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Growth, development and nutritional indices of tobacco caterpillar, *Spodoptera litura* (F) raised on bell pepper plants grown under elevated CO_2 and temperature conditions

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ABSTRACT

The experiment was conducted at experimental farm situated at 30°5'N latitude and about 77°11'E longitudes, an elevation of 1260 m above mean sea level of Department of Environmental Science, Dr YS Parmar University of Horticulture and Forestry Nauni, Solan, Himachal Pradesh during 2014 and 2015, to study the effect of elevated CO_2 (eCO_2) and elevated temperature (*e*T) on growth, development and nutritional indices of *Spodopteralitura* on bell pepper plant. Feeding trials with foliage feeding insect *S. litura* was conducted using foliage of bell pepper plants grown under four concentrations of CO_2 and temperature, viz. three Open Top Chambers, (T_1: OTC eCO_2 550±10 ppm; T_2: OTC with elevated temperature 1°C higher and eCO_2 550±10 ppm; T_3: OTC with ambient temperature and CO_2 and T_4: natural ambient air and temperature in open. Biochemical analysis of foliage revealed that plants grown under elevated CO_2 had lower nitrogen. The larvae fed on eCO_2 as well as eCO_2 and temperature foliage exhibited greater consumption compared to the larvae fed on foliage under ambient CO_2. Larval duration also increased by two days. The eCO_2 and eCO_2 and temperature foliage was more digestible with higher values of approximate digestibility. The relative consumption rate increased whereas the efficiency parameters, viz. efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), and relative growth rate (RGR) decreased in eCO_2 and eCO_2 and temperature. The consumption and weight gain of the larvae were negatively and significantly influenced by leaf nitrogen.

Key words: *Capsicum*, eCO_2, elevated temperature, open top chamber, nutritional indices

Climate change, especially rise in temperature and atmospheric carbon dioxide concentration, is a major concern today. The concentration of CO_2 in the atmosphere may rise above 500 ppm by the next decade. Changes in climate are expected to have significant impacts on crop yields through temperature and carbon dioxide induced changes which in turn are likely to influence insect-plant interactions in several ways. The effects of climate change on insect pests can be both direct and indirect. Many plant species respond to enriched atmospheric CO_2 by enhanced photosynthetic rates and increase in biomass as well as alterations in leaf quality factors. This might affect growth of leaf eating insects through altered consumption and digestibility. The tobacco caterpillar, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is polyphagous pest, damaging many economically important vegetables and ornamental plants and has a huge potential to invade new areas by adapting to wide range of ecological situations (Sharma and Brar, 2018). Temperature, which impacts the development time, longevity and fecundity of insects has a direct effect while elevated CO_2 (eCO_2) has an indirect host- mediated effect on growth and development of insect pests (Yadugiri, 2010). Because leaf nitrogen is considered essential for growth and reproduction of insects a reduction in nitrogen content of leaves grown under elevated CO_2 may elicit strong responses by them. As a consequence of these tight ecological linkages, the interplay between plants and herbivorous insects in the tropics can be affected by the perturbations of climate change. The most predicted effects of climate change, i.e., increase in atmospheric temperature and CO_2 concentration will have a significant effect on agriculture in general and on herbivore insect populations in particular. Temperature and CO_2 (Rao *et al*., 2012) are known to alter the growth and development of *S. litura.* Increasing level of CO_2 and temperature is affecting the growth, development and plant biochemistry of bell pepper in this region and hence influence insect-plant interactions which can cause drastic reductions in commercial yield and affect the livelihood of farmers. Among the damaging species, the tobacco armyworm, *S. litura,* is as a major pest, and can cause yield losses of 35-55%. The tobacco caterpillar, *S. litura* larvae feed on the foliage and complete their life cycle. Larvae feed gregariously on leaves, causing severe defoliation, leaving midrib veins only. Response of herbivory to elevated CO_2 is highly complex, and the interactions between legumes and insect-herbivores are unclear (Rao *et al*., 2012). The present study was aimed to elucidate the effect of *e*CO_2 and temperature on leaf quality of bell pepper and the cumulative effect on various growth indices of *S. litura* fed with *e*CO_2 grown bell pepper foliage.

MATERIALS AND METHODS

The present experiment was conducted at farm of Department of Environmental Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan India in the year 2014 and 2015. The Experimental farm is situated at 30°5'N latitude and about 77°11'E longitudes and at an elevation of 1260 m above mean sea level. Circular type open top chambers (OTC) of 4 x 4 m^2 dimension were used to raise the crop under elevated and ambient CO_2 and temperature conditions. An automatic CO_2 enrichment and temperature technology was developed by adapting software SCADA to automatically maintain the desired and accurate levels of CO_2 and temperature around crop canopy inside OTCs. Carbon dioxide gas was supplied to the chambers and maintained at set levels using manifold gas regulators, pressure pipelines, solenoid valves, rotameters, sampler, pump, CO_2 analyzer, PC linked Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA). The concentration of CO_2 in the chamber was monitored by a non dispersive infrared (NDIR) gas analyser. There were four treatmentsi.e.T_1: elevated CO_2 (550 ±10 ppm), T_2 :elevated CO_2 and temperature (CO_2:550 ±10 ppm, temperature: 1°C elevated than T_1), T_3: ambient temperature (reference) and T_4: natural air and temperature condition (control). The bell pepper crop was transplanted during crop growing seasons of the experiment by following recommended package of practices.

The growth, development as well as nutritional indices of *S. litura* F. in bell pepper was studied under all the four conditions. The adults of *S. litura* F. was collected from field. The pure culture of *S. litura* F. was maintained in the laboratory on bell pepper leaves. The freshly laid eggs were used for the experiment. After hatching of eggs larvae were placed on petri dishes with bell pepper leaves obtained from plants raised under different treatments. Each larva was provided with the leaves of plants grown in the same treatment throughout the life. Before placing the leaves in petri dishes a moistened filter paper was kept so that humidity was maintained to prevent desiccation (Hasan and Ansari 2011). The feeding of larvae was performed in four replications. Each replication consists of five larvae placed on single petri dish. The larvae were transferred to big transparent jar after four days to avoid congestion in petri dishes. Initially weight was measured for five larvae collectively and average value per larva was worked out. After fourth day onwards measurements were done independently. Before offering leaves to larvae the initial weight of larva as well as leaf were measured and then after 24 hours weight of remaining leaf, larval weight and faecal matter was recorded. The leaves from each treatment were provided to larvae till they undergo in pupation. Later on, pupal period and pupal weight was recorded.

Chemical analysis of leaves

For estimation of N, 0.5 g of plant material (leaf sample taken before flowering stage) was digested in concentrated H_2 SO_4 in the presence of a digestion mixture. After digestion, the N was determined by micro-kjeldahl method. Leaf nitrogen content was measured by adopting the standard procedure given by Jackson (1973).

Instruments/apparatus used

1. Infrared Digestion system (or Digestion Chamber having a fame exhaust system, if gas burners are used).
2. Automatic distillation-cum-titration system or distillation system.

Reagents

1. 0.1N H_2SO_4: Dilute suitable volume of cone. H_2SO_4 approximately 0.1N H_2SO_4 and standardize it against 0.1.*N* NaOH.
2. Boric acid (4%) with mixed indicator: Prepared 4% aqueous solution of boric acid, added mixed indicator and adjust pH at 4.5.
3. Digestion accelerator mixture: Mixed 20 parts of anhydrous Na_2SO_4 or K_2SO_4 with 1 part of $CuSO_4.5H_20$ or commercially available tablets composed of K_2SO_4 and $CuSO_4.5H_20$.

Procedure adopted for digestion of sample on digestion block

- Weigh the sample and wrap in filter paper and place in digestion tube. Added one tablet of the digestion accelerator or mixture and 6 mL of conc. H_2SO_4.

- Set the digestion system to attain a temperature of about 385 °C and then attached the digestion tube to the heating unit as per the instructions given in the operation manual.
- Run the tap water with desired flow rate for safe disposal of fumes and allowed the digestion to continue till completion (no black or brown colour), which takes about 60 to 75 minutes.
- Switched-off the system and removed the rack of sample tubes along with the exhaust system from the heating unit. Do not stop water flow as the fumes continue coming for some more time.
- Set the distillation unit to perform various steps, viz. dilution, addition of alkali, steam generation, titration etc.
- Keep boric acid in conical flask and run the distillation for 150 seconds then titrated the distillate against 0.1*N* H_2SO_4 until a purple color just starts appearing.
- Once the samples are digested, the subsequent titration can also be followed manually.
- Utmost care should be exercised during addition of 40% NaOH, if done manually.

Insect performance indices

Data on larval weight (g), larval period (days), fecal matter (g), pupal period (days), pupal weight (g), adult longevity (days), fecundity (number of eggs/female) was recorded. Data regarding amount of food consumed, fecal matter produced and weight gained was utilized to work out the various nutritional indices, on fresh weight basis (by taking into consideration the fresh weight of food, fecal matter and the insect) relative growth rate (RGR, larval weight gain per day as a fraction of body weight), relative consumption rate (RCR, weight of leaf ingested per day as a fraction of larval body weight), efficiency of conversion of ingested food (ECI, larval weight gain per unit weight of leaf ingested expressed as %), efficiency of conversion of digested food (ECD, larval weight gain per unit weight of leaf digested expressed as %) and approximate digestibility (AD, ratio of weight of leaf digested and weight of leaf ingested expressed as per cent . A pair of adults was kept in one chamber and the number of eggs laid by the female during entire life was counted.

RESULTS AND DISCUSSION

Biochemical analysis of leaf samples

Leaf nitrogen content was distinctly lower in *e*CO_2 foliage (4.08%) followed by *e*CO_2 and *e*T (4.39 %), *a*CO_2 and temperature (5.16%) and open condition (5.73%) (Fig. 1).

Growth performance of S. litura

Larval weight

It is evident from Table 1 that *S. litura* larvae fed on foliage of bell pepper grown under *e*CO_2 gained significantly maximum weight (0.51 g) while larval weight decreased under *e*CO_2 and temperature (0.44 g) and *a*CO_2 and temperature (0.32 g). *S. litura* larvae fed on foliage of natural condition gained minimum weight (0.29 g). Larval weight under *a*CO_2 and temperature was statistically at par with natural condition and overall average weight of 0.39g ± 0.10 (Stnd. Dev.). *S. litura* larvae gained maximum weight under *e*CO_2 compared to *a*CO_2 and temperature and natural condition which may be due to change in C/ N ratio. In present investigations, *S. litura* larvae fed on foliage of bell pepper plants grown under *e*CO_2 gained maximum weight compared to *a*CO_2 and temperature and natural condition which may be due to lower foliar nitrogen content under *e*CO_2 which causes an increase in food consumption by *S. litura* in order to compensate their nutritional requirement and hence resulted increased larval weight as compared to *a*CO_2 and temperature and natural condition. These results corroborate the findings of Rao *et al.* (2012) who reported that there was increased larval weight (18% and 32%) of *S. litura* with higher fecal matter release under both *e*CO_2 treatments over *a*CO_2 and temperature.

Larval period

Larval period *S. litura*i ncreased with foliage of *e*CO_2 (19.92 days) followed by *e*CO_2 and temperature (18.76 days) and *a*CO_2 and temperature (17.71 days). Minimum larval period was with foliage of natural condition (16.81 days). Overall average larval period was 18.30±1.34 (days). Longer larval period was observed when larvae were fed on foliage of *e*CO_2 which may be attributed to lower quality of foliage which resulted longer larval period and slower growth of larva. Foliage of bell pepper plants grown under *e*CO_2 concentrations contained lower leaf nitrogen due to which larvae consumed more food. So larvae got less nutrition by feeding on more bulk of food in order to compensate their nutritional demand and hence resulted longer larval period.

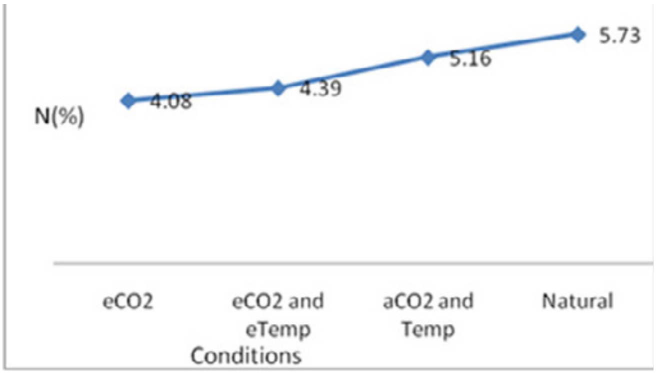

Fig. 1 : Nitrogen content (%) in leaf of bell pepper

These findings are in agreement with the findings of Rao *et al*. (2012) who stated that growth and development of the tobacco caterpillar, *S. litura* (F.) reared on peanut (*Arachis hypogea* L.) foliage grown under *e*CO_2 leads to longer larval duration and increased consumption of peanut foliage by *S. litura* larvae under *e*CO_2 compared with *a*CO_2. In the present findings, the larval period was less under*e*CO_2 and elevated temperature in comparison to elevated CO_2 which may be due to increase in temperature which interacted negatively against *e*CO_2 and caused increased nitrogen content and ultimately resulted reduced the larval duration.

Fecal matter

In all conditions average fecal matter 0.37±0.05 (g) was produced by larvae. Whereas when *S. litura* larvae fed on foliage of *e*CO_2 (0.42g) produced as much fecal matter as *e*CO_2 and temperature (0.40g) and differed statistically from *a*CO_2 and temperature (0.35g) and natural condition (0.31g). Lower foliar nitrogen content under *e*CO_2 caused increase in food consumption by *S. litura* in order to compensate their nutritional requirement and hence released more fecal matter as compared to *a*CO_2 and temperature and natural condition. The results are in consonance with findings of Rao *et al.* (2012) who have also reported that higher fecal matter release was observed under *e*CO_2 over *a*$CO_2$and temperature.

Pupal period

Significantly longest pupal period was recorded with *e*CO_2 (11.84 days) followed by *e*CO_2 and temperature (10.87 days), *a*CO_2 and temperature (9.34 days) while minimum pupal period was with natural condition (8.63 days). *a*CO_2 and temperature was statistically at par with pupal period under natural condition. An average of 10.17±1.45 pupal period was recorded in all conditions. In the present study, longest pupal period was recorded under *e*CO_2 which may be due to enhancement of overall growth period of insect under the influence of nutritionally poor food which slower the development at each stage of insect. The present results corroborate with the findings of Robinson *et al.* (2012) who reported that herbivores exposed to elevated concentrations of CO_2 had longer pupal period compared to ambient condition of CO_2.

Pupal weight

Over all 0.29±0.02 (g) pupal weight was recorded whereas Tobacco caterpillar, *S. litura* pupae gained significantly higher pupal weight under natural condition (0.32 g) which was statistically at par with *a*CO_2 and temperature (0.31 g) and differed statistically from *e*CO_2 (0.28 g) and *e*CO_2 and temperature. Lowest pupal weight was recorded with *e*CO_2 and temperature (0.27 g).In the present study, *e*CO_2 and temperature caused lowest pupal weight which may be due to reduction in food quality that might have caused the higher feeding by larvae and digestion was less which caused decreased pupal weight. Because nitrogen is the chief constituent of proteins so the plants grown under *e*CO_2 have lower in protein content, this resulted higher feeding by larvae but less digestion and caused decreased pupal weight. These results are in accordance with the findings of Rao *et al.* (2012) who reported that an increase in developmental time with decreased pupal weights was observed when larvae of *S. litura* were reared from hatching to pupation on *e*CO_2 grown peanut crop. In the present findings, it has also been observed that elevated temperature in combination with *e*CO_2 caused decreased pupal weight as compared to *e*CO_2 only and these results are in consonance with findings of Baeand Park (1999) who also reported that increase in temperature cause decreased in pupal weight by 3-13 per cent.

Fecundity

Maximum egg laying was observed under natural condition (886.19 eggs/female) followed by *a*CO_2 and temperature (799.71 eggs/female), *e*CO_2 (756.23 eggs/ female) and *e*CO_2 and temperature (733.54 eggs/ female). Elevated CO_2 and temperature was statistically at par with *e*CO_2. On an average 793.91±67.36 eggs/ female fecundity was recorded in all conditions.In present investigations, elevated CO_2 and temperature caused lower pupal weight which might have affected lower fecundity. Similar to present findings Bae and Park (1999) reported that lower pupal weight affected the fecundity.

Relative growth rate (RGR)

Higher growth rate (119.68) was with natural

Table 1: Effect of elevated CO_2 and temperature on growth, development of *S .litura* on bell pepper (pooled data)

Treatment	Larval weight (g)	Larval period (days)	Fecal matter (g)	Pupal period (days)	Pupal weight (g)
T_1 : Elevated CO_2 (550±10 PPM)	0.51	19.92	0.42	11.84	0.28
T_2 : Elevated CO_2 and elevated temp (550±10 PPM & 1°C)	0.44	18.76	0.40	10.87	0.27
T_3 : Ambient CO_2 and temperature	0.32	17.71	0.35	9.34	0.31
T_4 : Natural condition (control)	0.29	16.81	0.31	8.63	0.32
Mean ± SD	0.39±0.10	18.30±1.34	0.37±0.05	10.17±1.45	0.29±0.02
LSD (p = 0.05)	0.06	0.81	0.05	1.00	0.01

Table 2: Effect of elevated CO_2 and temperature on various nutritional indices of *S.litura* insect of bell pepper (pooled data)

Treatment	Fecundity (No of eggs/ female)	RGR	ECI (%)	RCR	AD (%)	ECD (%)
T_1 : Elevated CO_2(550±10 PPM)	756.23	92.93	36.14	64.86	84.24	43.23
T_2 : Elevated CO_2 and elevated temp (550±10 PPM & 1°C)	733.54	84.07	41.33	52.05	74.62	60.57
T_3 : Ambient CO_2 and temperature	799.71	103.01	36.41	43.50	74.20	49.78
T_4 : Natural condition (control)	886.19	119.68	47.79	31.63	70.92	68.42
Mean ± SD	793.91±67.36	99.92±15.27	40.42±5.46	48.01±14.01	75.99±5.74	55.50±11.19
LSD (p = 0.05)	51.74	26.07	10.63	12.61	11.43	21.34

condition was statistically at par with *a*CO_2 and temperature (103.01) and differed statistically from *e*CO_2 (92.93) and *e*CO_2 and temperature (84.07). Minimum relative growth rate was recorded with *e*CO_2 and temperature. Average of 99.92±15.27 RGR was found in all conditions. In this study, relative growth rate of *S. litura* reduced when larvae fed on foliage of bell pepper plants grown under *e*CO_2 as well as *e*CO_2 and temperature which may be due to less nutrition of foliage under *e*CO_2 and temperature. The present results are in accordance with the findings of Hatten schwiler and Schafellner (2004) who reported that relative growth rates of gypsy moth (*Lyman triadispar*) be reduced by 30 per cent when larvae fed on leaves of *Quercus petraea* exposed to high CO_2.

Relative consumption rate (RCR)

Relative consumption rate of *S. litura* larvae was at an average of 40.42±5.46 found in all conditions whereas higher under elevated CO_2 (64.86) which was statistically at par with elevated CO_2 and temperature (52.05) and differed statistically from ambient CO_2 and temperature (43.50) and natural condition (31.63). In the present investigations, higher relative consumption rate was observed under *e*CO_2 which probably may be due to changes in foliar chemical constituents which have caused more consumption of leaves by *S. litura*. The present results were in agreement with the findings of Coviella *et al*. (2000) who observed that when insects fed on *e*CO_2 grown plants showed increase in their individual consumption due to the poor food quality of these plants. Rao *et al*. (2012) also reported that relative consumption rate was significantly higher for *S. litura* larva fed on plants grown at 550 and 700 ppm than for larvae fed on plants grown at ambient treatment.

Efficiency of conversion of ingested food

Efficiency of conversion of ingested food was maximum (47.79%) with natural condition followed by *e*CO_2 and temperature (41.33%) and *a*CO_2 and temperature (36.41%) and least with *e*CO_2 (36.14%). An average of 48.01% ±14.01 was recorded in all conditions. In the present findings it has been observed that *S. litura* increased foliage consumption when fed on nitrogen-poor foliage which caused decreased efficiency of conversion of food into body mass. Similarly, Rao *et al*. (2012) reported that efficiency of conversion of ingested food by *S. litura* decreased in first and second generations under *e*CO_2 compared to ambient condition.

Approximate digestibility (AD)

Approximate digestibility was maximum (84.24%) with *e*CO_2 which was statistically at par with *e*CO_2 and temperature (74.62%), *a*CO_2 and temperature (74.20%). Least digestibility was with natural condition (70.92%). An average 75.99%±5.74 AD was recorded. Higher approximate digestibility was recorded under *e*CO_2which may be due to lower nitrogen content in the foliage grown under *e*CO_2. Higher approximate digestibility (AD) under elevated CO_2 may be attributed to accumulation of starch content and lower N content (Wang *et al*., 2008).The present findings indicated that the digestive efficiency (AD) increased and conversion efficiency (ECD) decreased for larvae fed on *e*CO_2 grown foliage.

Efficiency of conversion of digested food (ECD)

Maximum efficiency of conversion of digested food (68.42%) was with natural condition which was statistically at par with *e*CO_2 and elevated temperature (60.57%) and *a*CO_2 and temperature (49.78 %). Least efficiency of conversion of digested food was recorded with *e*CO_2 (43.23%) whereas overall average was 55.50%±11.19. In the present findings, it has been found that efficiency of conversion of digested food by *S. litura* was low under *e*CO_2 which may be due to low nutritional quality of food grown under *e*CO_2. Similar to present findings Rao *et al.* (2012) reported significantly lower efficiency of conversion of digested food (by 35% in 550 ppm and 32% in 700 ppm) of for *S. litura* larvae as compared to ambient condition. The present study is in agreement with the findings of Lawler *et al.* (1997) that herbivores responded to altered primary and secondary metabolism of plant under elevated CO_2 by increasing food consumption to counter balance for the lowered nutritional quality by decreasing food conversion efficiency.

CONCLUSION

Biochemical analysis of foliage revealed that plants grown under elevated CO_2 had lower nitrogen. The larvae fed on *e*CO_2 as well as *e*CO_2 and temperature foliage exhibited greater consumption compared to the larvae fed on foliage under ambient CO_2. Larval duration also increased by two days. The *e*CO_2 and *e*CO_2 and temperature foliage was more digestible with higher values of approximate digestibility. The relative consumption rate increased whereas the efficiency parameters, *viz*. efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), and relative growth rate (RGR) decreased in *e*CO_2 and *e*CO_2 and temperature. The growth and development of leaf eating insects certainly to be affected and changed through altered consumption and digestibility under ensuing climatic variability.

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