

INFLUENCE OF ELEVATED CO₂ ASSOCIATED WITH CHICKPEA ON GROWTH PERFORMANCE OF GRAM CATERPILLAR, *HELICOVERPA ARMIGERA* (HÜB.)

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Abstract. Influence of increased CO₂ concentrations (550 and 700 ppm) on host (Chickpea, *Cicer arietinum* L.) and its insect herbivore (Gram caterpillar, *Helicoverpa armigera* (Hüb.)) was studied in relation to ambient CO₂ (380 ppm) concentration under laboratory conditions. The foliar chemistry of chickpea under elevated CO₂ revealed low nitrogen and high carbon content with increased C: N ratio but no change in phenol content. This alteration in food quality significantly affected the growth parameters of *H. armigera* in the form of increased food consumption, gain in larval weight and more fecal matter production. Larval duration was also extended by one day under elevated CO₂ over ambient. Further, reduction in the fecundity (535 eggs/ female) was observed in the individuals raised under elevated conditions compared to ambient situation (580 eggs/ female). Diluted quality of food had a significant effect on growth performance indices of insect. Increase in approximate digestibility and relative consumption rate by the larva was observed under elevated CO₂ situation. The reduced efficiency of conversion of ingested food (35.88 %) and digested food (37.88 %) was observed under similar situation. As a result, the relative growth rate was down by 5.22 % and 6.20 % under both elevated CO₂ conditions. In a nutshell, it can be concluded that increased CO₂ concentrations has the negative effect on the growth and development of *H. armigera*.

Key words: CO₂ concentration, climate change, *Helicoverpa armigera*, chickpea, developmental biology

Introduction

Gram caterpillar, *Helicoverpa armigera* (Hub.) is a highly polyphagous pest known to cause serious economic damage to many field crops of arid and semiarid tropics across the globe. In India, it is a major pest of pigeon pea, chickpea, sunflower, sorghum and tomato crops. In Southern states of India, it breeds throughout the year completing over eight generations. The first generation usually starts in early June, coinciding with the onset of pre-monsoon showers either from the adults possibly emerging from diapausing pupae or from larvae that have been surviving on off season crops and weeds during summer. Initially, the species survives in low numbers on weeds, early-sown corn, sorghum, mung bean and groundnut but later builds up the population before shifting to pigeon pea in October-November and then to chickpea in November-March. Chickpea, the last notable crop of the season grown extensively across India, acts as a main breeding ground for *H. armigera* harbouring at least two generations. Thus, chickpea plays a crucial role in the mass breeding and sustenance of the pest for the next season (Bhatnagar, 1980). Success of carryover of the pest to the

next season mainly depends on fitness of the host on which it completes the last generation and the environmental condition during the transitional period.

The present change in climate is closely linked with the rise in atmospheric carbon dioxide (CO₂) levels from 280 to 387 ppm since the start of the Industrial Revolution. And current levels of CO₂ are expected to double by 2100 (IPCC 2007). Such rise in CO₂ levels affects the biological system of living organisms, including insects (Guerenstein and Hildebrand, 2008). Since fitness of any herbivorous insects depends on nutritional status of their host, any change in the quality of host plants can affect their growth, development, population dynamics and survival. The extent of growth, yield and biochemical responses of plants to elevated CO₂ depends on the photosynthetic pathway. Crops with C₃ photosynthesis respond markedly to increasing CO₂ concentrations by inhibiting photorespiration, making photosynthesis more efficient, however, leaf nitrogen and protein concentrations ultimately decrease by more than 12 % (Ainsworth and Long 2005). Such a loss of nitrogen and protein significantly diminishes the nutritional value of plant affecting growth and development of insect herbivores either directly or indirectly. In contrast, plants with C₄ photosynthesis will respond little to rising atmospheric CO₂ due to saturation of photosynthesis (Leon Hartwell and Vara Prasad, 2004).

Chickpea (*Cicer arietinum* L.) being a C₃ crop makes an interesting candidate to study the nutritional changes occurring due to altered CO₂ concentrations in its growth environment and its effect on the developmental and reproductive biology of one of its important pests, *H. armigera* to assess its pest status in next generations.

Materials and methods

Open top chambers (OTCs)

Three Open top chambers (OTCs) of 4 x 4 x 4 m dimensions, are established at Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad (17° 38' N; 78° 47' E). Elevated CO₂ concentrations of 550 ± 25 ppm (Condition I) and 700 ± 25 ppm (condition II) were maintained in two chambers and ambient CO₂ (380 ± 25 ppm) in the third chamber. Carbon dioxide gas supplied to these chambers was maintained at set levels using manifold gas regulators, pressure pipelines, solenoid valves, rotameters, sampler pump, CO₂ analyzer, PC linked Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA). The fully automated OTCs are first of their kind in India, which not only maintains the desired level of CO₂ but also the temperature and relative humidity (Vanaja et al., 2006).

Seeds of Chickpea Var. MNK-1 (Kabuli type) were sown in plastic pots (19 cm height, 17 cm diameter) and were kept in all the three OTCs during October 2011. Plant density and health were maintained by the adoption of proper agronomic practices.

Biochemical analysis of chickpea

Leaves of chickpea from respective OTCs were analyzed to estimate carbon, nitrogen, C: N ratio and phenol content through standard procedures. To estimate carbon, nitrogen and phenol content, leaf samples taken from 60 days old plants were dried at 80°C and subsequently ground to powder. Organic carbon was determined by Walkley Black method (1934) and nitrogen by Kjeldahl using block digestion and steam distillation method (McKenzie, 1994). C: N ratio was analyzed by using CHN

analyser (Elementar Analysen system GmbH, Germany) and total phenol was estimated by Folin-Denis method (Anderson and Ingram, 1993).

Feeding trials

Culture of *H. armigera* procured from CRIDA laboratory was maintained on artificial diet, which was prepared using chickpea leaves in a controlled chamber at 27° C with a 14-h day/ 10-h night cycle. Light intensity inside the chamber during the 14 h day period was maintained at 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with relative humidity of 60 % (day) and 70 % (night).

Eggs from the stock culture showing germ band development were surface sterilized with 10 per cent formalin for 10 minutes and washed under running tap water. Upon hatching, a day-old neonates was grouped into three batches; each batch containing 48 larvae. One batch was reared in the growth chamber maintained at ambient (380 ppm) CO₂ concentration on chickpea leaf composed artificial diet. Whereas, other two batches were reared separately in two different growth chambers maintained at 550 ppm and 700 ppm CO₂ concentrations. From fourth day onwards, larvae were bred individually by transferring them into multi cavity rearing trays (each tray having six well) on pre-weighed artificial diet prepared out of chickpea leaves harvested from OTCs maintained with respective CO₂ concentrations. Overall, 144 larvae were reared in all the three CO₂ condition with each individual larva representing a replication. On every alternative day, until the completion of the larval stage, weight of unfed artificial diet was recorded and removed. At the same time, fresh pre-weighed artificial diet was provided to individual larva. The larval and fecal matter weight (mg) was also recorded at the time of providing fresh food. After cessation of feeding which indicates the completion of larval stage, the larval duration was noted, and pupae were weighed and sexed. A pair of male and female pupae was kept separately in a plastic jar (22 cm diameter, 30 cm height) to study the reproductive biology.

In each condition, eight pairs of *H. armigera* moths were used to study the reproductive biology. Moths were provided with 10 per cent honey solution as adult food. The mouth of the container was closed with black cotton cloth to facilitate oviposition. Eggs laid on the cloth were collected everyday with the help of fine camel-hair brush and counted.

The insect performance indices were determined using the data generated from larval weight, quantity of food ingested and weight of fecal matter excreted (Waldbauer, 1968 and Srinivasa Rao *et al.*, 2009). Relative growth rate (RGR, in $\text{mg mg}^{-1} \text{d}^{-1}$), relative consumption rate (RCR, $\text{mg mg}^{-1} \text{d}^{-1}$), efficiency of conversion of ingested food (ECI, %), efficiency of conversion of digested food (ECD, %) and approximate digestibility (AD, %) were computed.

Data analysis

All the treatments were replicated forty eight times (n = 48), and results were presented as the mean value of each treatment \pm standard deviation. The effects of CO₂ concentrations on larval parameters were analyzed using one-way ANOVA. Treatment means were compared and separated using the least significant difference (LSD) at $p < 0.01$. The data on weight of food ingested, larval weight, fecal matter weight, larval duration, pupal weight and fecundity were analyzed using ANOVA with the help of STAR (Statistical tool for agriculture research), version 1.00.

Results

Biochemical analysis of chickpea leaves

In the present study, the nutritional quality of chickpea leaves differed significantly across all the three CO₂ concentrations. Leaf nitrogen content was distinctly lower (2.92 and 3.21 %) in elevated I (550 ± 25 ppm) and II (700 ± 25 ppm) CO₂ conditions compared to ambient (3.88%) (F_{2, 4} = 749.39; P < 0.01) (Fig. 1a) However, the carbon content of leaf tissue increased significantly (F_{2, 4} = 290.07; P < 0.01) to 43.13 % (700 ppm) and 40.46 % (550 ppm) under increased CO₂ over ambient CO₂ (33.99 %) (Fig. 1b) and resulted in a significant increase of C: N ratio (Fig. 1c). In contrast, the phenol content did not vary significantly across CO₂ conditions (F_{2, 4} = 3.00; P > 0.05) (Fig. 1d).

Larval growth performance

Total consumption of the food (diet) by *H. armigera* larvae differed significantly across CO₂ concentrations (F_{47, 94} = 5332.83; P < 0.01). The mean food consumption was substantially higher in larvae fed on the diet prepared from chickpea grown at 550 (1887.5 mg) and 700 ppm (1997.5 mg) over 380 ppm (1099.5 mg) of CO₂ concentrations. Similar trend was reflected in average weight gain by the larva (F_{47, 94} = 49.84; P < 0.01) under elevated I (381.25 mg), and elevated II (384.16 mg) condition compared to ambient (367.91 mg) condition. Fecal matter released by larvae was considerably more under elevated I (602.50 mg) and elevated II (597.70 mg) over ambient (372.29 mg) condition (F_{47, 94} = 4567.27; P < 0.01). The total larval development (time taken from hatching to pupation) was extended (15.16 and 15.27 days) significantly (F_{47, 94} = 184.60; P < 0.01) under elevated conditions over ambient (14.06 days). The pupal weight did not vary across CO₂ concentrations (Table 1).

Table 1. Effect of elevated CO₂ on growth and development of *H. armigera* on chickpea leaf mediated artificial diet

CO ₂ concentration (ppm)	Total diet consumed (mg)	Larval weight (mg)	FM produced (mg)	Larval duration (days)	Pupal weight (g)
380 (Ambient)	1099.50 ± 52.24	367.91 ± 9.66	372.29 ± 9.94	14.06 ± 0.24	0.257 ± 0.004
550 (Elevated-I)	1887.50 ± 24.45	381.25 ± 7.61	602.50 ± 18.73	15.16 ± 0.37	0.258 ± 0.005
700 (Elevated-II)	1997.50 ± 50.71	384.16 ± 4.98	597.70 ± 12.92	15.27 ± 0.44	0.259 ± 0.006
F _{47, 94}	5332.83	49.84	4567.27	184.60	NS
P	P<0.01	P<0.01	P<0.01	P<0.01	-
CV (%)	2.80	2.25	2.57	2.30	-

FM- Fecal matter

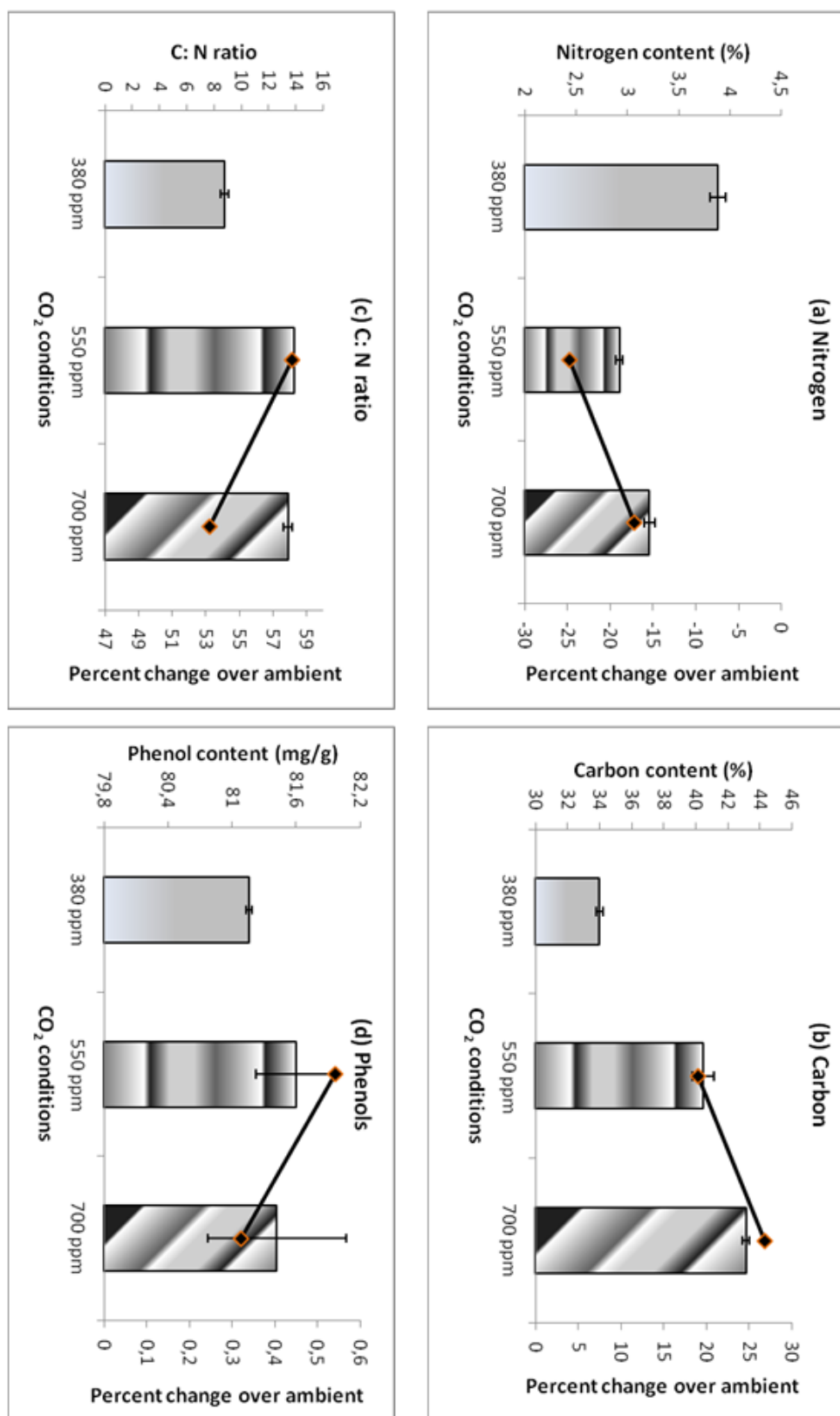


Figure 1. Biochemical changes in chickpea foliage under different CO₂ conditions (a) Nitrogen (b) Carbon (c) C: N ratio and (d) Phenol content

The approximate digestibility (AD) of the foliage by larvae was increased by about 2 % over ambient (67.81%) condition ($F_{47, 94} = 96.36$; $P < 0.01$). The efficiency of conversion of ingested food (ECI) was significantly lower (42.47 and 41.08 %) under elevated I and II conditions, compared to ambient (64.07 %). This was reflected in significant ($F_{47, 94} = 2824.30$; $P < 0.01$) lowering of efficiency of conversion of digested food (ECD) by the larvae under elevated conditions (61.54 and 58.71 %) compared to ambient (94.51 %). The relative consumption rate (RCR) was higher ($F_{47, 94} = 3174.26$; $P < 0.01$) under both elevated concentrations with an average of 52 - 53 mg/mg/day over ambient (36 mg/mg/day). The significant lower ($F_{47, 94} = 44.85$; $P < 0.01$) relative growth rates (RGR) of larvae were observed under elevated I and II concentrations (22.13 and 21.90 mg/mg/day) compared to ambient (23.35 mg/mg/day) (Table 2, Fig. 2). The females emerged from both elevated CO₂ conditions produced the lower number of eggs 535 and 538 eggs/female compared to 580 eggs/female under ambient CO₂ concentration ($F_{7, 14} = 17.56$; $P < 0.01$) (Fig. 3).

Table 2. Effect of elevated CO₂ on growth performance or indices of *H. armigera*

CO ₂ conc. (ppm)	Life history parameters/ indices				
	AD (%)	ECI (%)	ECD (%)	RGR (mg/mg/day)	RCR (mg/mg/day)
380 (Ambient)	67.81 ± 1.08	64.07 ± 2.48	94.51 ± 4.27	23.35 ± 0.96	36.46 ± 0.87
550 (Elevated-I)	69.02 ± 0.62	42.47 ± 1.34	61.54 ± 1.97	22.13 ± 0.88	52.16 ± 1.13
700 (Elevated-II)	69.98 ± 0.60	41.08 ± 1.16	58.71 ± 1.69	21.90 ± 0.97	53.30 ± 1.73
F _{47, 94}	96.36	2979.59	2524.30	44.85	3174.26
P Value	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
CV (%)	1.11	3.32	3.83	3.60	2.45

AD = Approximate Digestibility; ECI = Efficiency of Conversion of Ingested food; ECD = Efficiency of Conversion of Digested food; RGR = Relative Growth Rate; RCR = Relative Consumption Rate.

Discussion

Alteration in phytochemistry of plants under the elevated CO₂ concentrations is well documented (Hunter 2001). Irrespective of the biochemical pathway (C₃ and C₄), crop exhibit reduced 'N', increased 'C' and C: N ratio due to rapid photosynthesis and growth (Norby *et al.*, 1999) of the plant. Similar change was observed in the present study also wherein, biochemical analysis of chickpea foliage, a C₃ plant revealed a significant reduction in leaf nitrogen (over 24.74 %) when grown under elevated CO₂ concentrations compared to ambient.

A significant increase in 'C' (over 26.9 %) and C: N ratio (over 58.17 %) was observed in chickpea foliage under elevated CO₂ conditions than ambient condition, which might be due to increased carbon intake by the plants when grown under elevated CO₂ conditions. Similar observation was reported by Hughes and Bazzaz (1997) in a common milkweed *Asclepia syriaca*. The phenolic content in chickpea foliage did not

differ significantly across CO₂ concentrations (Fig. 1). Present study indicated the poor nutritional quality of the food for larvae under elevated CO₂ condition over ambient and is in agreement with Srivastava et al. (2002); Goverde and Erhardt (2003).

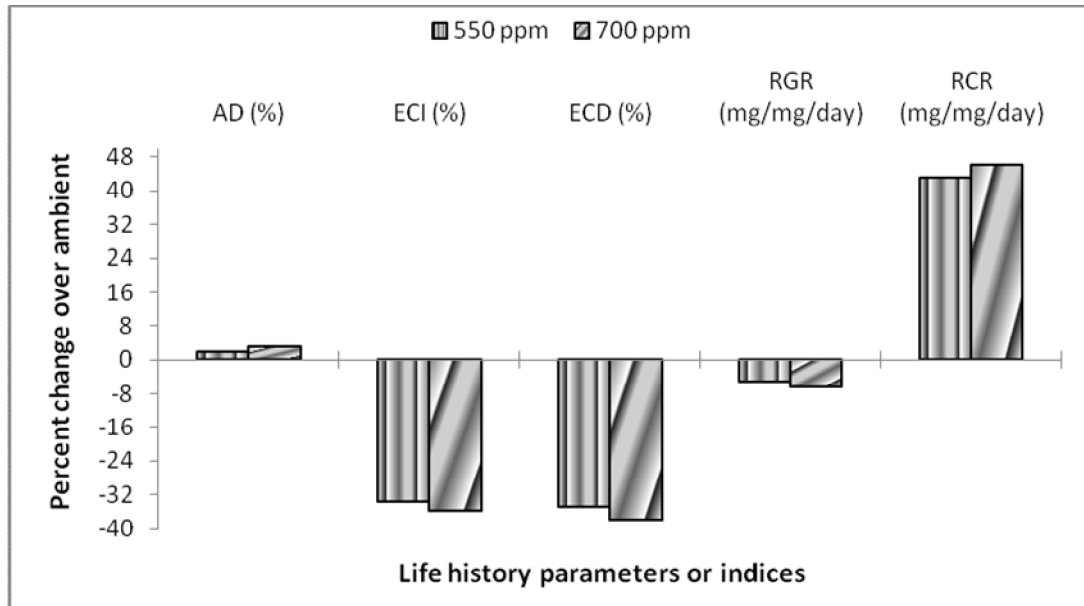


Figure 2. Impact of elevated CO₂ on life history parameters or indices of *H. armigera*

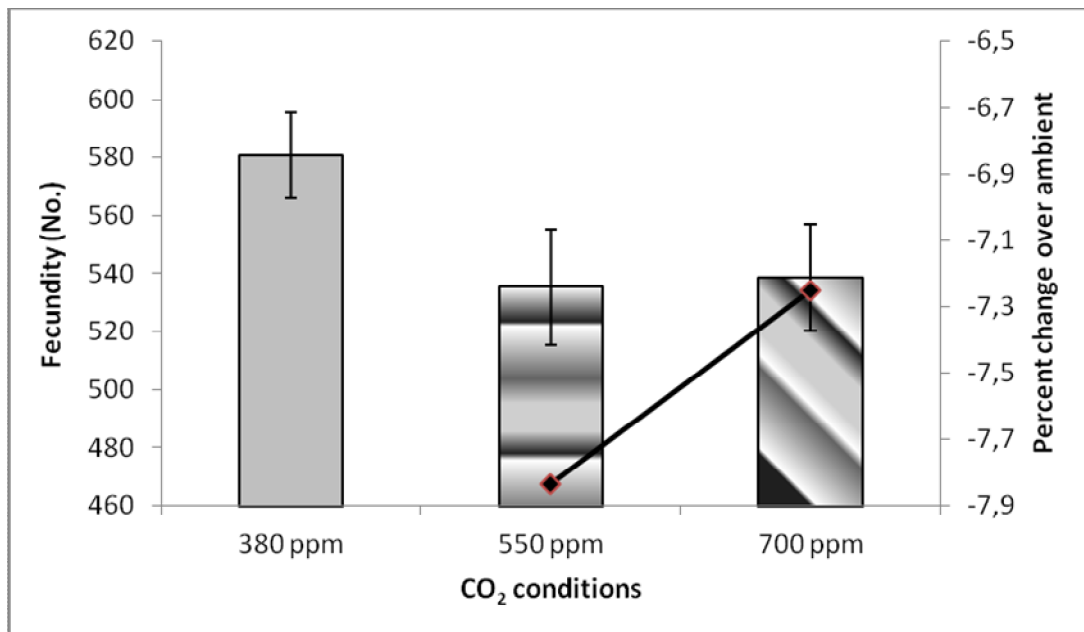


Figure 3. Impact of elevated CO₂ on fecundity of *H. armigera*

Since nitrogen is the chief constituent of proteins and is evident that chickpea plants grown under elevated CO₂ concentrations have lower protein content in their tissues, which might result in poor nutritional quality of food. Gram caterpillar, a herbivore

needs higher protein content for better growth and development. If the nutritional quality of the food is low, it would try to compensate through higher consumption and intake.

In the present study, increased food consumption of 81.67 % by the larvae under elevated CO₂ conditions was noticed over ambient. This resulted in increased RCR by 46.18 % in larvae reared under elevated CO₂ concentrations. Similar observation was also made by Wu et al (2006). Increase in larval RCR under elevated CO₂ concentrations was attributed to production of fewer allelochemicals reducing the nutritional quality of food thus, promoting phago-stimulatory responses (Scriber and Slankly, 1981). Further, in spite of increased food consumption, RCR and AD (by 3.2 %), the gain in the larval weight was mere 4.41 % over ambient. This could be attributed to lower protein content and higher 'C' content and 'C: N' ratio in the plant tissues resulting reduced efficiency in the conversion of ingested food (33 %) and digested food (38 %) by the larvae grown under elevated CO₂ concentrations over ambient.

Due to poor efficiency of both ingested and digested food, much of the food consumed was resulted in higher quantity release of fecal matter (about 60 %) compared to ambient CO₂. Similar observations were made by Chen et al, 2007 who recorded 46.3 and 37.8 per cent increased food consumption and feces production, respectively by *H. armigera* when fed on spring wheat (C₃ plant) under elevated CO₂. Further, in spite of higher consumption by larvae under elevated CO₂ condition, larvae took approximately one day more to complete their period over ambient. As a result, the relative growth rate (RGR) was reduced by 5 - 6 % in elevated conditions.

There is a general prediction that the fecundity is the most common parameter for determining the effect of larval food quality on performance of the insect. A significant reduction of fecundity (6.6 %) was recorded under elevated CO₂ over ambient and similar fewer fecund females were reported by Wu et al (2006).

Succinctly, if we put together the above results, it is understood that the dilution of bio-chemical constituents of chickpea foliage caused the poor growth, development and fecundity of *H. armigera* under elevated CO₂ conditions. Based on the present study, it can be speculated that, the growth performance of gram caterpillar under elevated CO₂ conditions, affects badly resulting in poor perpetuation of the population which might reduce its fitness in subsequent generations.

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