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Research Paper

Impact of climate change (elevated CO_2 and temperature) on growth and development of cowpea pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae)

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ABSTRACT

Climate change has widespread influence on agricultural productivity and relying upon the fact that responses vary between crops. Any changes in the nutritional quality of host plant will have direct influences on associated insect pest and the damage that it causes. In this context, study was conducted on to measure the impact of elevated CO_2 and temperature on growth and development of cowpea (C_3 plant) and its herbivore pod borer, *Maruca vitrata* (Fabricius) under open top chambers at Center for Agro-climatic Studies, UAS, Raichur, Karnataka. Elevated CO_2 and temperature had a positive impact on cowpea growth by encouraging crop boom along with vital changes in its phytochemistry. The increased supply of CO_2 resulted in higher concentrations of 'C' and C-based metabolites and leaf pigments like chlorophyll (39.81 µg cm^{-2}) and nitrogen balancer index (104.52). However, 'N' and N-based compounds were reduced. Larvae fed upon such nutrient-deficient food increased development period (36.07 ±0.42days) and compensatory feeding inflicting more damage (7.72 webbings/plant). Although the larvae consumed more food, it decreased the body weight of the larvae and pupae, which in turn decreased the percent of moth emergence, ultimately decreased the fecundity (60.09 ± 0.20/female) and fitness of the pest in the long run.

Keywords: Cowpea, growth and development, eCO_2 and temperature, *Maruca vitrata* and phytochemistry

Climate change and agriculture are interconnected, at the global level, climate change is expected to have both positive as well as negative effects on agricultural systems, with the negative effects outweighing the favorable ones. The increase in the emission of CO_2 causing a worldwide temperature rise wherein, the CO_2 concentration in the atmosphere has increased dramatically to 416 ppm, and is likely to double in 2100 (Skendzic *et al.,* 2021). Increased CO_2 and temperature may interact to affect herbivores by modifying the biomass of plant material accessible to herbivorous insects, as pests rely on their hosts for development and survival (Ahmed *et al.,* 1993; Pooja *et al*., 2022; Kumari and Verma, 2021).

The cowpea, *Vigna unguiculata* L. being a photo insensitive carbon responsive C_3 legume, its production is hindered by insect infestation which causes loss to an extent of 90 per cent. Legume pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera, Crambidae) is pre harvest pest of cowpea causes typical yield losses ranging from 20-88 per cent at flowering stage (Jayasinghe *et al.,* 2015). Hence, an investigation carried out with an aim to know how cowpea crop responds to high levels of CO_2 and heat stress intern its effect on pod borer growth and development.

MATERIAL AND METHODS

Studies on the host mediated effect of climate change at varied levels of CO_2 and temperature was made on pod borer, *M. vitrata* by growing cowpea in the open top chambers at Centre for Agro-climatic Studies, Main Agricultural Research Station (MARS), University of Agricultural Sciences, Raichur, Karnataka.

Experimental setup under OTCs

Required concentration of carbon dioxide and temperature inside OTCs surrounding the cowpea canopy was provided using Supervisory Control and Data Acquisition (SCADA) software system. Standard climate change treatments were set in OTCs as described (IPCC, 2018).

Cowpea seeds were dibbled in 10 cement pots (size 42 × 32 sq. cm). Each pot having five plants making a total of 50 plants. Pots containing mixture of FYM, vermicompost and soil in the ratio of 1:1:2 and pots were placed in respective OTCs. All agronomic

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practices for raising crop was followed as per the package of the University of Agricultural Sciences, Raichur. When the seedlings were 10 days old, each pot was covered with nylon cage (0.15 × 0.15 sq. cm) to avoid infestation of other insect pests. Out of 10 pots, five pots containing 25 plants were used for releasing *M. vitrata* larvae while reaming five pot (25 plants) were used for recording the plant growth, physiological and biochemical parameter at 30 and 60 days.

Observations

Cowpea growth parameters *viz.,* plant height, number of leaves, branches and yield parameters (number of flowers or pods/ plant, yield/plant and test weight of seeds) were recorded at 30 and 60 days after sowing. Physiological parameters *viz.,* chlorophyll, flavonoids and Nitrogen Balanced Index (NBI) were estimated using Dualex scientific sensor, a hand tool leaf clip combining the use of fluorescence as well as light transmission of a leaf. Biochemical constituents such as organic carbon (dry combustion method / ash method using Muffle furnace), leaf nitrogen and seed protein (Micro-Kjeldahl technique, McKenzie, 1994), C:N ratio, reducing and total sugars (Nelson Somogyi method, Marais *et al.,* 1966), tannins (Folin-Dennis method, Malick and Singh, 1980) and phenols (Folin-Ciocalteau method, Malick and Singh, 1980).

Sufficient population of pod borer, *M. vitrata* larvae was collected from the infested field of cowpea, field bean, red gram and black gram and maintained on semi synthetic diet (Baramappa, 2015) for one generation and pure culture was obtained by rearing first generation larvae on natural cowpea flowers and pods in the National Food Security Mission laboratory, UAS, Raichur, Karnataka.

After attaining the age of 40 days old, plants in OTCs were released with two 2nd instar larvae of pod borer, *M. vitrata* (total of 50 larvae per treatment) and covered with nylon cage which helped the plants to prevent escape of released larvae. Later on they were examined for damage by pod borer (webbings) for 12 days (pod borer larval duration is 12 days). Further all the webbings containing pupae were collected separately from each treatment. The pupae were sexed and moths (1:1) were placed in plastic containers (10 cm diameter) having 10 percent honey solution for adult emergence by placing containers in BOD incubator at 26 ± 1°C and 60 (±10 %) RH. A total of 20 pairs of adults are maintained for each treatment and the following observations were recorded.

Pest damage: Number of webbings per plant was recorded on weekly basis till the emergence of adult from the webbing. Larval period and pupal period were calculated. Adult longevity was worked out by transferring individual moth into wooden cages (size 60 x 30 x 30 cm) with twenty males and twenty female adults with four replications were maintained separately.

The direct effect of elevated CO_2 and temperature on biotic potential of *M. vitrata* under laboratory conditions was studied. Wherein, flowers and/or pods were collected from cowpea plants grown under different OTCs which fed to *M. vitrata* neonates (1st instar) separately in bread boxes (size 8 × 4'') and reared under growth chambers. The growth chambers were previously set with standard climate change treatments matching with the respective OTC conditions. For each treatment three replications were maintained and in each replication with 10 neonates (1st instar) (totally 30 larvae/ treatment) were released.

Number of instars, total larval and pupal period (days), larval and pupal weight (mg), adult longevity, fecundity per female, total life cycle and morphometric measurements of pod borer were recorded at regular interval.

Data analysis

Combined effects of CO_2 and temperature on growth and biochemical parameters were analyzed using one-way analysis of variance. Treatment means were compared and separated using least significant difference (LSD) at $p < 0.01$. The data on larval weight, larval duration, pupal weight, pupal duration, adult longevity, fecundity and other biological parameters of different instars were analyzed using ANOVA. Statistical analysis was done by using SPSS software (version 16.0).

RESULTS AND DISCUSSION

Effect of eCO$_2$ and temperature on growth and development of cowpea

Growth parameters such as plant height (43.10 cm), number branches (6.06/plant) and number of leaves (16.15/plant) recorded at 30 DAS was significantly higher in elevated CO_2 treatments. Whereas, treatment with ambient CO_2 + 2°C rise in temperature recorded lower plant height (35.57cm), lesser number of branches (3.92/plant) and leaves (14.13/plant). Similar trend was observed at 60 DAS, even for the number of flowers and pods per plant (Table 1).

Nitrogen Balance index (NBI) was significantly maximum (95.63) in by elevated CO_2 with rise in temperature of 2°C. Chlorophyll content was significantly higher (32.82 µg cm^{-2}) under elevated CO_2 alone. However, the leaf flavonoid content was found to be maximum in reference plot (0.67 µg cm^{-2}) at 30 days after sowing. The trend was similar for 60 days after sowing analyzed samples (Table 2)

Plant biochemical parameters

Percent leaf nitrogen was maximum under reference plot both at 30 days after sowing (DAS) (5.24%) and 60 DAS (7.94%). However, per cent seed protein content at harvest was highest in reference plot (33.10%) and found lowest in elevated CO_2 with rise in temperature of 2°C with (25.07%). Significantly maximum organic carbon (43.19%), C:N ratio (10.82), phenols (4.07 mg g^{-1}), tannins (3.63 mg g^{-1}), total sugars (3.84 mg g^{-1}) and reducing sugars (2.18 mg g^{-1}) at 30 DAS under eCO$_2$ treatments. Likewise, analysis at 60 DAS showed the same trend (Table 3).

Variation in CO_2 and temperature has great influence on plant growth parameters with increased plant height, number of branches, leaves, flowers and pods per plant intern increasing the plant yield, which is in line with the findings of Bhattacharya *et al*. (1985) and Das *et al*. (2020). The present findings with decreased

Table 1: Effect of eCO_2 and temperature on growth parameters of cowpea under open top chambers.

Treatment details	Growth parameters							
	Plant height (cm)		Number of branches/plant		Number of leaves/ plant		Number of flowers/ plant	Number of pods/ plant
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	60 DAS	60 DAS
T_1: eCO_2(550 ppm) +aTemp (33 °C)	43.10[a]	77.36[a]	6.06[a]	16.43[a]	16.15[a]	25.51[a]	20.30[a]	6.22[a]
T_2: eCO_2(550 ppm)+ eTemp (33+2°C)	41.99[b]	75.10[b]	5.72[b]	15.76[b]	15.73[b]	24.76[b]	19.13[b]	5.89[b]
T_3: aCO_2(410 ppm)+eTemp (33+2°C)	35.57[d]	66.56[d]	3.92[d]	14.39[d]	14.13[d]	23.13[d]	14.14[d]	4.26[d]
T_4: aCO_2(410 ppm)+ aTemp (33°C) (reference OTC)	37.90[c]	69.58[c]	4.61[c]	14.75[c]	15.28[c]	23.93[c]	17.12[c]	5.4[c]
T_5: Reference plot (open plot)	38.09[c]	72.84[c]	4.84[c]	14.87[c]	15.42[c]	24.32[c]	17.95[c]	5.48[c]
S.Em (±)	0.01*	0.02*	0.03*	0.01*	0.01*	0.01*	0.02*	0.03*
CD (p=0.01)	0.03	0.07	0.1	0.03	0.03	0.03	0.07	0.1
CV(%)	0.30	0.43	2.51	0.40	0.49	0.33	0.87	2.43

DAS: Days after sowing eCO_2: elevated Carbon dioxide aCO_2: ambient Carbon dioxide
*Significant @ 1%eTemp: elevated temperature eTemp: elevated temperature aTemp: ambient temperature
Means denoted by same letters in vertical column are not significantly different by DMRT

Table 2: Effect of eCO_2 and temperature on physiological parameters of cowpea under open top chambers.

Treatments	Physiological parameters								
	NBI (30 DAS)	NBI (60 DAS)	Mean	Chlorophyll (µg cm^{-2}) (30 DAS)	Chlorophyll (µg cm^{-2}) (60 DAS)	Mean	Flavanoids (µg cm^{-2}) (30 DAS)	Flavanoids (µg cm^{-2}) (60 DAS)	Mean
T_1: eCO_2 @ 550 ± 25 ppm with normal temperature	89.19[b]	119.84[b]	104.51	32.82[a]	46.79[a]	39.81	0.33[c]	0.75[c]	0.54
T_2: eCO_2 @ 550 ± 25 ppm with 2°C rise in temperature	95.63[a]	123.86[a]	109.75	28.99[b]	42.01[b]	35.5	0.30[d]	0.64[d]	0.47
T_3: aCO_2 @ 410 ± 25 ppm with 2°C rise in temperature	86.31[c]	113.62[c]	99.96	24.15[d]	30.96[d]	27.56	0.62[a]	0.96[a]	0.79
T_4: aCO_2 @ 410 ± 25 ppm with normal temperature (reference OTC)	80.71[d]	100.34[d]	90.53	26.86[c]	32.05[c]	29.46	0.49[b]	0.89[b]	0.69
T_5: Reference plot (open plot)	87.30[c]	115.08[c]	101.19	27.42[c]	32.41[c]	29.92	0.67[a]	1.02[a]	0.85
S.Em (±)	0.03*	0.04*	-	0.05*	0.06*	-	0.02*	0.01*	-
CD (p=0.01)	0.1	0.14	-	0.18	0.22	-	0.07	0.03	
CV (%)	0.77	0.93	-	1.91	2.05	-	6.09	1.52	

DAS: Days after sowing eCO_2: elevated Carbon dioxide aCO_2: ambient Carbon dioxide
*Significant @ 1%eTemp: elevated temperature eTemp: elevated temperature aTemp: ambient temperature
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flavonoids and slightly increased chlorophyll content resulted in increased NBI content. Carbon dioxide enrichment with high C:N ratio increased photosynthesis resulting in higher assimilation of foliar C based compounds (phenols and tannins along with higher level of carbohydrate-sugars) in the plant tissue which decreased the nitrogen concentration. Seed protein content negatively affected by elevated CO_2 which was directly linked with availability of foliar N. Results are resemblance with the studies conducted by Stiling and Cornelissen, (2007).

Cowpea mediated effect of eCO_2 and temperature on pod borer, M. vitrata under OTCs

The weekly observation on number of webbings per plant under different climate change treatments showed that highest number of webbings was recorded 7.72 per plant under eCO_2 conditions. Whereas, significantly less number of webbings registered under ambient CO_2 with 6.70 per plant (Fig. 2).

Effect of eCO_2 and temperature on yield of cowpea in absence of insects (control) found to be maximum under elevated CO_2 with ambient temperature with 61.59 g per plant with 35.03 per cent over control. Wherein, test weight of seeds ranged from 14.96 to 22.69 g per plant (Fig. 1). However, pod borer infested plants were recorded yield of (12.52 g/plant) under ambient conditions of CO_2 which is found to be with maximum compare to treatment with eCO_2 with 10.11 g per plant (Fig. 1).

Results clearly indicate that elevated climatic treatments registered more damage to plants by increasing the feeding rate of insects. Insect response to manage the nutrient deficient plants resulted in increased consumption through compensatory feeding

Table 3: Effect of eCO_2 and temperature on biochemical constituents of cowpea under open top chambers

Treatment details	Biochemical constituents														
	Leaf nitrogen (%)		Carbon (%)		C:N ratio		Phenols (mg g^{-1})		Tannins (mg g^{-1})		Total sugars (mg g^{-1})		Reducing sugars (mg g^{-1})		Seed protein at harvest (%)
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	
T_1: eCO_2 (550 ppm) + aTemp (33 °C)	4.06^{d} (11.62)	6.82^{e} (15.14)	43.19^{a} (41.09)	45.57^{a} (42.46)	10.82^{a}	6.74^{a}	4.07^{a}	5.75^{a}	3.63^{a}	4.08^{a}	3.84^{a}	7.04^{a}	2.18^{a}	4.14^{a}	26.21^{d} (30.14)
T_2: eCO_2 (550 ppm) + eTemp (33+2°C)	4.17^{c} (11.79)	7.06^{d} (15.41)	41.02^{b} (39.82)	44.63^{b} (41.92)	10.08^{b}	6.34^{b}	3.77^{b}	4.93^{b}	3.42^{b}	3.75^{b}	3.53^{b}	6.77^{b}	1.96^{b}	3.89^{b}	25.07^{e} (30.05)
T_3: aCO_2 (410 ppm) +eTemp (33+2°C)	4.29^{b} (11.95)	7.25^{c} (16.65)	38.33^{c} (38.25)	43.06^{c} (41.01)	9.39^{c}	5.95^{c}	3.58^{c}	4.54^{c}	2.87^{c}	3.54^{c}	2.94^{d}	5.16^{e}	1.76^{d}	3.00^{e}	26.61^{c} (31.06)
T_4: aCO_2 (410 ppm) + aTemp (33°C)(Reference OTCs)	5.16^{a} (13.13)	7.79^{b} (16.21)	37.88^{d} (37.99)	40.99^{d} (39.81)	7.66^{d}	5.38^{d}	3.47^{d}	4.32^{d}	2.22^{d}	3.20^{d}	3.15^{c}	5.23^{d}	1.83^{c}	3.62^{c}	32.46^{b} (34.73)
T_5: Reference plot (open plot)	5.24^{a} (13.24)	7.94^{a} (16.37)	35.63^{e} (36.65)	40.05^{e} (39.26)	7.12^{d}	4.98^{e}	3.76^{b}	4.73^{b}	3.40^{b}	3.65^{b}	3.33b^{c}	5.38^{c}	1.90^{b}	3.49^{d}	33.10^{a} (35.12)
S. Em (±)	0.05*	0.03*	0.06*	0.06*	0.04*	0.01*	0.02*	0.01*	0.02*	0.02*	0.02*	0.01*	0.01*	0.01*	0.08*
CD (p=0.01)	0.18	0.11	0.22	0.22	0.14	0.03	0.06	0.03	0.07	0.06	0.07	0.03	0.03	0.03	0.3
CV (%)	0.82	0.40	0.32	0.30	2.68	0.58	1.77	0.82	2.10	1.87	2.18	0.75	1.39	1.02	0.65

DAS: Days after sowing eCO_2: elevated Carbon dioxide aCO_2: ambient Carbon dioxide
*Significant @ 1% eTemp: elevated temperature aTemp: ambient temperature
Means denoted by same letters in vertical column are not significantly different by DMRT

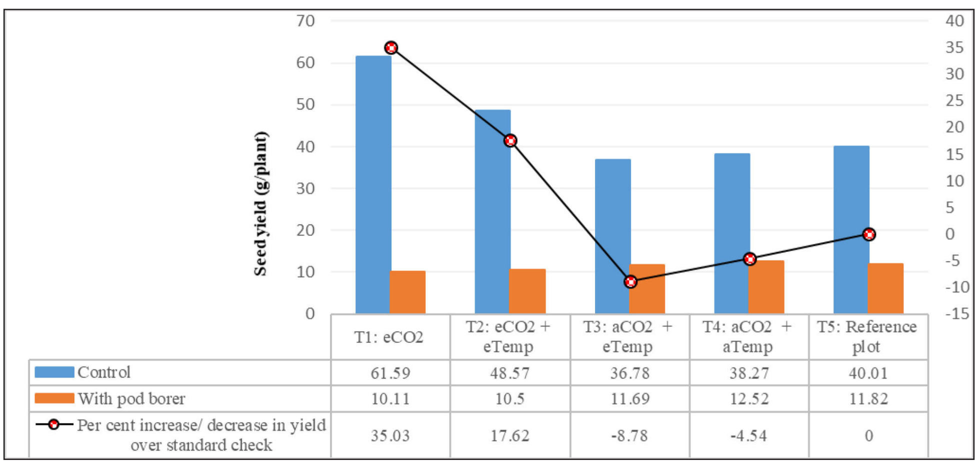

Fig. 1: Effect of eCO_2 and temperature on yield components of cowpea a grown under OTCs

implicating higher crop damage. Wherein, it would be expected if inducibility of constitutive phenolic were constrained by nitrogen availability under elevated CO_2 then the systemic defense will be nullified by the herbivore causing more crop damage in similar with reports of Karowe and Grubb (2011) and Xie *et al.,* 2015).

Biotic potential of pod borer

Developmental period under OTCs: Significantly maximum larval and pupal duration was recorded (22.63±0.04days) under eCO_2. However, significantly lower duration was recorded 20.30 ±0.06 days under ambient CO_2 with 2 °C. Larval and pupal duration under eCO_2 + elevated temperature was 21.51 ±0.11 days (Fig. 2). Adult longevity was significantly highest in reference plot (10.69 ±0.13 days). Whereas, significantly least adult longevity (9.40 ±0.05 days) recorded under eCO_2 + elevated temperature.

Development period under laboratory condition: The larval duration of first, second and third instar larva did not vary much among different treatments wherein, duration of fourth and fifth instar larva varied significantly among different climate change treatments. The total larval period was significantly maximum under eCO_2 (17.20±0.12 days). However, the total larval period was significantly less under ambient CO_2 (14.52±0.17 days). Significantly highest pupal duration was recorded under eCO_2 (8.21±1.31 days). Adult longevity was significantly highest under reference plot for both male (10.49±0.16 days) and female (11.24±0.12 days). However, total life cycle of pod borer varied among treatments significantly and maximum duration (36.07±0.42 days) was recorded under eCO_2 (Table 4).

Percent moth emergence (74.40 ±0.22%) and oviposition

Table 4: Direct effect of eCO_2 and temperature on biotic potential of *M. vitrata* under plant growth chambers.

Treatments	Total larval period (days)	Total pupal period (days)	Adult longevity (days)		Oviposition period (days)	Fecundity/ Female	Incubation period (days)	Egg hatching (%)	Moth emergence (%)	Larval weight (mg)	Pupal weight (mg)	Total life cycle (days)
			Male	Female								
T_1: eCO_2 (550 ppm) + aTemp (33 °C)	17.20 ±0.12^{a}	8.21 ±1.31^{a}	7.03 ±0.09^{c}	8.44 ± 0.09^{c}	3.03 ±0.04^{d}	54.20 ±0.25^{e}	4.40 ±0.55^{a}	66.13 ±0.83^{c}	63.66 ±0.19^{c}	38.26 ±0.19^{bc}	36.38 ±0.44^{c}	36.07 ±0.42^{a}
T_2: eCO_2 (550 ppm) + eTemp (33+2°C)	16.30 ±0.15^{b}	8.04 ±1.00^{a}	7.09 ±0.07^{c}	8.48 ±0.12^{c}	3.09 ±0.04^{d}	54.56 ±0.12^{d}	3.80 ±0.84^{ab}	58.74 ±0.44^{e}	59.32 ±0.43^{e}	38.15 ±0.23^{c}	34.21 ±0.11^{d}	35.43 ±0.48^{ab}
T_3: aCO_2 (410 ppm) + eTemp (33+2°C)	16.11 ±0.11^{b}	7.21 ±0.45^{b}	9.67 ±0.36^{b}	10.92 ±0.10^{b}	3.48 ±0.12^{b}	58.20 ±0.12^{c}	3.24 ±0.68^{bc}	61.71 ±0.26^{d}	60.08 ±0.04^{d}	42.10 ±0.06^{ab}	44.74 ±0.13^{b}	33.82 ±0.54^{b}
T_4: aCO_2 (410 ppm) + aTemp (33°C) (Reference OTCs)	14.52 ±0.17^{e}	6.62 ±0.55^{bc}	9.63 ±0.12^{b}	10.64 ±0.16^{b}	3.27 ±0.05^{c}	58.57 ±0.18^{b}	2.62 ±0.55^{c}	70.46 ±0.27^{b}	68.90 ±0.40^{b}	41.93 ±0.09^{b}	44.98 ±1.36^{b}	30.53 ±0.26^{c}
T_5: Reference plot (open plot)	15.23 ±0.13^{c}	6.40 ±0.55^{c}	10.49 ±0.16^{a}	11.24 ±0.12^{a}	3.76 ±0.08^{a}	60.09 ±0.20^{a}	2.84 ±0.45^{c}	83.43 ±0.21^{a}	74.40 ±0.22^{a}	44.85 ±0.18^{a}	50.03 ±0.21^{a}	30.86 ±0.23^{c}
S.Em (±)	0.02*	0.07*	0.02*	0.01*	0.01*	0.01*	0.06*	0.13*	0.08*	0.01*	0.02*	0.02*
CD (p=0.01)	0.09	0.28	0.09	0.04	0.03	0.02	0.23	0.51	0.32	0.03	0.09	0.07
CV (%)	1.25	5.70	1.63	0.62	1.01	0.17	10.29	0.51	0.33	0.23	0.78	0.64

*Significant @ 1% eCO_2: elevated Carbon dioxide aCO_2: ambient Carbon dioxide
eTemp: elevated temperature aTemp: ambient temperature
Means denoted by same letters in vertical column are not significantly different by DMRT

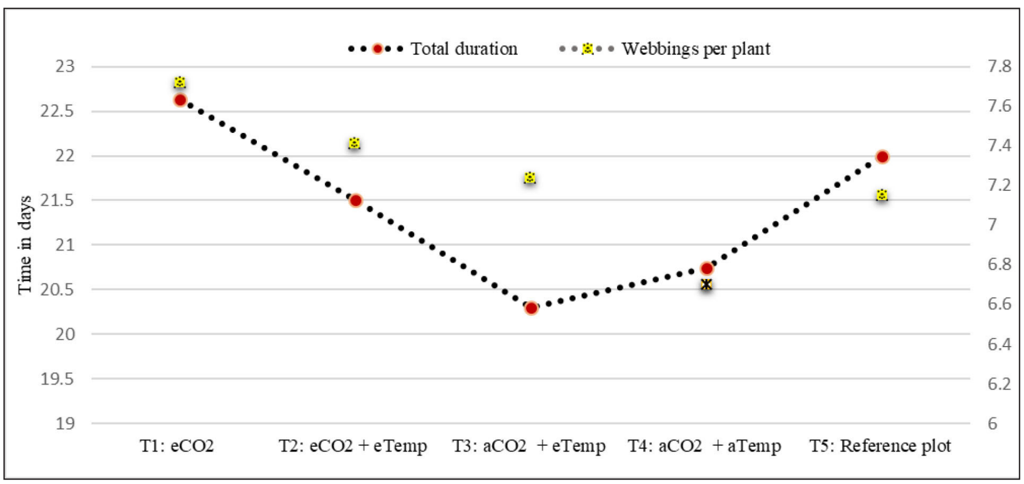

Fig. 2: Cowpea mediated effect of eCO_2 and temperature on growth and development of *M.vitrata* under OTCs

period (3.76 ±0.08 days) were maximum at reference plot. Significantly maximum larval weight (44.85 ±0.18 mg) was registered by the larva which fed upon the flowers/pods grown under reference plot. However, significantly least larval weight was recorded (38.15±0.23mg) by the larva fed upon plants raised under eCO_2 + elevated temperature. Fecundity of pod borer was significantly maximum under reference plot (60.09± 0.20/ female). Similarly, per cent hatching of eggs was significantly highest under reference plot (83.43 ±0.21%). However, incubation period of eggs was significantly more under eCO_2 (4.40 ±0.55 days) (Table 4).

Results shows that a slight reduction in foliar N content would have profound effects on the herbivore performance reducing body weight and population size. As a result, herbivore is more prone to prolong the development time, devouring more tissue and have greater mortality. Results also indicated that the decreased adult longevity and reduced fitness of adults under elevated climatic environment resulted in reduced reproductive success with decreased fecundity, survival and increased mortality of the insects under eCO_2 level compared to ambient condition. The findings of Adati *et al.* (2004); Abdul *et al*. (2014); Swetha *et al*. (2017) and Gouri Shankar *et al.* (2022) support for understanding present results.

CONCLUSION

Climate factors created ideal conditions for cowpea development, resulting in major changes in the plant's phytochemical makeup. As a result, it's been proven that monotrophic interaction has favored the crop in the face of climate change. On the other hand, increased CO_2 and temperature had a significant negative impact on both herbivores and the host plant in general. Herbivores increased compensatory feeding on undernourished plants with weak N-based supplements, resulting in greater crop damage and lower crop yields. Furthermore, increased CO_2 and temperature resulted in a prolonged growth time and lower larval and pupal body weight. As a result, adults become weaker and have lower fertility. This suggests that future herbivore generations will be less fit, causing less damage to the host plants.

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

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