and by 36 % (*e*CO_2 +*e*Temp) at 60 DAS. It shows the impact of interactive effect on drastically decreasing protein levels of Bt cotton foliage, and that temperature is an indispensible factor in climate change investigations on crop pests. Similarly, Wu *et al*. (2009) observed that protein content was not significantly different between transgenic Bt cotton and non-transgenic Bt cotton. Wang *et al*. (2015) observed that soluble protein content was reduced and amino acid concentration was enhanced in bolls at temperatures above 38 °C below which they were not affected. The decline rates of the temperatures from 38 to 44 °C were 10.0 to 22.2 % for Sikang-1 (Bt cultivar) and 8.2 to 21.0 % for Sikang-3 (Bt Hybrid), respectively.

4.3.2 Bt endotoxin

Bt protein is the insecticidal toxin which is a kind of soluble protein. Leaf Bt toxin content is related to amino acid and soluble protein, and other enzymes (Chen *et al*. 2007). Its synthesis and degradation is linked to nitrogen metabolism (Wang *et al*., 2015). Oosterhuis and Brown, (2004)., Wang *et al.* (2012) and Chen *et al.* (2018) reported that the concentration of Bt protein in plant tissues was also significantly positively related with total soluble protein and total nitrogen.

To maintain optimum Bt toxin, nitrogen fertilization was resorted to by Coviella *et al.* (2002) and Chen *et al.* (2019). Coviella *et al.* (2002) reported that production of nitogen based toxin was effected by an interaction between CO_2 and nitrogen. Elevated CO_2 decreased nitrogen allocation to Bt, but the reduction was largely alleviated by the addition of nitrogen.

Cry 1Ac and Cry2Ab concentrations in Bt-cotton leaves were 2 and 100 µg g^{-1} dry weight (Meissle and Romeis, 2018, Hagenbucher *et al*. 2017). Bahar *et al*. (2019) reported that only 1 % of *H. armigera* larvae survived after 6 days on greenhouse grown Bollgard II plants compared to 31 % on non-Bt cotton plants. Many larvae survived on reproductive parts than on vegetative parts on Bollgard II plants. The concentration of Cry1Ac toxin did not differ between plant structures, whereas Cry2Ab toxin differed significantly. However they could not find relationship between the level of expression and the location of larvae.

4.3.2.1 Cry1Ac content: The Bt endotoxin Cry1Ac is present in higher amounts in early stage of crop growth. As time progresses the toxin quantity decreases. With increase in temperature also there is a corresponding decrease in endotoxin content at both *a*CO_2 and *e*CO_2. The data on Cry1Ac content in the cotton foliage under *e*CO_2 and *e*Temp was presented in the Table 4.77 and Fig 4.61 and it significantly varied across different conditions. At 30 DAS, the Cry1Ac content was lower under *e*CO_2 (3.71 to 3.19 µg g^{-1}) at all temperatures (28 to 35 ºC) as compared to *a*CO_2 conditions (4.86 to 4.38 µg g^{-1}).

At 45 DAS, lower Cry1Ac content was recorded at temperatures (28 to 35 ºC) under *e*CO_2 (3.03 to 2.32 µg g^{-1}) than at *a*CO_2 (4.10 to 3.61 µg g^{-1}). At 60 DAS, the Cry1Ac content was more under *a*CO_2 (3.17 to 2.68 µg g^{-1}) at temperatures (28 to 35 ºC) compared to *e*CO_2 (2.21 to 1.85 µg g^{-1}). At 75 DAS, higher per cent Cry1Ac was found under *a*CO_2 (2.47 to 2.07 µg g^{-1}) compared to *e*CO_2 (1.92 to 1.39 µg g^{-1}). Similarly, at 90 DAS, the Cry1Ac content in the cotton foliage was higher under *a*CO_2 (1.86 to 1.48 µg g^{-1}) than at *e*CO_2 (1.18 to 0.66 µg g^{-1}) at corresponding temperatures. Interaction effect has non-significant influence at 30 DAS interval and significant influence at all other intervals of cotton foliage. At 60 DAS, Bt endotoxin Cry1Ac content decreased by 30 % in CO_2 and by 42 % in *e*CO_2 + *e*Temp. At 90 DAS, Cry1Ac content decreased by 37 % in CO_2 and by 65 % in *e*CO_2 + *e*Temp.

Table 4.77. Effect of *e*CO_2 and *e*Temp on Bt endotoxin (Cry1Ac) content in Bt cotton foliage

Temperature	Cry1Ac (µg g^{-1}) content in Bt cotton foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	4.86	3.71	**4.28**	4.10	3.03	**3.57**	3.17	2.21	**2.69**	2.47	1.92	**2.20**	1.86	1.18	**1.52**
29 ± 1°C	4.77	3.51	**4.14**	4.01	2.71	**3.36**	3.01	2.12	**2.57**	2.35	1.81	**2.08**	1.77	1.10	**1.44**
31 ± 1°C	4.61	3.39	**4.00**	3.88	2.66	**3.27**	2.88	2.09	**2.48**	2.23	1.66	**1.94**	1.65	0.99	**1.32**
33 ± 1°C	4.47	3.32	**3.90**	3.72	2.53	**3.12**	2.74	1.98	**2.36**	2.13	1.48	**1.81**	1.55	0.78	**1.17**
35 ± 1°C	4.38	3.19	**3.79**	3.61	2.32	**2.97**	2.68	1.85	**2.27**	2.07	1.39	**1.73**	1.48	0.66	**1.07**
Mean	**4.61**	**3.42**		**3.86**	**2.65**		**2.89**	**2.05**		**2.25**	**1.65**		**1.66**	**0.94**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	4878.84*	0.01	0.04	7811.02*	0.01	0.03	3643.35*	0.01	0.03	1652.76*	0.01	0.03	2359.55*	0.011	0.031
Temperature (°C)	105.24*	0.02	0.06	223.56*	0.02	0.05	113.96*	0.02	0.05	136.54*	0.02	0.05	125.87*	0.017	0.049
Interaction (CO_2 + Temp(°C))	1.44	0.03	NS	8.962*	0.02	0.06	6.603*	0.02	0.07	3.58*	0.02	0.07	5.01*	0.024	0.069
CV	1.16%			1.16%			1.55%			2.07%			3.13%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

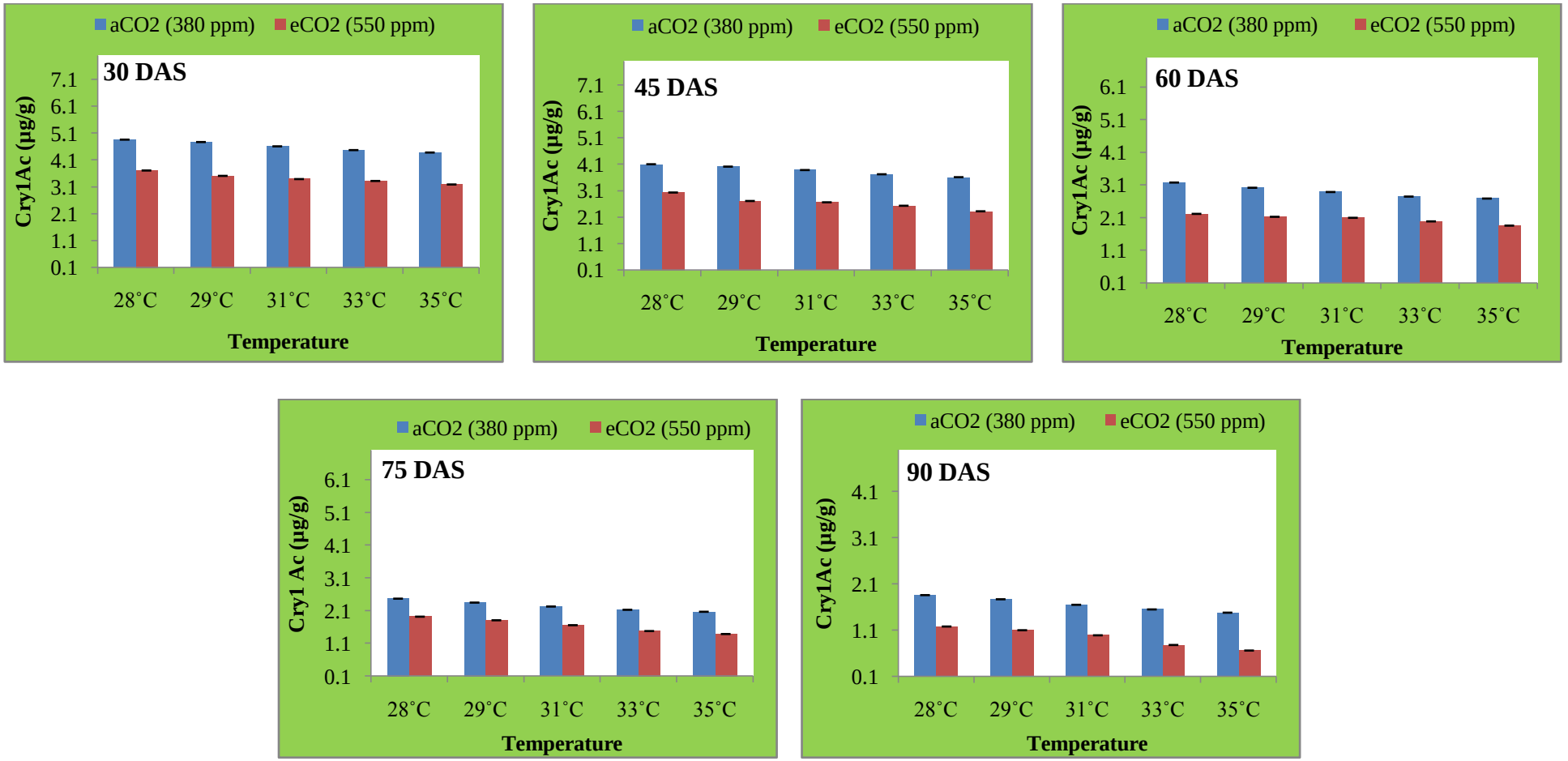


Fig. 4.61. Effect of *e*CO_2 and *e*Temp on Bt endotoxin (Cry1Ac) content in Bt cotton foliage

The data projects the impact of interactive effect on drastically reducing Cry1Ac levels of foliage.

Rana *et al*. (2015) recorded that the highest expression of Bt proteins in cotton were observed at 31-35 °C and declined rapidly at temperatures higher than 40 °C. Supriya *et al*. (2018) estimated the Bt toxin Cry 1Ac in various Bt cotton hybrids at 90-120 DAS, and found that the quantity of the toxin was highest in the leaves over squares and bolls (4.44, 2.09 and 1.09 ppm in leaf, squares and bolls of NCS -207 Bt I hybrid). Cry 1Ac content in the leaves of other Bt I hybrids is as follows: 3.02 ppm in NCS-145, 3.01 ppm in RCH-134 and 2.65 ppm in NCS-954. Dong *et al*. (2000) and Chen *et al*. (2003) demonstrated that nitrogen metabolism is associated with the level of the insecticidal protein in Bt cotton. Chen *et al*. (2005a) reported significant positive correlation between the leaf insecticidal protein and soluble protein content ($r = 0.75^{*}$), and negative correlation between the leaf insecticidal protein and free amino acid content at the boll period ($r = -0.79^{*}$). He showed that high temperature stimulated higher protease activity that resulted in leaf soluble protein along with leaf insecticidal protein i.e. Bt toxin during peak boll development phase.

4.3.2.2 Cry2Ab content: The Bt endotoxin Cry2Ab is present in higher amounts in early stage of crop growth with highest toxin content at *a*CO_2 at 30 DAS interval and temperature 28 °C with 318.55 µg g^{-1} and lowest at 90 DAS and 35 °C at *e*CO_2 with 103.05 µg g^{-1}. The data on Cry2Ab content in the cotton foliage under *e*CO_2 and *e*Temp was presented in the Table 4.78 and Fig 4.62 and it significantly varied across set conditions.

At 30 DAS, the Cry2Ab content was lower under *e*CO_2 (247.76 to 207.04 µg g^{-1}, respectively) at five temperatures (28 to 35 ºC) as compared to *a*CO_2 conditions (318.55 to 260.81 µg g^{-1}). At 45 DAS, low Cry2Ab content was recorded at temperatures (28 to 35 ºC) under *e*CO_2 (214.80 to 173.95 µg g^{-1}) than at *a*CO_2 (270.05 to 227.75 µg g^{-1}). At 60 DAS, the Cry2Ab content was more under *a*CO_2 (236.19 to 191.07 µg g^{-1}) at temperatures (28 to 35 ºC) compared to *e*CO_2 (180.79 to 145.23 µg g^{-1}). At 75 DAS, higher per cent Cry2Ab was found under *a*CO_2 (201.55 to 172.85 µg g^{-1}) compared to *e*CO_2 (162.49 to 121.96 µg g^{-1}). Similarly, at 90 DAS, the

Table 4.78. Effect of *e*CO_2 and *e*Temp on Bt endotoxin (Cry2Ab) content in Bt cotton foliage

Temperature	Cry2Ab (µg g^{-1}) content in Bt cotton foliage after														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	318.55	247.76	**283.16**	270.05	214.80	**242.42**	236.19	180.79	**208.49**	201.55	162.49	**182.02**	152.65	116.16	**134.41**
29 ± 1°C	299.10	242.51	**270.81**	261.11	208.01	**234.56**	217.80	169.42	**193.61**	193.64	151.93	**172.78**	145.63	112.69	**129.16**
31 ± 1°C	289.24	231.46	**260.35**	248.76	195.95	**222.36**	215.72	157.67	**186.70**	187.34	144.65	**165.99**	138.40	109.19	**123.8**
33 ± 1°C	277.50	217.34	**247.42**	239.47	186.53	**213.00**	207.03	148.35	**177.69**	177.51	130.12	**153.82**	128.59	104.15	**121.37**
35 ± 1°C	260.81	207.04	**233.93**	227.75	173.95	**200.85**	191.07	145.23	**168.15**	172.85	121.96	**147.41**	119.51	103.05	**111.28**
Mean	**289.04**	**229.22**		**249.42**	**195.85**		**213.56**	**160.29**		**186.57**	**142.22**		**136.95**	**111.04**	
	F value	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**	**F value**	**SE m**	**CD (p=0.05)**
CO_2	610.37*	1.71	5.05	614.76*	1.53	4.51	524.57*	1.65	4.85	497.16*	1.41	4.15	135.32*	1.575	4.645
Temperature (°C)	50.735*	2.71	7.99	47.164*	2.42	7.13	35.035*	2.60	7.67	39.635*	2.22	6.56	12.247*	2.49	7.345
Interaction (CO_2 + Temp(°C))	1.47	3.83	NS	0.04	3.42	NS	1.26	3.68	NS	1.13	3.15	NS	3.965*	3.521	10.387
CV	2.56%			2.66%			3.41%			3.31%			4.92%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant
DAS – Days after sowing

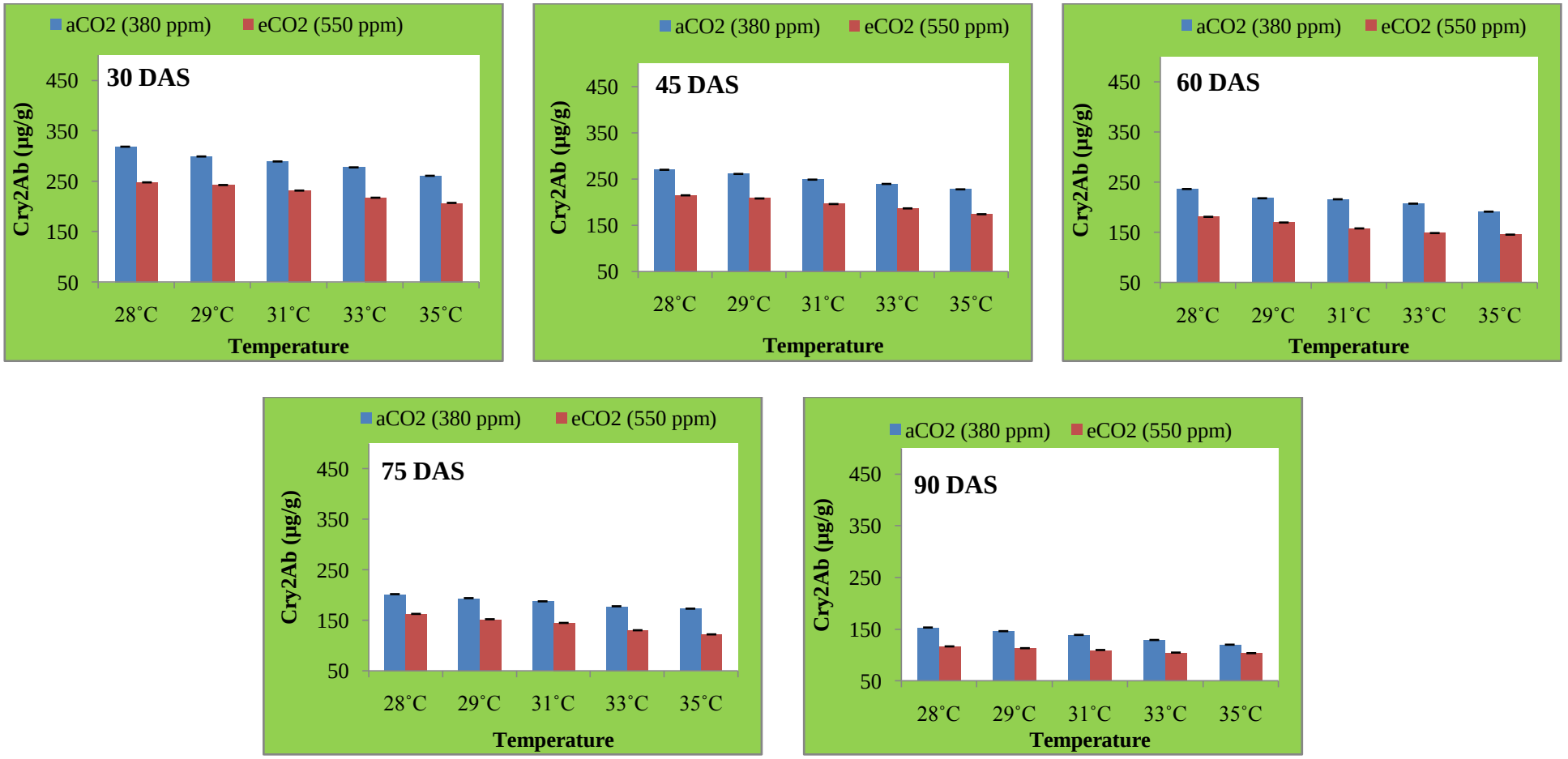


Fig. 4.62. Effect of eCO_2 and *e*Temp on Bt endotoxin (Cry2Ab) content in Bt cotton foliage

Cry2Ab content in the cotton foliage was higher under *a*CO_2 (152.65 to 119.51 µg g^{-1}) than at *e*CO_2 (116.16 to 103.05 µg g^{-1}) at corresponding temperatures. Interaction effect has non-significant influence at all intervals except at 90 DAS of cotton foliage.

At 60 DAS, Bt endotoxin Cry2Ab content decreased by 23 % in CO_2 and by 39 % in *e*CO_2 + *e*Temp. At 90 DAS, Cry2Ab content decreased by 24 % in CO_2 and by 33 % in *e*CO_2 + *e*Temp. The data projects the impact of interactive effect on drastically reducing nitrogen levels of foliage. Supriya *et al*. (2018) reported that the quantity of Cry2Ab toxin is relatively lesser in the leaves (126.20, 210.71 ppm) than that in squares (213.24, 318.14 ppm) and bolls (456.36, 767.08 ppm) in Bt-II hybrids NCS-207 and RCH-134 respectively during 90-120 DAS. In the leaves of the hybrids NCS-145, 950 and 954, Cry1Ac content was 2.63, 1.63 and 2.65 ppm respectively and Cry2Ab was 162.81, 138.31 and 122.78 ppm respectively. In leaves of RCH-2 and 134, the Cry1Ac (1.62, 1.59 ppm) was as per other hybrids but Cry2Ab quantities (201.23 and 210.71 ppm) were the highest among the tested hybrids.

4.3.2.3 Mortality of test insects on Bt cotton foliage

At *a*CO_2, *H. armigera* fed with Bt cotton foliage had highest mortality at 28 °C (69.32 %) and lowest at 35 °C (16.00 %). The mortality of *H. armigera* in *e*CO_2 decreased by 5.77, 9.28, 8.83, 16.63 and 41.75 % compared to *a*CO_2 at 28, 29, 31, 33 and 35 °C respectively (Fig. 4.63). There was no mortality in non-Bt cotton foliage. The decrease in mortality of *H. armigera* at *e*CO_2 is probably explained by the decrease in Bt toxin content of the foliage at *e*CO_2 and *e*Temp.

At *a*CO_2, *S. litura* fed with Bt cotton foliage had highest mortality at 28 °C (64.00 %) and lowest at 35 °C (21.32 %). The mortality of *S. litura* in *e*CO_2 decreased by 4.19, 4.95, 17.65, 29.67 and 49.91 % compared to *a*CO_2 at 28, 29, 31, 33 and 35 °C respectively. There was no mortality in non-Bt cotton foliage. The decrease in mortality of *S. litura* at *e*CO_2 is probably explained by the decrease in Bt toxin content of the foliage at *e*CO_2 and *e*Temp.

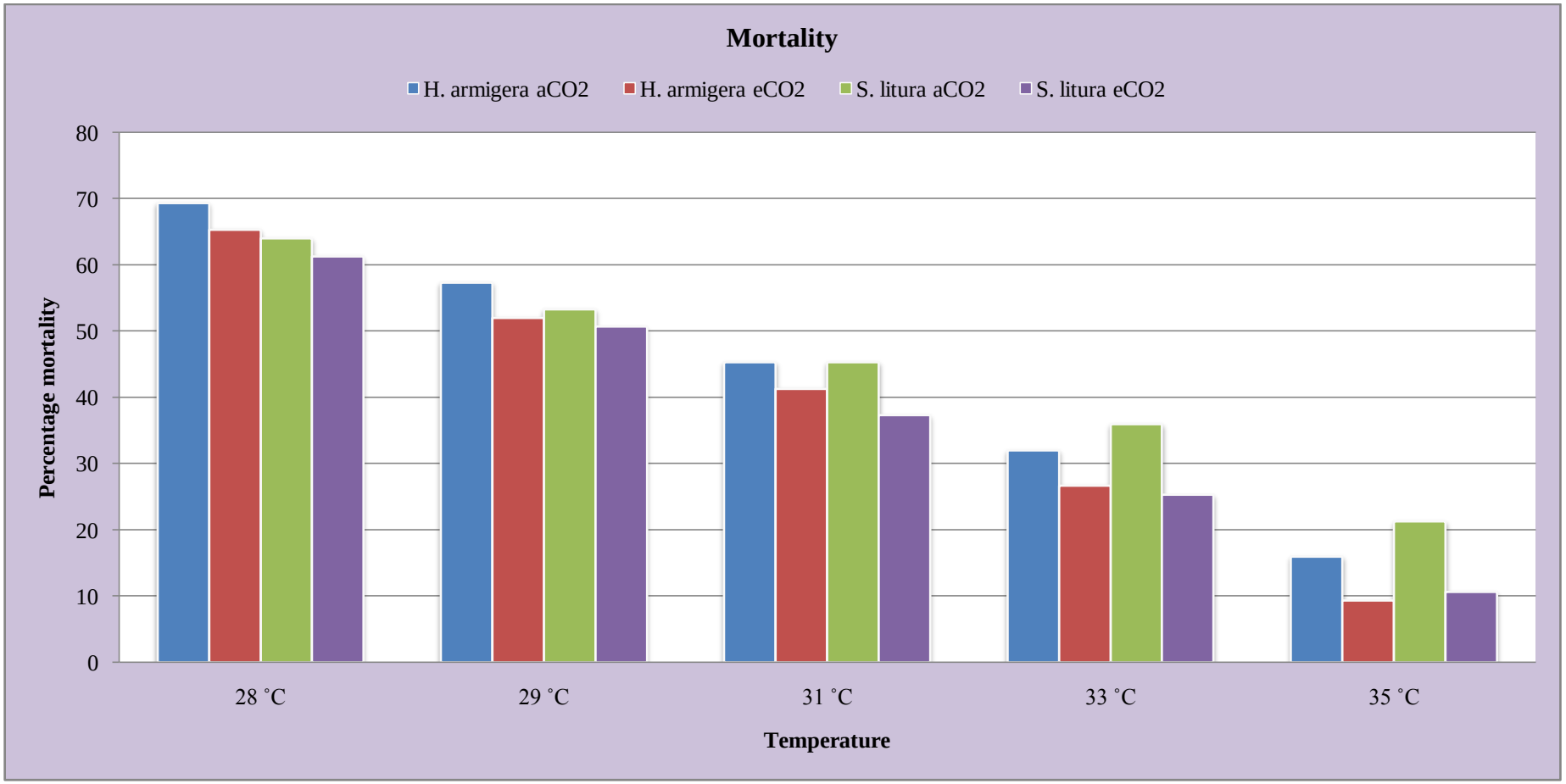


Fig. 4.63. Effect of eCO_2 and *e*Temp on mortality in *H. armigera* and *S. litura* larvae

4.3.3 Biochemical Estimation in Test Insects

4.3.3.1 Carbohydrate estimation

***Helicoverpa armigera*:** The carbohydrate content in *H. armigera* has increased with elevation in carbon dioxide concentration (Table 4.79 and Fig. 4.64). In non-Bt cotton, the carbohydrate content was lower under aCO_2 with 3.88 mg g^{-1} compared to eCO_2 conditions with 4.23 mg g^{-1}. The temperature has inverse relationship with carbohydrate content of insect fed with non-Bt cotton. With increase in temperature there is a decrease in carbohydrate content. The values of carbohydrate content in insects reared at varied temperatures (28 to 35 ºC) are 4.41 to 3.65 mg g^{-1}.

In case of *H. armigera* raised on Bt cotton also similar effect was observed. There was a significant effect of eCO_2 and *e*Temp on carbohydrate content. With increase in concentration of carbon dioxide there was corresponding increase in carbohydrate content in *H. armigera.* High carbohydrate content was recorded at eCO_2 (3.04 mg g^{-1}) than at aCO_2 (2.48 mg g^{-1}). With increase in temperature (28 to 35 ºC) there is a corresponding decrease in carbohydrate content from 3.11 to 2.43 mg g^{-1}.

Succinctly, in non-Bt cotton, carbohydrate content of *H. armigera*, increased by 8 % in eCO_2 and by 9 % in eCO_2 + *e*Temp. Whereas in Bt cotton, it increased by 17 % in eCO_2 and by 6 % in eCO_2 + *e*Temp. The data conveys that though carbohydrate level in *H. armigera* on Bt cotton increases with eCO_2, the cumulative effect of eCO_2 + *e*Temp would not bring forth abrupt spike in carbohydrates in insect body.

***Spodoptera litura*:** The carbohydrate content in *S. litura* has increased with elevation of carbon dioxide (Table 4.79 and Fig. 4.64). In non-Bt cotton, the carbohydrate content was lower under aCO_2 with 3.43 mg g^{-1} as compared to eCO_2 conditions with 3.80 mg g^{-1}. The temperature has negative effect on carbohydrate content of insect fed with non-Bt cotton. With increase in temperature there is a decrease in carbohydrate content. The values of carbohydrate content in insects reared at varied temperatures (28-35 ºC) are 3.96 to 3.30 mg g^{-1}.

In case of *S. litura* grown on Bt cotton also similar effect was observed. There was a significant effect of eCO_2 and *e*Temp on carbohydrate content. With increase in concentration of carbon dioxide there was corresponding increase in carbohydrate

Table 4.79. Effect of *e*CO_2 and *e*Temp on carbohydrate content in *H. armigera* and *S. litura*

Temperature	*H. armigera* carbohydrate (mg g^{-1})						*S. litura* carbohydrate (mg g^{-1})					
	NON-BT			BT			NON-BT			BT		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	4.24	4.59	**4.41**	2.87	3.35	**3.11**	3.75	4.17	**3.96**	2.77	3.18	**2.98**
29 ± 1°C	4.07	4.47	**4.27**	2.62	3.19	**2.91**	3.60	3.97	**3.79**	2.54	3.04	**2.79**
31 ± 1°C	3.94	4.20	**4.07**	2.43	3.09	**2.76**	3.42	3.76	**3.59**	2.39	2.93	**2.66**
33 ± 1°C	3.74	4.07	**3.90**	2.33	2.89	**2.61**	3.29	3.66	**3.47**	2.32	2.85	**2.58**
35 ± 1°C	3.43	3.87	**3.65**	2.16	2.70	**2.43**	3.13	3.48	**3.30**	2.18	2.72	**2.45**
Mean	**3.88**	**4.23**		**2.48**	**3.04**		**3.43**	**3.80**		**2.44**	**2.94**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	129.688*	0.02	0.07	1812.811*	0.01	0.03	413.795*	0.01	0.04	779.554*	0.01	0.04
Temperature (°C)	75.681*	0.04	0.10	315.748*	0.02	0.04	161.433*	0.02	0.06	99.470*	0.02	0.06
Interaction (CO_2 + Temp(°C))	1.00	0.05	NS	4.991*	0.02	0.06	0.57	0.03	NS	1.84	0.03	NS
CV	2.10%			1.31%			1.38%			1.83%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant

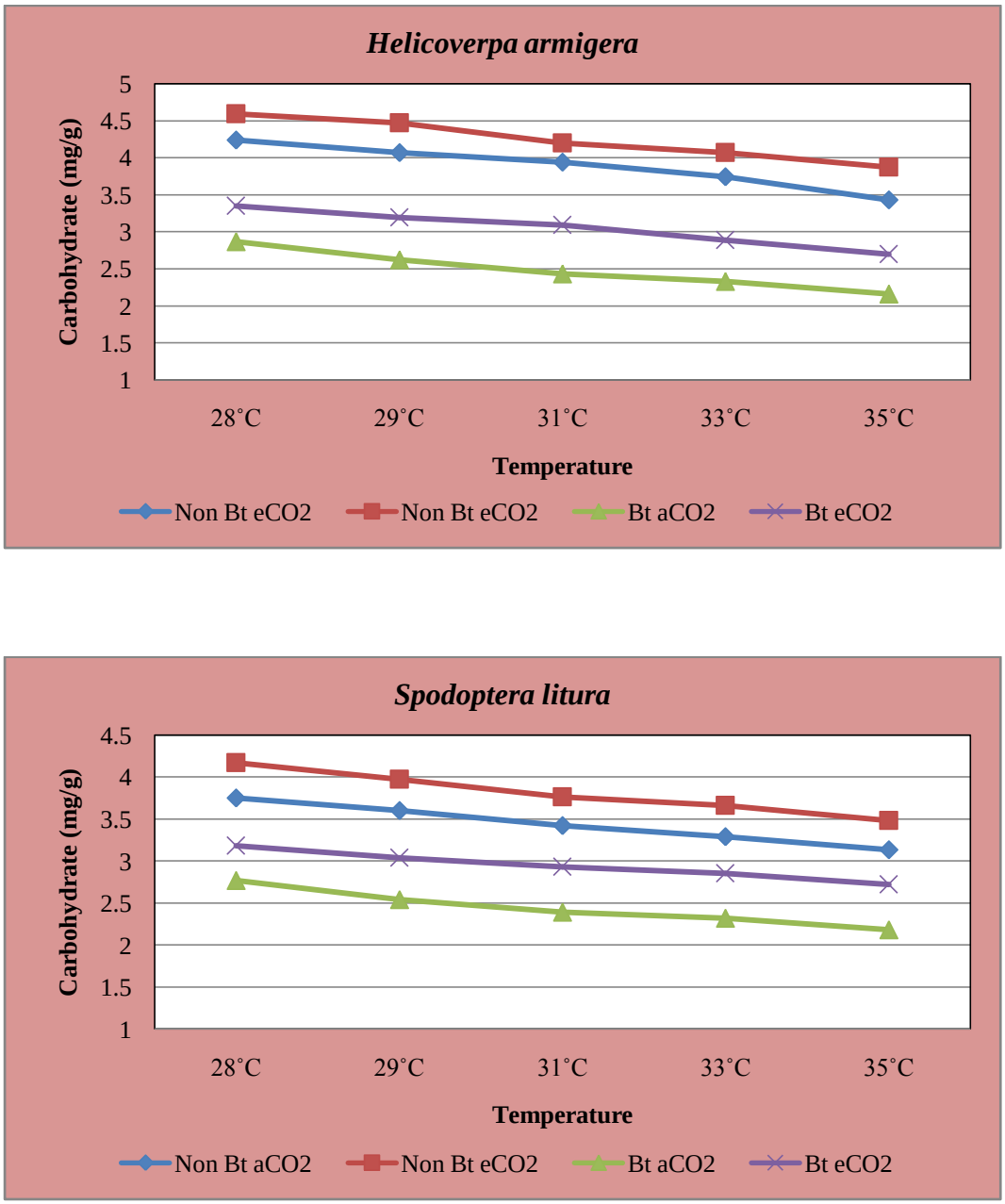


Fig. 4.64. Effect of eCO_2 and *e*Temp on carbohydrate content in *H. armigera* and *S. litura* larvae

content in *S. litura*. High carbohydrate content was recorded at eCO_2 (2.94 mg g^{-1}) than at aCO_2 (2.44 mg g^{-1}). With increase in temperature (28-35 ºC) there is a corresponding decrease in carbohydrate content from 2.98 to 2.45 mg g^{-1} body weight.

Succinctly, in non-Bt cotton, carbohydrate content of *S. litura*, increased by 11 % in eCO_2 and by 7 % in eCO_2 + *e*Temp. Whereas in Bt cotton, it increased by 15 % in eCO_2 and by 2 % in eCO_2 + *e*Temp. The data conveys that though carbohydrate level in *S. litura* on Bt cotton increases with eCO_2, the cumulative effect of eCO_2 + *e*Temp would negate that and may not bring huge sugar imbalance in insect body. Thus on Bt cotton, insects may seem to accumulate higher carbohydrates in eCO_2, but it may not happen in real situation where there is a concomitant increase in temperature. Kamel *et al.* (2018) reported decrease in the carbohydrate content of *S. littoralis* from 25 to 35 °C (9.63-4.77 mg g^{-1} bodyweight). Ismail (2021) reported that carbohydrate content decreased by 75 % with temperature in *S. littoralis* from 28.32 to 6.86 mg g^{-1} body weight when temperature increased from 25 to 35 °C.

4.3.3.2 Protein estimation

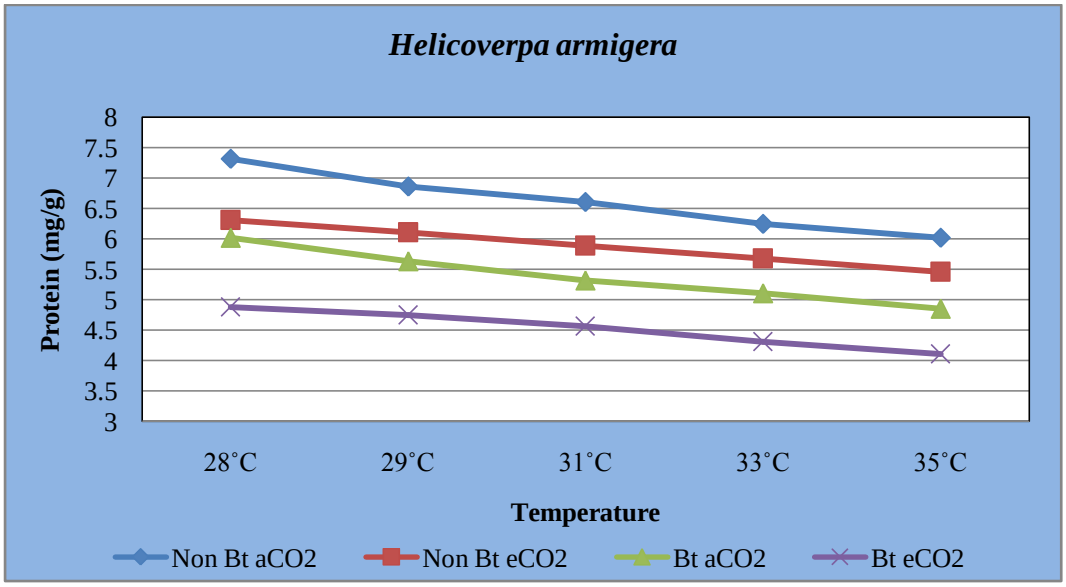
***Helicoverpa armigera*:** The protein content in *H. armigera* decreased with elevation in carbon dioxide and temperature (Table 4.80 and Fig. 4.65). In non-Bt cotton, the protein content decreased with temperature from 28-35 °C under aCO_2 (7.32-6.02 mg g^{-1}) and eCO_2 (6.31-5.46 mg g^{-1}). In case of those larvae fed with Bt cotton also, there was a significant effect of eCO_2 and *e*Temp on insect protein content. With increase in carbon dioxide and temperature, there was corresponding decrease in protein content in *H. armigera.* High protein content was recorded at aCO_2 (6.02-4.85 mg g^{-1}) than at eCO_2 (4.88-4.11 mg g^{-1}). This indicates that protein content is comparatively lesser in Bt fed *H. armigera* than those fed with non-Bt cotton foliage.

Succinctly, in non-Bt cotton, protein content of *H. armigera* decreased by 14 % in eCO_2 and by 25 % in eCO_2 + *e*Temp. Whereas in Bt cotton, it decreased by 19 % in eCO_2 and by 32 % in eCO_2 + *e*Temp. Hachiya *et al*. (2007) reported that proteins denature more rapidly at higher temperatures, which, in turn, requires greater rates of protein synthesis and repair to maintain basic cellular function. Malik and Malik (2009) observed that protein level decreased significantly in mulberry silkworm larvae as well as in pupae, at 36 °C temperature.

Table 4.80. Effect of *e*CO_2 and *e*Temp on protein content in *H. armigera* and *S. litura* larval midgut

Temperature	*H. armigera* larval protein (mg g^{-1})						*S. litura* larval protein (mg g^{-1})					
	NON-BT			BT			NON-BT			BT		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	7.32	6.31	**6.81**	6.02	4.88	**5.45**	7.59	6.71	**7.15**	6.13	5.21	**5.67**
29 ± 1°C	6.86	6.11	**6.48**	5.63	4.75	**5.19**	7.33	6.44	**6.88**	5.96	4.95	**5.46**
31 ± 1°C	6.61	5.89	**6.25**	5.32	4.56	**4.94**	7.03	6.18	**6.60**	5.75	4.74	**5.25**
33 ± 1°C	6.25	5.68	**5.97**	5.11	4.31	**4.71**	6.62	5.88	**6.25**	5.50	4.50	**5.00**
35 ± 1°C	6.02	5.46	**5.74**	4.85	4.11	**4.48**	6.28	5.66	**5.97**	5.19	4.26	**4.73**
Mean	**6.61**	**5.88**		**5.38**	**4.52**		**6.96**	**6.17**		**5.70**	**4.73**	
	F value	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**	**F value**	**SE m**	**CD(p=0.05)**
CO_2	1276.454*	0.01	0.04	2668.578*	0.01	0.04	1134.263*	0.02	0.05	1307.112*	0.02	0.06
Temperature (°C)	346.573*	0.02	0.07	419.628*	0.02	0.06	321.956*	0.03	0.08	1307.112*	0.03	0.09
Interaction (CO_2 + Temp(°C))	16.731*	0.03	0.10	19.121*	0.03	0.08	4.550*	0.04	0.11	0.65	0.04	NS
CV	0.89%			0.93%			0.99%			1.42%		

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant



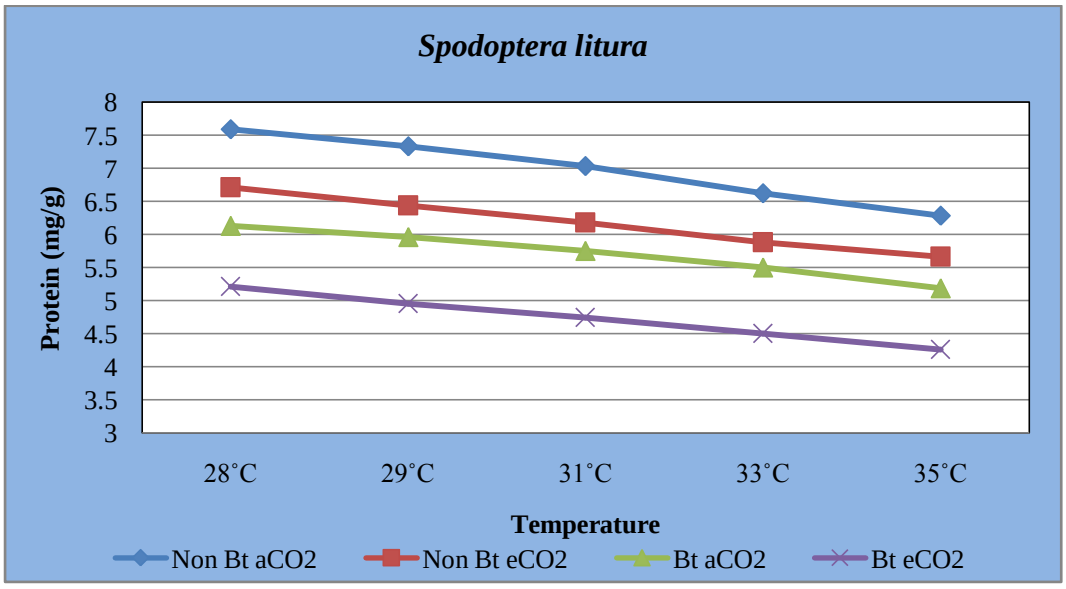


Fig. 4.65. Effect of eCO_2 and *e*Temp on protein content in *H. armigera* and *S. litura* larval midgut

Zeng *et al*. (2012) reported that nymphs of *Nilaparvata lugens* had significantly lower protein and higher glucose content in the *e*CO_2 treatment compared to *a*CO_2.

***Spodoptera litura*:** The protein content in *S. litura* also decreased with elevation of carbon dioxide and temperature (Table 4.80 and Fig. 4.65). In non-Bt cotton, the protein content was higher under *a*CO_2 (7.59-6.28 mg g^{-1}) compared to *e*CO_2 (6.71-5.66 mg g^{-1}) at 28 and 35 °C respectively. Protein content of *S. litura* grown on Bt cotton also had similar influence of CO_2 and temperature. High protein content was recorded at *a*CO_2 (6.13-5.19 mg g^{-1}) than at *e*CO_2 (5.21-4.26 mg g^{-1}). *S. litura* had lower protein levels when provided with Bt cotton foliage compared to non-Bt foliage.Succinctly, in non-Bt cotton, protein content of *S. litura* decreased by 12 % in *e*CO_2 and by 25 % in *e*CO_2 + *e*Temp. Whereas in Bt cotton, it decreased by 15 % in *e*CO_2 and by 31 % in *e*CO_2 + *e*Temp. Similarly, Lemoine and Shantz (2016) reported decrease of protein in *S. exigua* at 30 °C due to decline in nitrogen digestibility by almost 25 % at 30 °C compared with 25 °C. Reduced nitrogen digestibility, coupled with increased cellular division and somatic growth rates, may be responsible for the observed protein limitation at high temperatures. Kamel *et al*. (2018) reported decrease in protein content of *S. littoralis* from 62.33 (20 °C) to 22.67 mg g^{-1} bodyweight (35 °C). Ismail (2021) reported that protein content decreased by 64 % with temperature in *S. littoralis* from 59.62 to 20.88 mg g^{-1} body weight when temperature increased from 25 to 35 °C.

4.3.3.3 Endotoxin estimation in Insects

The Bt endotoxin content in the test insect larvae fed with Bt cotton were very meager (0.02 to 0.04 µg g^{-1}), may be because of the rapid metabolic rate of endotoxins in the insects concerned. Thus, Zhao *et al.* (2016) studied the distribution and metabolism of Bt endotoxin Cry1Ac in various tissues and organs of *H. armigera* and found that Bt endotoxin gets eliminated completely from the *H. armigera* body within 4 – 48 hrs. Meissle and Romeis (2018) stated that concentration of Cry2Ab was proportionally lower in herbivores and their natural enemies, than Cry1Ac, indicating relative instability of Cry2Ab.

Chapter – V

Summary and Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

The present investigations on '**Interactive Effects of Elevated Carbon Dioxide and Temperature on *Helicoverpa armigera* Hub. and *Spodoptera litura* Fab. on Bt cotton**' of Department of Entomology, Agricultural College, Bapatla, Acharya N. G. Ranga Agricultural University (ANGRAU) were conducted during the period 2018-2020 at ICAR – Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad. The results obtained from the investigations are summarized hereunder.

5.1 EFFECT OF *e*CO_2 AND *e*Temp ON GROWTH AND DEVELOPMENT OF HELICOVERPA ARMIGERA AND SPODOPTERA LITURA

The data from the studies on impact of ambient CO_2 (*a*CO_2) and elevated CO_2 (*e*CO_2) (380 and 550ppm ± 25 ppm, respectively) at ambient and elevated temperatures (*a*Temp and *e*Temp) of 28, 29, 31, 33 and 35 ± 1°C on growth and development of test insects has significant effect across three successive generations.

In *H. armigera*, fed with leaves of non-Bt cotton, food ingestion increased by 17.35, 13.63, 19.67, 20.10 and 23.59 %; 18.69, 14.92, 14.00, 18.84 and 18.86 %; and 16.98, 15.75, 14.04, 18.12 and 18.75 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Where as in Bt cotton, an increase of 23.20, 29.69, 33.78, 52.25 and 66.76 %; 20.90, 28.34, 35.50, 46.35 and 65.29 %; and 21.84, 26.84, 31.29, 43.24 and 64.45 % in food ingestion was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Where as in *S. litura*, fed with leaves of non-Bt cotton, food ingestion increased by 13.68, 12.42, 13.10, 17.74 and 23.04 %; 12.61, 11.12, 11.66, 14.73 and 18.69 %; and 12.82, 12.01, 12.00, 13.06 and 18.84 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. But in Bt cotton, an increase of 25.89, 27.98, 33.18, 38.73 and 42.73 %; 23.43, 25.98, 32.68, 33.73 and 41.95 %; and 20.65, 26.62, 32.15, 29.24 and 36.02 % in food ingestion was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

Larval weight of *H. armigera* in non-Bt cotton, increased by 19.76, 18.87, 18.97, 18.43 and 21.90 %; 17.74, 17.14, 17.80, 18.90 and 19.26 %; and 15.74, 16.35, 17.85, 19.07 and 18.51 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton an increase of 32.06, 32.30, 28.21, 31.33 and 31.95 %; 34.62, 31.91, 33.09, 33.89 and 34.94 %; and 31.03, 30.42, 29.88, 31.31 and 33.54 % in larval weight was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Larval weight of *S. litura* in non-Bt cotton, increased by 13.66, 14.34, 15.58, 12.61 and 15.60 %; 14.02, 14.78, 14.72, 14.80 and 17.55 %; and 14.20, 14.72, 15.64, 15.07 and 15.99 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 30.58, 33.45, 37.06, 38.37 and 41.75 %; 25.16, 32.13, 35.96, 34.96 and 38.70 %; and 25.37, 29.40, 35.23, 35.61 and 39.46 % in larval weight was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

Larval excretion of *H. armigera* in non-Bt cotton, increased by 25.42, 17.06, 17.97, 21.48 and 15.11 %; 28.47, 18.23, 9.34, 18.48 and 15.11 %; and 23.89, 21.26, 14.28, 19.86 and 13.47 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 23.05, 27.96, 26.35, 41.61 and 56.32 %; 22.46, 25.41, 33.34, 43.09 and 58.11 %; and 22.43, 23.05, 31.68, 43.27 and 54.43 % in larval excretion was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Larval excretion of *S. litura* in non-Bt cotton, increased by 21.36, 14.29, 16.18, 17.84 and 17.09 %; 20.42, 12.74, 10.51, 14.95 and 16.89 %; and 17.22, 11.78, 10.29, 15.04 and 19.63 % was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 28.42, 31.85, 33.97, 46.79 and 54.71 %; 26.36, 25.43, 34.59, 35.68 and 47.70 %; and 22.45, 22.97, 34.40, 35.86 and 40.79 % in larval excretion was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

Pupal weight of *H. armigera* in non-Bt cotton, decreased by 11.77, 13.51, 10.93, 9.38 and 2.58 %; 9.93, 8.31, 3.92, 7.87 and 3.28 %; and 8.46, 8.44, 6.18, 5.69 and 6.90 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 9.11, 11.36, 11.32, 10.59 and 4.53 %; 9.18, 6.15, 7.32, 8.32 and 5.39 %; and 8.23, 5.95, 3.28, 6.51 and 4.73 %

in pupal weight was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Pupal weight of *S. litura* in non-Bt cotton, decreased by 10.60, 9.30, 9.52, 8.17 and 2.94 %; 8.28, 5.44, 6.43, 8.32 and 5.06 %; and 7.53, 4.96, 4.53, 6.50 and 4.09 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 8.61, 8.73, 10.51, 11.58 and 3.18 %; 7.79, 6.27, 7.04, 8.41 and 5.28 %; and 7.75, 6.45, 3.44, 5.47 and 8.26 % in pupal weight was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

Larval duration of *H. armigera*, in non-Bt cotton, increased by 13.15, 13.80, 14.76, 15.06 and 17.83 %; 13.32, 14.25, 13.57, 14.87 and 17.12 %; and 14.44, 13.85, 14.12, 15.72 and 16.61 % was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, larval duration increased by 8.05, 7.42, 12.15, 10.61 and 16.95 %; 8.21, 8.59, 12.05, 11.09 and 17.63 %; and 9.25, 7.31, 11.11, 11.25 and 17.02 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Larval duration of *S. litura* in non-Bt cotton increased by 12.53, 14.17, 14.16, 13.43 and 16.56 %; 12.63, 13.93, 13.73, 14.12 and 15.65 %; and 12.79, 13.59, 13.97, 14.04 and 15.09 % was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 16.86, 16.02, 16.63, 15.33 and 13.74 %; 15.61, 15.98, 17.31, 15.36 and 13.72 %; and 15.73, 15.36, 16.43, 14.86 and 12.95 % in larval duration was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

Pre-pupal duration of *H. armigera* in non-Bt cotton increased by 15.91, 6.98, 7.50, 13.51 and 11.43 %; 15.22, 21.43, 11.90, 12.20 and 17.14 %; and 5.66, 20.00, 13.95, 14.29 and 15.79 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 12.28, 4.12, 7.01, 4.00 and 15.00 %; 9.00, 5.78, 2.96, 10.53 and 10.96 %; and 9.39, 10.98, 2.22, 7.19 and 12.26 % in pre-pupal duration was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Pre-pupal duration of *S. litura* in non-Bt cotton, increased by 11.76, 12.50, 12.50, 6.67 and 14.29 %; 22.22, 11.76, 12.50, 20.00 and 23.08 %; and 9.09, 10.53, 11.11, 12.50 and 6.67 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and

third generations, respectively. In Bt cotton, an increase of 9.20, 7.74, 6.67, 6.12 and 9.42 %; 8.19, 5.70, 4.64, 8.33 and 11.76 %; and 10.56, 5.99, 11.73, 13.73 and 11.49 % in pre-pupal duration was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

Pupal duration of *H. armigera* in non-Bt cotton, increased by 5.98, 8.66, 11.52, 9.48 and 9.09 %; 4.96, 8.37, 13.06, 8.60 and 8.29 %; and 6.82, 5.86, 12.02, 9.91 and 7.11 % was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 5.27, 6.92, 9.07, 8.11 and 9.55 %; 1.85, 7.69, 7.87, 6.51 and 4.03 %; and 1.73, 4.18, 8.58, 4.67 and 3.45 % in pupal duration was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Pupal duration of *S. litura* in non-Bt cotton, increased by 5.16, 9.57, 10.55, 9.00 and 10.15 %; 4.94, 8.33, 7.79, 10.14 and 6.70 %; and 6.54, 2.86, 9.57, 10.23 and 6.98 % was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 3.44, 7.08, 9.67, 6.26 and 5.48 %; 1.08, 9.00, 6.67, 8.18 and 5.40 %; and 2.43, 4.88, 8.29, 8.05 and 3.27 % in pupal duration was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

Pre-ovipositional duration of *H. armigera* in non-Bt cotton, decreased by 29.82, 31.46, 30.80, 29.00 and 25.84 %; 28.01, 28.57, 31.46, 28.40 and 29.00 %; and 25.93, 27.60, 29.03, 24.02 and 27.54 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 20.00, 27.08, 27.27, 25.00 and 20.00 %; 10.00, 28.00, 28.33, 22.75 and 22.00 %; and 9.09, 25.09, 26.56, 19.09 and 19.53 % in pre-ovipositional period was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Pre-ovipositional duration of *S. litura* in non-Bt cotton, decreased by 18.82, 19.38, 23.87, 24.79 and 18.18 %; 18.95, 15.73, 20.00, 22.36 and 12.39 %; and 28.01, 28.57, 31.46, 28.40 and 29.00 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 11.11, 19.35, 16.50, 11.17 and 14.59 %; 12.00, 9.09, 11.74, 10.53 and 11.35 %; and 4.00, 6.55, 11.11, 0.00 and 8.85 % in pre-ovipositional period was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

Ovipositional duration of *H. armigera* in non-Bt cotton decreased by 9.25, 12.16, 9.18, 9.14 and 10.70 %; 9.74, 9.43, 9.00, 8.19 and 9.39 %; and 8.12, 7.65, 6.06, 6.15 and 8.14 % at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively. In Bt cotton a decrease of 8.00, 9.72, 11.11, 8.26 and 10.76 %; 10.00, 12.00, 11.32, 8.86 and 10.41 %; and 9.68, 11.34, 10.89, 10.56 and 10.17 % in ovipositional duration was recorded at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively. Ovipositional duration of *S. litura* in non-Bt cotton, decreased by 4.59, 6.74, 5.96, 7.20 and 8.97 %; 4.07, 5.22, 3.28, 3.67 and 6.66 %; and 2.95, 5.22, 4.40, 4.50 and 6.21 % at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 6.90, 7.14, 7.99, 4.44 and 7.03 %; 6.67, 5.56, 3.63, 3.03 and 4.33 %; and 6.13, 5.79, 5.79, 5.71 and 6.50 % in ovipositional duration was recorded at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively.

Post-ovipositional duration of *H. armigera* in non-Bt cotton, decreased by 35.60, 41.67, 41.82, 38.98 and 36.12 %; 35.00, 40.31, 40.07, 42.91 and 41.06 %; and 33.99, 39.94, 42.02, 41.44 and 42.49 % at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 33.33, 37.50, 38.46, 40.00 and 37.39 %; 18.18, 25.09, 26.80, 30.04 and 28.44 %; and 16.67, 22.26, 16.80, 24.40 and 29.00 % in post-ovipositional duration was recorded at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively. Post-ovipositional duration of *S. litura* in non-Bt cotton, decreased by 32.24, 30.67, 37.76, 39.03 and 38.43 %; 29.59, 30.67, 28.83, 34.30 and 39.22 %; and 29.18, 31.83, 32.47, 34.28 and 33.97 % at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 30.77, 36.91, 33.45, 33.20 and 40.34 %; 26.15, 31.25, 30.56, 28.00 and 29.00 %; and 21.92, 26.14, 22.33, 22.22 and 25.61 % in post-ovipositional duration was recorded at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively.

Fecundity of *H. armigera* in non-Bt cotton, decreased by 11.84, 11.42, 15.80, 11.91 and 11.43 %; 8.87, 9.55, 9.27, 7.63 and 7.17 %; and 15.28, 14.16, 11.30, 13.30 and 11.91 % at eCO$_2$ over aCO$_2$ at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 7.48, 10.89, 10.05, 10.16

and 9.02 %; 4.34, 5.97, 6.67, 7.05 and 1.88 %; and 9.99, 10.27, 11.95, 12.49 and 8.16 % in fecundity was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. Fecundity of *S. litura* in non-Bt cotton, decreased by 6.41, 7.12, 2.82, 2.26 and 3.26 %; 4.34, 4.82, 3.36, 0.15 and 0.01 %; and 9.59, 9.08, 8.75, 6.44 and 5.26 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, decrease of 4.82, 6.14, 6.60, 1.02 and 0.10 %; 0.42, 0.43, 2.17, 0.20 and 1.11 %; and 1.31, 4.85, 6.72, 5.58 and 5.72 % in fecundity was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

5.2 EFFECT OF *e*CO_2 AND *e*TEMP ON THE FEEDING INDICES OF HELICOVERPA ARMIGERA AND SPODOPTERA LITURA

AD in *H. armigera* in non-Bt cotton, increased by 1.27, 1.56, 2.29, 4.01 and 2.30 %; 0.61, 0.02, 0.60, 1.64 and 1.21 %; and 1.70, 0.93, 1.36, 2.19 and 0.40 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 1.68, 2.26, 2.92, 2.01 and 3.12 %; 0.86, 1.17, 1.51, 1.14 and 2.10 %; and 1.22, 1.50, 0.12, 0.55 and 1.46 % in AD was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. AD of *S. litura* in non-Bt cotton, increased by 4.41, 2.12, 3.15, 2.85 and 3.92 %; 1.05, 3.21, 1.93, 1.25 and 0.73 %; and 1.08, 1.48, 2.11, 0.64 and 0.97 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 1.99, 1.66, 1.27, 1.81 and 2.11 %; 2.00, 1.71, 0.76, 2.44 and 2.66 %; and 2.21, 0.88, 1.23, 1.98 and 1.25 % in AD was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

RCR in *H. armigera* in non-Bt cotton, increased by 17.47, 14.87, 16.38, 17.29 and 8.37 %; 17.33, 16.61, 16.80, 17.72 and 13.43 %; and 16.22, 13.51, 14.00, 18.96 and 15.43 % was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 5.08, 1.18, 9.51, 11.50 and 17.45 %; 1.98, 1.56, 7.78, 10.46 and 22.42 %; and 2.34, 8.22, 4.82, 8.56 and 22.22 % in RCR was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. RCR of *S. litura* in non-Bt cotton, increased by 12.24, 15.51, 15.92, 8.82 and 8.80 %; 0.67, 4.27, 4.07,

2.42 and 2.36 %; and 4.31, 3.15, 7.58, 10.39 and 2.23 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, an increase of 19.90, 18.00, 19.38, 20.36 and 26.50 %; 18.84, 21.89, 31.24, 35.31 and 19.86 %; and 22.04, 32.19, 30.58, 25.44 and 19.21 % in RCR was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

ECI of *H. armigera* in non-Bt cotton, decreased by 1.36, 1.41, 0.54, 4.43 and 2.01 %; 1.27, 1.12, 3.24, 1.94 and 0.60 %; and 5.55, 1.72, 2.92, 6.52 and 4.08 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 18.03, 13.82, 4.14, 1.96 and 6.73 %; 11.68, 9.48, 1.82, 2.59 and 5.76 %; and 11.31, 6.98, 5.66, 9.72 and 6.30 % in ECI was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. ECI of *S. litura* in non-Bt cotton, decreased by 6.59, 4.23, 4.55, 2.02 and 1.54 %; 7.85, 1.12, 2.50, 3.48 and 5.83 %; and 13.07, 7.26, 8.34, 4.05 and 2.95 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease in ECI by 0.63, 1.44, 1.43, 3.39 and 3.85 %; 4.70, 0.58, 2.51, 4.82 and 3.09 %; and 2.12, 4.03, 8.56, 5.40 and 3.98 % was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

ECD in *H. armigera* in non-Bt cotton, decreased by 2.01, 0.88, 1.44, 4.01 and 1.13 %; 4.96, 4.27, 3.41, 11.04 and 2.35 %; and 1.39, 6.27, 1.08, 1.76 and 8.06 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 4.32, 1.50, 2.91, 1.69 and 1.12 %; 9.32, 8.39, 1.03, 3.84 and 3.87 %; and 11.92, 8.42, 2.13, 3.85 and 4.19 % in ECD was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. ECD of *S. litura* in non-Bt cotton, decreased by 6.03, 1.55, 6.93, 6.34 and 5.15 %; 6.08, 1.92, 0.97, 2.31 and 6.38 %; and 7.72, 7.16, 5.05, 1.97 and 5.75 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 3.19, 2.69, 3.25, 5.65 and 5.23 %; 12.38, 11.89, 10.48, 9.55 and 11.79 %; and 7.69, 9.34, 5.68, 7.87 and 7.38 % in ECD was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

RGR of *H. armigera* in non-Bt cotton, decreased by 16.02, 13.94, 13.73, 13.01 and 12.48 %; 14.59, 12.91, 11.92, 12.43 and 8.51 %; and 13.40, 12.74, 11.55, 11.32 and 11.74 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease in RGR by 15.91, 10.11, 11.41, 7.29 and 7.80 %; 14.89, 9.88, 10.57, 7.80 and 7.71 %; and 13.66, 9.46, 9.21, 6.30 and 7.90 % was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. RGR of *S. litura* in non-Bt cotton, decreased by 8.51, 12.71, 13.32, 13.35 and 4.07 %; 13.50, 12.34, 12.05, 12.21 and 11.19 %; and 12.29, 11.49, 11.41, 11.12 and 4.74 % at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively. In Bt cotton, a decrease of 12.65, 14.05, 15.27, 14.97 and 15.30 %; 11.22, 13.23, 14.85, 13.69 and 13.52 %; and 10.72, 12.06, 13.16, 12.42 and 12.59 % in RGR was recorded at *e*CO_2 over *a*CO_2 at corresponding temperatures in first, second and third generations, respectively.

5.3 BIOCHEMICAL CONSTITUENTS IN COTTON FOLIAGE

The biochemical components (nitrogen, carbon, C:N ratio, proteins, carbohydrates and polyphenols) increased with advancement of crop age till 60 DAS and then decreased at 75 and 90 DAS and this decrease indicates translocation of those compounds to the reproductive parts and concomitant reduction in nutritive quality in senescent leaves.

In non-Bt cotton, at *e*CO_2 nitrogen content decreased by 5.64, 8.06, 7.82, 8.75 and 9.82 % and carbon content increased by 25.00, 17.08, 13.38, 14.13 and 12.73 % . The C:N ratio also increased by 32.36, 27.45, 23.12, 24.87 and 24.96 % at *e*CO_2. With increase in CO_2 concentration, carbohydrates increased by 7.04, 5.19, 4.65, 5.28 and 5.95 % and protein content decreased by 10.23, 23.23, 18.19, 19.02 and 13.97 %. The polyphenol content was seen to increase at *e*CO_2 by 8.72, 10.54, 8.74, 5.00 and 4.20 % compared to *a*CO_2. In Bt cotton at *e*CO_2, nitrogen content decreased by 4.13, 3.58, 3.33, 5.63 and 2.56 % and carbon content increased by 28.30, 19.69, 10.92, 14.17 and 16.06 %. The C:N ratio increased by 31.89, 19.91, 14.65, 21.01 and 19.33 % at *e*CO_2 over *a*CO_2. With increase in CO_2 concentration, carbohydrates increased by 4.35, 4.43, 3.29, 4.91 and 7.64 % and protein content decreased by 10.09, 18.17, 18.68, 13.08 and 18.37 %. The polyphenol content also increased at *e*CO_2 by 8.74, 3.49,

4.71, 6.62 and 3.86 % than *a*CO_2. The two Bt endotoxins, Cry1Ac content decreased by 30.28, 29.57, 27.43, 27.74 and 30.97 %; and Cry2Ab decreased by 23.46, 22.21, 26.91, 28.34 and 23.99 %, respectively with increase in CO_2 concentration.

5.4 BIOCHEMICAL CONSTITUENTS IN TEST INSECTS

The carbohydrate content in the larvae of *H. armigera* and *S. litura* fed on non-Bt cotton increased in *e*CO_2 at all the test temperatures by 8.25, 9.83, 6.60, 8.82 and 12.83 %; and 11.20, 10.28, 9.94, 11.25 and 11.18 %, respectively compared to *a*CO_2. Whereas in Bt cotton, an increase of 16.72, 21.76, 27.16, 24.03 and 25.00 %; and 14.80, 19.69, 22.59, 22.84 and 24.77 % was recorded at *e*CO_2.

Midgut protein content in the larvae of *H. armigera* and *S. litura* in non-Bt cotton, decreased by 13.80, 10.93, 10.89, 9.12 and 9.30 %; and 11.59, 12.14, 12.09, 11.08 and 9.87 %, respectively with increase in CO_2 concentration. In Bt cotton, protein level decreased by 18.94, 15.63, 14.29, 15.66 and 15.26 %; and 15.01, 16.95, 17.57, 18.18 and 17.92 % at *e*CO_2.

CONCLUSIONS

Climate change is largely driven by CO_2 and temperature. CO_2 affects insect pests indirectly by host mediated effects. A short period of extreme temperature is unlikely to cause mortality but may modify population dynamics by impacting life history traits. From the perusal of data obtained and findings across the test conditions in Bt and non-Bt cotton for *H. armigera* and *S. litura*, it can be concluded that:

- All the growth parameters were relatively higher in case of test insects fed with non-Bt cotton compared to Bt cotton, perhaps due to the inherent antibiosis in transgenic plants that does not allow for normal growth of the herbivores
- In both non-Bt and Bt cotton, with increase in CO_2 and temperature, the growth parameters and feeding indices of *H. armigera* and *S. litura*, change abnormally with certain peculiar differences
- Food ingestion (13-65 %), larval weight (15-40 %) and excretion (10-54 %) increased with *e*CO_2 and decreased with *e*Temp, however they showed mixed responses in the interaction treatment. *S. litura,* generally considered as the most sturdy pest consumed relatively higher quantity of foliage in each successive generation
- Significantly lower foliar nitrogen and higher carbon and C: N ratio seems to influence the feeding behaviour of *H. armigera* and *S. litura* to ingest more foliage
- In addition, carbohydrates often considered as phagostimulants stimulated higher food ingestion by *H. armigera* and *S. litura* under *e*CO_2 + *e*Temp
- Food ingestion in the interaction treatment (*e*CO_2 + *e*Temp) has a greater influence of temperature, where both the larvae of *H. armigera* and *S. litura* had decreased their food ingestion in non-Bt cotton.
- The apparent increase in food ingestion capacity with every next generation, in *H. armigera* in *e*CO_2 + *e*Temp on Bt cotton is a point of concern, in indicating a greater chance for adaptation to climate change with advancement of generations.
- Larval excretion was higher for *S. litura* in *e*CO_2 + *e*Temp in fibercomparison to *H. armigera*. In both non-Bt and Bt cotton, larval excretion decreased for both the insects with increase in temperature

- Larval weights under *e*CO_2 + *e*Temp increased in both non-Bt and Bt cotton, except for *S. litura* in non-Bt cotton

- Similarly larval and pupal weights were relatively higher for *S. litura*, probably due to the higher amount of leaf ingested

- Pupal weights (3-13 %) decreased across all the test conditions, pronouncing their vulnerability to variation in CO_2 and temperature. Healthy pupae considered as indicators of adult moth fitness, seems to get disturbed under *e*CO_2 + *e*Temp

- Under *e*CO_2 and *e*Temp, in non-Bt and Bt cotton, larval duration increased significantly in both the test insects, which seems to have been necessarily prolonged to consume sufficient nitrogen for attaining a balanced C: N ratio

- Larval (8-17 %) and pupal (2-10 %) duration appears to increase with *e*CO_2, but in combination with *e*Temp (*e*CO_2 + *e*Temp), duration decreases in both the larvae in non-Bt and Bt cotton

- Reproductive biology parameters *viz*., pre-ovipositional, ovipositional and post-ovipositional durations reduced across all conditions in both the insects in either of the cotton types, where decreased pre-ovipositional and ovipositional durations drastically affected fecundity of the female moths.

- Fecundity decreased in elevated conditions in both the test insects in both non-Bt and Bt cotton. Fecundity of *H. armigera* was by and large higher than that of *S. litura. H. armigera* was also observed to excrete more probably including Bt toxins and other harmful substances and escape from mortality. Further, relative growth rate (RGR) is comparatively higher for *H. armigera*. With advancement of generations, larval duration increased for improved sequestration of nutrients to attain more weight in pupal stage. Hence CO_2, temperature and food ingestion influences fecundity through their effects on larval duration and pupal weight. Ultimately these activities increased the chances to transform into healthy pupae and to accommodate and produce more eggs in the adult stage.

- AD (0.5-4 %) and RCR (2-35 %) increased across all the test conditions in both the insects in non-Bt and Bt cotton, commensurate with the increase in food ingestion and the excretion under *e*CO_2 and *e*Temp conditions

- ECI (2-13 %) and ECD (1-11 %) decreased across all the conditions in *H. armigera* and *S. litura* on both non-Bt and Bt cotton foliage which has a direct influence on having lower RGR (5-16 %)

- At elevated conditions, in comparison with *S. litura*, *H. armigera* has higher ECI in Bt cotton, and high RGR in both non-Bt and Bt cotton. It implies that *H. armigera* could be a potential pest in future climate stress scenarios

- Temperature played an important role over and above CO_2 with regard to many growth, development and feeding indices. This was evident by the outcome opposing that of CO_2 in the interaction treatment (eCO_2 + *e*Temp), due to strong exertion by temperature

- In both non-Bt and Bt cotton, under eCO_2 and *e*Temp, nitrogen (3-9 %) and protein (10-23 %) content decreased coupled with increase in carbon (12-28 %), C:N ratio (19-32 %), carbohydrates (4-8 %) and polyphenols (4-9 %) across different temperatures

- Total polyphenols increased in cotton foliage which could have played an important role in the indigestibility of foliage grown under eCO_2+*e*Temp

- Leaf Bt toxins *viz.*, Cry1Ac (27-31 %) and Cry2Ab (22-38 %) quantities reduced with eCO_2+*e*Temp causing decreased mortality of test insects which can pose challenge to both cotton growers and scientific community

- The larval carbohydrate content was relatively higher for *H. armigera* in non-Bt cotton. It was almost equal in Bt cotton for both the larvae. It tends to increase with eCO_2, but ultimately decreased with eCO_2 + *e*Temp, showing a greater influence of temperature over CO_2

- Larval midgut protein content was relatively higher for *S. litura* in non-Bt and Bt cotton. It was observed to decrease indefinitely at all test conditions, as protein get unstable with drastic changes in CO_2 and temperature

- In totality, CO_2 escalates the necessity for the test insects to attain higher nitrogen from the anticipated under-nutritious foliage. Insects would consume more quantities as evident from the increased food ingestion and the enhanced AD and RCR in both the polyphagous pests. Apart from the other growth and feeding indices, higher foliage consumption itself can threaten crop production. Increased larval and pupal durations may play a key role in gaining biomass and sufficient energy for transforming into adults with improved fitness. Besides, they could adapt to changing climate as evident from the higher values generation after generation under elevated conditions.

- Another intriguing issue is the decrease in Bt toxin production in the leaves, at *e*CO_2 + *e*Temp conditions which may increase the vulnerability of Bt cotton hybrids to *H. armigera*. Hence, Bt plants with reduced expression of endotoxins behave similar to non-Bt cotton. At ambient conditions, larval growth and development parameters, and feeding indices were higher in non-Bt cotton. However at elevated conditions of CO_2 and temperature, the per cent increase in these parameters appears to be relatively higher for Bt cotton.

FUTURE LINE OF WORK

- To meet the challenges of agriculture in the backdrop of global warming cumulative simulation studies with interactive abiotic factors *viz.*, CO_2, temperature, humidity and even U.V. radiations and biotic factors like pest natural enemies are essential to be continued and even with crop management problems like drought, floods, weeds etc.

- As plant phenotypic expression is a result of biochemical constitution, molecular studies to assess the multifarious roles of biochemical constituents need to be focused under climatic stress conditions.

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