



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2017; 5(6): 34-37

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Received: 08-09-2017

Accepted: 09-10-2017

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## Impression of elevated CO<sub>2</sub> on the herbivory of tomato fruit borer *Helicoverpa armigera* (Hubner)

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**Abstract**

The present study was conducted to observe the influence of elevated CO<sub>2</sub> concentrations i.e. 550 ppm, 700 ppm on host (Tomato, *Lycopersicon esculentum*) and its insect herbivore (Tomato Fruit Borer, *H. armigera*, Hubner) in relation to ambient CO<sub>2</sub> i.e. 398 ppm concentration in open top chambers and laboratory conditions between September to December, 2015. Biochemical analysis was done for the tomato leaves raised under elevated CO<sub>2</sub> conditions, which included nitrogen, carbon, C:N ratio and phenol content analysis using different methods. The results of biochemical analysis showed that nitrogen per cent was 2.80 per cent and 2.52 per cent as compared with ambient condition 3.78 per cent, carbon content did not affected by elevated CO<sub>2</sub> concentrations. The C:N ratio was observed in increasing trend with the elevated CO<sub>2</sub> level i.e. 11.8, 12.8 and 13 per cent in ambient, 550 ppm and 700 ppm respectively. However, no change was observed in phenol content of the leaves. While growth performance on *H. armigera* showed larval weight and larval duration were in increasing trend i.e. 0.72g (700ppm) and 20.15 days (700ppm) respectively. Moreover, pupal weight and fecundity were found in decreasing trend i.e. 0.28g and 424 eggs/female/day respectively.

**Keywords:** Tomato fruit borer, Elevated CO<sub>2</sub>, Biochemical analysis, C:N ratio

**1. Introduction**

The mass balance analysis shows that net global carbon uptake has increased significantly by 0.05 billion tonnes of carbon per year and global carbon uptake doubled, from 2.4±0.8 to 5.0±0.9 billion tonnes per year, between 1970 to 2010 and is anticipated to reach 550 ppm by 2050<sup>[1]</sup>. Climate Change reasserted that the atmospheric concentrations of CO<sub>2</sub>, methane, and nitrous oxide greenhouse gases have increased markedly since 1750<sup>[2]</sup>. The concentration of CO<sub>2</sub> have been steadily rising, from approximately 315 ppm in 1959 to a current atmosphere concentration of 403.47 ppm on September 5, 2017 and is expected to reach 550 ppm by 2050<sup>[3]</sup>. This increase is likely to affect biology indirectly via climate change, and directly by producing changes in plant growth, plant tissue chemical composition as well as impact on insect's life cycle and its herbivory<sup>[4]</sup>. Tomato plants grown under elevated CO<sub>2</sub> concentration have greater total root length, root surface area, root diameter, root volume and number of lateral roots than those under ambient CO<sub>2</sub><sup>[5]</sup>. Elevation in CO<sub>2</sub> increases the carbon to nitrogen (C: N) ratio and reduces the N content in the tissue of plants, it alters the synthesis of phenols, terpenes and other secondary metabolites. Such changes in C: N ratio and secondary metabolites alter the nutritional quality and palatability of host plant for herbivorous insects<sup>[6]</sup>. Tomato is one of the most preferred vegetable crops in the world with the total global area of 45.82 lakh ha and production of around 1500 million tonnes while productivity around 32.8 metric tonnes/ha<sup>[7]</sup>. While, India ranks second accounting the total area of 8.79 lakh hectares, production of about 182.26 lakh tonnes and productivity of 20.7 tonnes/ha, while in Uttar Pradesh state the area under tomato crop is 8.74 thousand hectare with a total production of 358.18 thousand tonnes and the productivity nearly 40.98 tonnes/ha<sup>[8]</sup>. The tomato crop is attacked by many species of insects viz., tomato fruit borer, green house whitefly, serpentine leaf miner, etc. due to its tenderness and softness as compared to other crops. Among them, the tomato fruit borer, *Helicoverpa armigera* (Hubner) is main bottle neck and causes heavy loss in yield<sup>[9]</sup>. So, the present study was aimed to elucidate the insect-herbivore (*Helicoverpa armigera*, Hubner) and plant (tomato) interactions under elevated CO<sub>2</sub> with following objectives:

1. Growth performance of *Helicoverpa armigera*
2. Biochemical analysis of tomato foliage

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## 2. Materials and Methods

### 2.1 Experiment details

For raising tomato plants three square type open top chambers (OTCs) of 3.5x3.5x3.5 m dimensions were constructed. Two chambers were maintained at elevated CO<sub>2</sub> level of 550 ppm and 700 ppm using gas regulators and analyzer on the CO<sub>2</sub> cylinder and third one was maintained at ambient CO<sub>2</sub>. Seedlings were raised in coco-peat pro-tray and covered with polyethene sheet till germination. Seedlings were transplanted from the coco-peat pro-tray to main experiment site in the mid of September 2015 and maintained throughout the season. While *Helicoverpa armigera* population was maintained at the entomology laboratory by collecting eggs from the field. Culture was maintained at 25 °C in 12:12, Day: Night cycle. The freshly hatched neonates were cultured in petriplates fed with respective chamber young leaves. Weighed quantity of the leaves was provided and after 24 hours of feeding larval weight was taken. In the same manner process was repeated for five days with the fresh leaves. Later the larvae were transferred to individual jar mouth covered with muslin cloth. Water soaked cotton was placed at the leaf peduncle to maintain the leaf moisture. The mean larval duration, mean larval weight, later mean pupal weight was taken according to the treatment and statistical analysis was performed. After emergence of the adult moths were fed on honey solution and kept separately for pairing. Observation on egg masses was taken and calculation on fecundity was done.

### 2.2 Biochemical Analysis

The biochemical analysis of foliage was done for carbon, nitrogen (C: N) and phenols. Nitrogen estimation was done by using Macro Kjeldahl's Method given by Association of Official Agricultural Chemists [10]. Leaf sample was dried at 80°C further; 0.2g grinded sample was transferred in 100ml conical flask, after adding H<sub>2</sub>SO<sub>4</sub> it was left over night. Next

day flask was kept on hot plate till fumes generates, later the sample was cooled down and 2ml chromic acid was added. Cool it down and transfer the content into nitrogen distillation flask by giving 4-5 washes with distilled water. Carbon per cent was estimated by Walkley-Black Method (CWB), in which the samples were ground to pass through 0.2-mm sieve [11]. The indicator used was diphenylamine. Total phenol content was determined by Folin-Ciocalteu procedure, in which absorbance was measured at 650 nm using spectrophotometer [12].

### 2.3 Statistical Analysis

The impression of CO<sub>2</sub> was analyzed by using one way ANOVA. Six replications were taken for the experiment. Treatment means were compared and separated using least significant difference (LSD) at  $p < 0.01$  and  $p < 0.05$ . The data on larval weight, pupal weight, larval duration and fecundity were analysed using SPSS version 16.0.

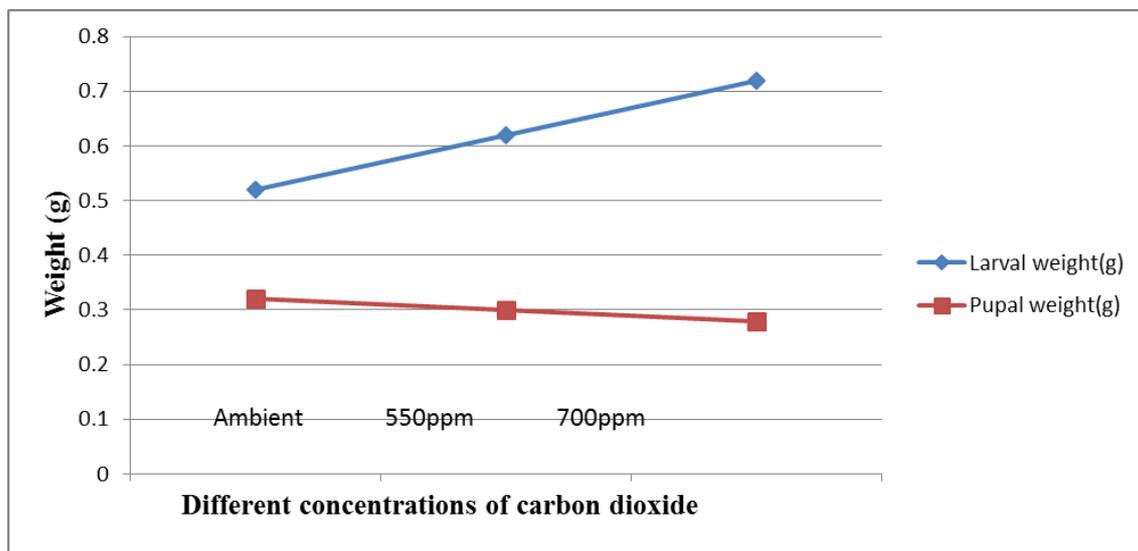
## 3. Results

### 3.1 Growth performance of *Helicoverpa armigera*

The relative larval weights 0.62g and 0.72g differ significantly ( $F_{5,10} = 7.55$ ;  $p < 0.01$ ) with elevated CO<sub>2</sub> concentrations than ambient CO<sub>2</sub> (0.52g). The higher larval weights were recorded in 550 ppm and 700 ppm CO<sub>2</sub> concentrations. Oppositely the lower pupal weights were recorded in elevated CO<sub>2</sub> concentrations than ambient (Fig 1). While the larval duration was expanded significantly under elevated CO<sub>2</sub> concentrations than ambient ( $F_{5, 10} = 3.57$ ;  $p < 0.05$ ). Most importantly less adult fecundity was observed under elevated CO<sub>2</sub> concentrations (550 ppm and 700 ppm) i.e. 430 and 424 eggs per female per day respectively as compared with ambient condition i.e. 470 eggs per female per day (Table 1).

**Table 1:** Impact of elevated CO<sub>2</sub> on growth parameters of *Helicoverpa armigera* on tomato.

CO <sub>2</sub> condition	Larval weight (g)	Pupal weight (g)	Larval duration (days)	Fecundity (eggs/female/day)
Ambient	0.52	0.32	19	470
550 ppm	0.62	0.3	20	430
700 ppm	0.72	0.28	20.15	424
$F_{5,10}$	7.55	6.75	3.57	10.1
$p$	<0.01	<0.05	<0.05	<0.01



**Fig 1:** Impact of elevated CO<sub>2</sub> on larval weight and Pupal weight of *Helicoverpa armigera* on tomato.

### 3.2 Biochemical analysis of tomato foliage

Biochemical analysis of tomato leaves under elevated CO<sub>2</sub> revealed low nitrogen than ambient. It was observed 2.80 per cent and 2.52 per cent as compared with ambient condition 3.78 per cent. While carbon content did not affected by elevated CO<sub>2</sub> concentrations. The C: N ratio was observed in increasing trend with the elevated CO<sub>2</sub> level i.e. ambient (11.8), 550 ppm (12.8) and 700 ppm (13). However, No change was observed in phenol content of the leaves (Table 2).

**Table 2:** Biochemical elements of tomato foliage under elevated CO<sub>2</sub> conditions.

Nutrient	Ambient	550 ppm	700ppm
Nitrogen %	3.78	2.80	2.52
Carbon %	33	35	34
C:N	11.8	12.8	13
Total Phenols (mg/100g)	0.53	0.52	0.53

### 4. Discussion

The photosynthesis and growth of many plants are stimulated when plants are grown under elevated CO<sub>2</sub> condition and reduction in leaf N content in plants grown at elevated CO<sub>2</sub>, due to faster growth of the plant [13]. Biochemical analysis of peanut foliage shows a significant reduction of 8% in leaf N under elevated CO<sub>2</sub> conditions w.r.t. the ambient. It is understood that leaf N content of legumes decreased on average by only 7% under elevated CO<sub>2</sub>, which was less than half the decrease exhibited by the non-legumes C3 plants [14]. Elevated CO<sub>2</sub> dramatically increased leaf photosynthesis as well as shoot and root biomass. Increased biomass is a general feature of CO<sub>2</sub> responses in C3 crops [15]. Legumes capable of N fixation are less likely to suffer reduction in N, but may exhibit lower leaf N during early growth stages in soybean [16]. Increase in atmospheric levels of CO<sub>2</sub> can cause increases in plant growth rates, and changes in the physical and chemical composition of their tissues [17]. In addition, most herbivorous insects appear to be negatively affected by elevated CO<sub>2</sub> because of the reduction in foliar N and increase in C: N ratio [18]. In the studies 13 per cent increase in C: N ratio was observed under elevated CO<sub>2</sub> conditions. The reduction in protein content, and increase of C: N ratio in leaves under elevated CO<sub>2</sub> [19], imply a reduction in food quality that might have caused the higher feeding by larvae. The CO<sub>2</sub> mediated changes in the tomato foliage (i.e., decreased N and increased C:N ratio) in the present study affected the growth and development of *H. armigera*, causing increase in larval weight due to heavy feeding while fecundity decreased to 424-430 eggs per female from ambient situation i.e. 470 eggs/female. Carbon, C: N ratio, phenol and tannin was significantly highest in eCO<sub>2</sub> (550 ppm) and least in aCO<sub>2</sub> + e Temperature (390 ppm + 2 °C) treatment. Similar result was observe in chickpea [20]. Most of chewing insects exhibit compensatory increase in food consumption [21]. Insects, when fed on plants grown under elevated CO<sub>2</sub>, were shown to increase their individual consumption due to poor food quality of plants [22]. Substantial influence of elevated CO<sub>2</sub> on *S. litura* was noticed, such as longer larval duration, higher larval weights, and increased consumption of peanut foliage by *S. litura* larvae under elevated CO<sub>2</sub> compared with ambient CO<sub>2</sub>. Relative consumption rate was significantly higher for *S. litura* larva fed plants grown at 550 and 700 ppm than for larvae fed plants grown at ambient condition.

### 5. Conclusion

In the present studies growth performance of *Helicoverpa armigera* significantly varied in the bracket of three CO<sub>2</sub> levels. The larvae under elevated CO<sub>2</sub> showed alteration in growth parameters, in form of gain in larval weight because of heavy feeding. The development time of the larva increased and pupal weight decreased. Further, fecundity decreased to 424-430 eggs per female from ambient situation i.e. 470 eggs/female. Thus the studies conclude that increased CO<sub>2</sub> concentrations have positive effect on C: N ratio and the negative effect on the growth and development of *H. armigera*.

### 6. Acknowledgement

The authors are thankful to the chairman Mewar University, Chittorgarh Rajasthan for providing necessary facilities and financial support in the form of mini project on climate change. Authors are also thankful to reviewers for their useful suggestions that helped in improving the article.

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