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Elevated CO_2 and temperature effect on growth and physiology of *Chenopodium album* L.

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ABSTRACT

A study was conducted in open top chambers (OTCs) to understand the effect of elevated temperature (ambient+2±0.5^{o}C) and elevated CO_2 (550±50 ppm) individually and in combination on *Chenopodium album*. Impact of the climate variables was studied in terms of selected plant attributes, viz., leaf area, RGR etc. Study showed that elevated temperature as well as elevated CO_2 individually and in combination had significant positive effect on growth and development, rate of photosynthesis, and water use efficiency of the *Chenopodium album*. Rate of transpiration and stomatal conductance increased marginally in plants grown at elevated temperature, but a marked decrease was evident at elevated CO_2 individually and in combination with elevated temperature as compared that in plants grown in ambient conditions in the *Chenopodium album*. No significant changes were observed in relative water content and relative stress injury under any of the *Chenopodium album*. Treatments changes were evident with respect to the activity of antioxidant enzymes and nitrate reductase and peptide banding pattern using SDS-PAGE. This research was conducted to examine the joint effects of increased temperature and elevated CO_2 level on*Chenopodium album* (C3 weed). Results from this experiment suggested that rising (CO_2) could alter physiochemical response for growth and development of *Chenopodium album* and it is well defined competitors with different crops in current changing climate conditions.

Keywords: Climate change, protein profiling, elevated CO_2, elevated temperature, SDS-PAGE

INTRODUCTION

Chenopodium album (L) belongs to the *Chenopodiaceae* family, which comprises around 250 species that are cosmopolitan, annual weed species of significant economic significance distributed worldwide over semi-arid areas, including India. Their biological characteristics, such as high reproductive capacity, seed dormancy, high persistence in the soil seed bank, ability to germinate and develop under a wide range of environmental conditions, and abiotic stress tolerance, allow this species to infest a wide range of cropping systems. High temperature, strong sunlight during summers, high evaporation, little precipitation and increased salinity in the soil surface are some suitable conditions for its growth (Medina 1996). The *Chenopodium* plant has traditionally been used as an herbal medicine to treat a variety of ailments, including stomach pains, eye disease, throat problems, piles, blood, heart, and spleen diseases, and biliousness (Bhatia*et al.,* 2020).

C. album grows tall and absorbs nutrients very efficiently. Since this species is allelopathic, it prevents native vegetation and/or crop plants from germinating and growing. Many agronomic and horticultural crops are infested by this weed species, which can result in crop yield losses of up to 90%. *C. album* is more troublesome because it is more common and infests a greater number of crops, as well as acting as an alternative host for a variety of crop pests (Bajwa *et al.*, 2019).Apart from basic nutritional benefits, *C. album* is an under utilised vegetable with a lot of functional potential. The plant is used in the diet to provide nutrients, fibre, vitamins, and essential fatty acids, as well as to improve the food's sensory and functional value. The plant has been used as a blood purifier, diuretic, sedative, hepatoprotective, antiscorbutic laxative, and anthelmentic against round and hookworms for thousands of years (Poonia and Upadhayay 2015).The RCPs identify four separate 21st century routes of GHG emissions and ambient concentrations, air pollutant emissions, and land use, according to the IPCC's 5th Assessment Report. The increase in global mean surface temperature by 2080–2100 is in the range 0.3–1.7°, 1.1–2.6°, 1.4–3.1°C and 2.6–4.8°C under RCP 2.6, 4.5, 6.0 and 8.5 respectively. Climate change has an effect on not only the production of individual plant species, but also on interactions with other

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species at various stages of development **(**Sarathambal *et al.,* 2016). The two most significant causes of climate change are an increase in atmospheric CO_2 and a rise in temperature. Domestication of a weedy/wild species necessitates in-depth analyses of the species' adaptive ability to stress and climatic conditions, in addition to a detailed evaluation of yield potential and nutritional aspects. Plant growth and metabolism are directly linked to increasing CO_2 concentrations and rising global surface temperatures. Since CO_2 is a primary raw material in photosynthesis, an increase in atmospheric CO_2 concentration has been shown to have a fertilisation effect, resulting in increased crop biomass and yields, particularly in C3 plants (Taub, 2010; Lenka and Lal,2012). This has been due to the increased rate of photosynthesis as a result of increased CO_2 levels. However, increased surface temperature conditions can alter the CO_2 fertilisation effect.

C. album is important weed/ crop in major parts of central India. However, there is a scarcity of data from field studies on the effect of climate change on *C. album* in Indian conditions. As a result, the present study was conducted to investigate the individual and combined effects of elevated CO_2 and elevated temperature on *C. album* growth and development, physiological and biochemical attributes in field conditions using Open Top Chambers (OTCs).

MATERIALS AND METHODS

The research was carried out in open top chambers (OTCs) at the ICAR-Directorate of Weed Research in Jabalpur, Madhya Pradesh, India (23°102 N 79°562 E). *C. album* plants were grown in OTC. Ambient, elevated temperature (ambient + 2°C), elevated CO_2 (550 ± 50 ppm), and elevated temperature + elevated CO_2were among the treatments. Elevated temperature was achieved using infrared heaters installed within the OTC chambers, which were precisely controlled using an automatic control system with an on/off mechanism that took ambient temperature as a reference point at any given time. The desired temperature was maintained round the clock throughout the experiment. Elevated CO_2 treatment was maintained only during sunshine hours only. Different treatments were imposed from 10 days after sowing (DAS) till the end of the experiment. Sampling for different growth parameters was done at 45 and 90 days after treatments (DAT), while physiological and biochemical observations were also taken at 45 and 90 DAT only. All the observations were made at least three times and analyzed using completely randomized design.

The plant's leaves were separated and the total leaf area was calculated using an area meter (LI-3100CR, Lincoln, Nebraska, USA). RGR (mg/plant/day) = $(W_2 - W_1)/(t_2 - t_1)$, where W_1 and W_2 are the dry weights of the plant's aboveground sections at time t_1 (45 DAT) and t_2 (90 DAT), respectively. Using an integrated portable photosynthesis system, rates of photosynthesis, transpiration, and stomatal conductance were measured (LI-COR, LI-6400, Lincoln, NE, USA). All of the measurements were made in the second leaf from the top between 10 a.m. and 11 a.m.RSI was calculated using the formula:

$$RSI = \frac{EC1}{EC2} \times 100$$

The relative water content (RWC) was calculated using the following formula:

$$RWC = \frac{(FW-DW)}{(TW-DW)} \times 100$$

For Photosynthetic pigments estimation, a standard curve was prepared according to Hiscox and Israelstam (1979). Quantity of these pigments were calculated in mg g^{-1} tissue fresh weight and expressed as µmoles g^{-1} tissue fresh weight (Welburn 1994).For biochemical observations, leaf samples were taken from second leaf from top and snapfrozen in liquid nitrogen, and stored at -80°C till further use. A 0.1g of leaves were harvested and ground to a fine powder in liquid nitrogen. Ground powder was homogenized in 1.5 ml of cold phosphate buffer (100 mM, pH 7.0) containing 1% polyvinylpyrrolidone (PVP) and 1mM EDTA and then centrifuged at 4^oC for 15 min at 10000g. The supernatant was separated and stored on ice till the assay of enzyme activity. This extract was used for the enzyme assay with spectrophotometer of all the enzymes except APX for which extraction buffer was supplemented with 2 mM L-ascorbate. Protein

content of extract was determined using dye binding method. Activity of catalase (EC 1.11.1.6) was determined by monitoring H_2O_2 removal as the decrease in absorbance at 240 nm as suggested by Aebi (1983). The enzyme activity was expressed as units/mg protein/min and a change of 0.1 absorbance corresponds to one unit of enzyme activity. Ascorbate peroxidase (EC 1.11.1.11) activity was determined by monitoring the oxidation of ascorbate according to the method suggested by Nakano and Asada (1981) with slight modification. The change of 0.1 absorbance corresponds to one unit of enzyme activity and enzyme activity was expressed as units/ mg protein/min.

Guaiacol peroxidase (EC 1.11.1.7) activity was measured as suggested by Rao *et al.*, (1996).The increase in absorbance was recorded for 2 min at 470 nm and change of 0.1 absorbance has been taken as one unit and enzyme activity was expressed as units/mg protein/min. Superoxide dismutase (EC 1.15.1.1) activity was estimated using xanthine-xanthine oxidase system as suggested by Beyer and Fridovich (1987). The enzyme activity was calculated as units (amountof enzyme required to inhibit NBT reduction by 50%) and expressed as units/mg protein/min.Glutathione reductase (EC 1.11.1.9) activity was estimated by the method as suggested by Smith *et al.*, (1988). The change of 0.1 absorbance is taken as one unit and enzyme activity was expressed as units/mg protein/min. The *in vivo* assay of nitrate reductase (EC 1.7.1.2) in leaf was done according to the procedure of Ahmad *et al.*, (2010). Nitrite was estimated by the method of Evans and Nason (1953). The enzyme activity was expressed as mole NO_2/mg protein/ min.

Changes in peptide profile of the leaves were determined by SDS-PAGE using discontinuous buffer system.

Results and Discussion

Effect of elevated temperature and elevated CO_2 on growth and development

Growth and development of *C. album* were affected intreatments. Elevated temperature (ET) as well as elevated CO_2 (EC) individually and in combination (ET + EC) had significant positive effect on growth of *C. album* ascompared that plant grown in ambient conditions (Plate 1).

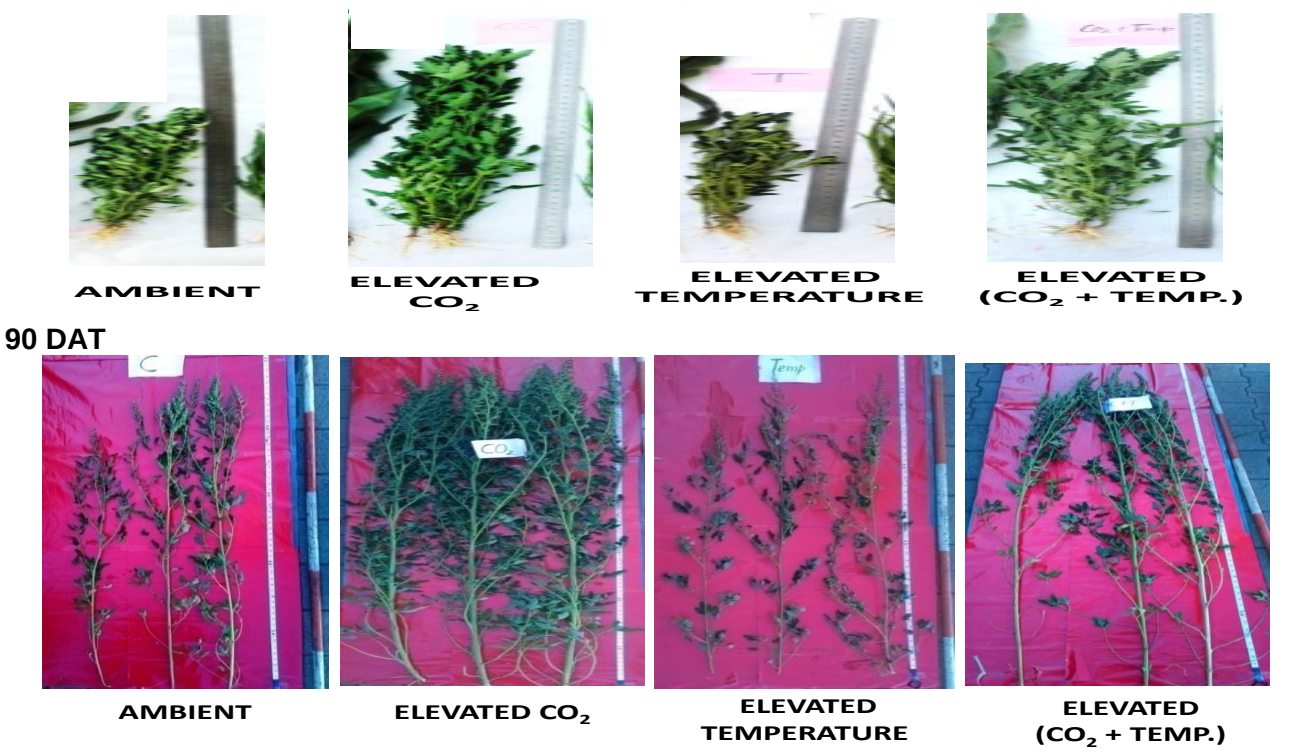


Plate 1: Effect of elevated temperature and elevated CO_2on growth and development of *C. album* at 45 and 90 DAT

At 45 and 90 DAT, different growth parameters, such as leaf area per plant, plant dry weight, and relative growth rate (RGR) was measured in *C. album*. Plants grown under elevated temperature, elevated CO_2 individually and in combination increased marginally in leaf area per plant as compared to plants grown in ambient conditions in *C. album* and at both sampling levels, but the difference found was to be significant. In comparison to *C. album* under ambient and climate change conditions, elevated CO_2, combination (ET+EC) treatment resulted in significantly higher leaf area, while elevated temperature resulted in significantly lower leaf area (45 and 90 DAT) (Table 1).

Table 1: Effect of climate change on Leaf area and Total dry weight, RSI, RWC and relative growth rate (RGR) of *Chenopodium album* L

Treatment	Leaf area (cm^2)		Total Dry weight (g/plant)		RSI(%)		RWC (%)		RGR (mg dry wt/ plant/day)
	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	
Temperature									
Ambient	276^{b}	269^{a}	3.68^{a}	6.56^{b}	18.82^{b}	23.84^{a}	79.73^{a}	79.96^{a}	64.11^{b}
Elevated	343^{a}	302^{a}	2.98^{b}	8.28^{a}	25.72^{a}	23.70^{a}	75.42^{b}	72.64^{b}	117^{a}
Carbon dioxide									
Ambient	302^{a}	203^{b}	2.37^{b}	6.40^{b}	21.79^{b}	25.67^{a}	79.26^{a}	78.15^{a}	89.41^{a}
Elevated	317^{a}	368^{a}	4.29^{a}	8.45^{a}	22.75^{a}	21.87^{b}	75.88^{b}	74.45^{b}	92.41^{a}
Temperature and Carbon dioxide									
C: Ambient (AT+AC)	244^{b}	197^{b}	3.11^{b}	5.96^{b}	16.61^{d}	26.16^{a}	81.88^{a}	83.26^{a}	63.41^{b}
AT + EC	309^{ab}	341^{a}	4.25^{a}	7.16^{ab}	21.03^{c}	21.52^{b}	77.58^{b}	76.66^{b}	64.81^{b}
ET + AC	361^{a}	208^{b}	1.64^{c}	6.83^{b}	26.98^{a}	25.18^{a}	76.65^{bc}	73.05^{b}	115^{a}
ET+ EC	325^{a}	395^{a}	4.33^{a}	9.73^{a}	24.47^{b}	22.22^{b}	74.19^{c}	72.24^{b}	120^{a}

The values with same letter cases are not significantly different at p ≤ 0.05 level.
DAT- days after treatment, AT: Ambient temperature, AC: Ambient CO_2, ET: Elevated Temperature, EC: Elevated CO_2

Similarly, plant dry weight of above ground biomass was decreased by elevated temperature but increased by elevated CO_2 and combination of elevated CO_2+ temperature as compared to *C. album* under ambient conditions at 45 DAT. But at 90 DAT, plant dry weight of above ground biomass was substantially increased under elevated CO_2 + temperature combination and marginally increased under elevated CO_2 and elevated temperature as compared to *C. album* under ambient conditions. For the Relative Growth Rate (RGR), the effect of combination of elevated CO_2 + temperature of *C. album* was significantly greater than the effect of other treatments. Plants grown under elevated CO_2 had only minor variations in RGR as compared to plants grown in ambient conditions. However, in *C. album* plants grown under elevated CO_2+temperature conditions, the RGR difference was substantially higher than in *C. album* plants grown under elevated temperature conditions (Table 1).

Plants had a greater number of branches under elevated CO_2, resulting in increased leaf biomass, which may explain the growth enhancement in *C. album* in this study. Second, increased growth may be due to a higher rate of photosynthesis in the presence of elevated CO_2, resulting in the accumulation of more assimilates in the plant (Zhu *et al.*, 2016). CO_2 levels are thought to be an essential factor that affects plant growth and increases aboveground biomass, which is good for them. In agreement with our results, stimulation of plant growth in response to high CO_2 has been reported in many crops such as wheat, soybean and rice. Zhu *et al.*, (2016) reported a significant increase in stem and leaf yields of two varieties of *Artemisia annua* under elevated CO_2 due to enhanced rate of photosynthesis. Promotion of growth and development has also been reported in weeds species. Price *et al.*, (2009) reported positive responses in growth of six weed species to elevated CO_2, suggesting that they may become more problematic as atmospheric CO_2 continues to rise. However, there are opinions that an increased CO2 effect on plant growth is not universal (Bader *et al.*, 2013). Plant growth stimulation in response to high CO_2 has been documented in many crops, including wheat (Wu *et al.*, 2004), soybean (Rogers *et al.,* 2004), and rice (Long *et al.,*

2006), which agrees with our findings. Because of the increased rate of photosynthesis, Zhu *et al.,* (2016) recorded a substantial increase in stem and leaf yields of two varieties of *Artemisia annua* under elevated CO_2. Weed species have also been found to promote growth and development. Price *et al.,* (2009) found that six weed species responded positively to increased CO_2, implying that they may become more troublesome as atmospheric CO_2 levels rise. However, some argue that the impact of increased CO_2 on plant growth is not universal.

Ziska (2003) investigated the impact of elevated CO_2 on the growth of six weed species, including *Cirsium arvense, Convolvulus arvensis, Euphorbia esula, Sonchus arvensis, Centaurea maculosa,* and *Centaurea solstitialis,* and discovered a 46 percent increase in plant biomass accumulation from ambient to elevated CO_2. Franzaring *et al.* (2008) used a FACE method to study *Brassica napus* and found that CO_2 enrichment significantly increased plant height, shoot weight, and dry weight of reproductive organs, suggesting that plant growth and the transition from vegetative to generative organs were accelerated. It was also suggested that elevated CO_2 had a greater impact on plant phenology at all stages of development (Lee 2011). In tomato plants, Yelle *et al.*, (1990) found that two weeks of high CO_2 (900 ppm) exposure resulted in 55 and 33 percent increases in leaf area and real leaf weight, respectively. Many crop species have recorded adverse effects as a result of these climate events. Temperature plays an important role in phonological production, which includes flowering (Bahuguna and Jagadish 2015). Temperature and CO_2 are predicted to have a major effect on key plant physiology and phenology processes when combined. The beneficial effects of elevated CO_2 have been reported for many crops, however, it is also suggested that elevated temperature would counterbalance the beneficial effects of CO_2. As a result, studying individual organisms' responses to CO_2 and high temperatures is crucial for determining the effects of future climate change. The biomass and seed weights harvested from *C. album* were significantly decreased, by 47.3% and 14.6%, respectively, under 4°C elevated temperature with ambient CO_2 conditions compared to the control, whereas they were dramatically increased, by 33.9% and 114.4%, respectively, in combined conditions of 4 °C elevated temperature and 1.8 times ambient CO_2 conditions compared to the control. The biomass of *S. viridis* grown under combined conditions of 4 °C elevated temperature and 1.8 times ambient CO_2 conditions did not differ significantly from that of the control, although it was slightly increased under 4°C elevated temperature with ambient CO_2 conditions.

Table 2: Effect of climate change on Chlorophyll a, Chlorophyll b, Total Chlorophyll content and Carotenoid of *Chenopodium album* L.

Treatment	Chlorophyll a (µg/ml)		Chlorophyll b (µg/ml)		Total Chlorophyll Content (µg/ml)		Carotenoid (µg/ml)	
	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT
Temperature								
Ambient	1.46^{b}	3.53^{a}	0.32^{a}	1.01^{b}	1.78^{b}	4.55^{a}	0.89^{a}	1.39^{a}
Elevated	1.94^{a}	3.14^{b}	0.36^{a}	1.38^{a}	2.30^{a}	4.52^{a}	0.88^{a}	1.01^{b}
Carbon dioxide								
Ambient	1.31^{b}	3.12^{b}	0.32^{a}	1.32^{a}	1.64^{b}	4.45^{b}	0.79^{b}	1.05^{b}
Elevated	2.09^{a}	3.55^{a}	0.36^{a}	1.07^{b}	2.45^{a}	4.63^{a}	0.98^{a}	1.35^{a}
Temperature and Carbon dioxide								
C: Ambient (AT+AC)	0.72^{c}	3.12^{b}	0.30^{a}	0.94^{b}	1.02^{c}	4.06^{b}	0.69^{c}	1.44^{a}
AT + EC	2.19^{a}	3.95^{a}	0.35^{a}	1.09^{b}	2.55^{a}	5.04^{a}	1.09^{a}	1.33^{a}
ET + AC	1.90^{b}	3.12^{b}	0.35^{a}	1.71^{a}	2.25^{b}	4.83^{a}	0.90^{ab}	0.66^{b}
ET+ EC	1.98^{b}	3.16^{b}	0.36^{a}	1.06^{b}	2.35^{ab}	4.22^{b}	0.86^{bc}	1.36^{a}

AT: Ambient temperature, AC: Ambient CO_2, ET: Elevated Temperature, EC: Elevated CO_2
The values with same letter cases are not significantly different at $p \leq 0.05$ level, DAT- days after treatment

Our findings indicate that increased temperature has a significant impact on biomass production in annual grasses during the reproductive stage versus the vegetative growth

stage, and that these effects may be stronger in C3 plants than in C4 plants. However, the negative effects of warming may be mitigated significantly by increased CO_2, especially for C3 grasses (Jae-Seok Lee 2011). Our observations and data revealed that elevated temperature and CO_2 have a substantial positive impact on various aspects of growth and development in *C. album*, both individually and in combination.

Effect of elevated temperature and elevated CO_2 on physiological aspects

Rates of photosynthesis increased marginally in plants grown at elevated temperature and significantly at elevated CO_2 individually and in combination with elevated temperature as compared that in plants grown in ambient conditions in *C. album*. Significantly higher rates of photosynthesis were noticed in *C. album* under elevated conditions as compared to ambient condition as well under climate change conditions. However, increase in photosynthesis rates was more in *C. album* under elevated CO_2 individually and in combination with elevated temperature (Table 3). Stomatal conductance is a measure of opening of stomata through which gas exchange takes place to external environment. In *C. album*, a significant increase in stomatal conductance was noticed at elevated temperature; however, it decreased significantly at elevated CO_2 as compared to that under ambient conditions. A marginal increase in stomatal conductance was noticed in combination treatment (elevated temperature + elevated CO_2) (Table 3). Rates of transpiration increased marginally in plants grown at elevated CO_2, but a marked decrease in the rate of transpiration was evident at elevated temperature individually and in combination with elevated CO_2 + temperature as compared that in plants grown in ambient as well under climate change conditions in *C. album* (Table 3).

Table 3: Effect of Climate Change on Photosynthesis, Stomatal Conductance, Transpiration, Water Use Efficiency of *Chenopodium album* L. in OTC

Treatment	Photosynthesis (µmoles/m/s)	Stomatal Conductance (mmoles/m2/s)	Transpiration (mmoles/m2/s)	Water Use Efficiency
Temperature				
Ambient	23.87^{b}	0.037^{b}	1.89^{a}	14.63^{b}
Elevated	28.12^{a}	0.06^{a}	1.28^{b}	22.07^{a}
Carbon dioxide				
Ambient	22.79^{b}	0.06^{a}	1.84^{a}	14.60^{b}
Elevated	29.20^{a}	0.03^{b}	1.32^{b}	22.10^{a}
Temperature and Carbon dioxide				
C: Ambient (AT+AC)	18.14^{c}	0.05^{b}	2.42^{a}	7.52^{b}
AT + EC	29.60^{a}	0.02^{d}	1.36^{b}	21.75^{a}
ET + AC	27.44^{b}	0.08^{a}	1.27^{b}	21.69^{a}
ET+ EC	28.80^{ab}	0.04^{c}	1.28^{b}	22.45^{a}

The values with same letter cases are not significantly different at $p \leq 0.05$ level.
DAT- days after treatment, AT: Ambient temperature, AC: Ambient CO_2, ET: Elevated Temperature, EC: Elevated CO_2

Instantaneous Water Use Efficiency is higher in plant grown at elevated CO_2, elevated temp and its combination however, No significant difference in Instantaneous Water Use Efficiency was observed in any of the treatments as compared to ambient as well under climate change conditions in *C. album* (Table 3). Relative stress injury (RSI) is an indicator of membrane damage. Significantly higher values of RSI were noticed in *C. album* at elevated temperature as compared to ambient however, no significant difference in RSI was observed in any of the treatments at 45 DAT (Table 1). But higher values of RSI were noticed in all treatment along with ambient as well under climate change conditions in *C. album* at 90DAT (Table 1). No significant difference in relative water content (RWC) of *C. album* at 45 and 90 DAT was observed with respect to treatments. Chlorophyll 'a' content was increased by elevated temperature and combination of elevated CO_2 + temperature but slightly decreased by elevated CO_2 as compared to *C. album* under ambient conditions at 45 DAT. But

at 90 DAT, Chlorophyll 'a' content was significantly highly increased under elevated CO_2 and slightly difference under elevated temp and as combination of elevated CO_2 + temperature as compared to *C. album* under ambient conditions.

No significant difference in Chlorophyll 'b' content was observed in any of the elevated treatments as compared to ambient as well under climate change conditions in *C. album* at 45DAT. (Table 2) But Chlorophyll 'b' content was increased by elevated temperature and no significant difference was noticed in any other treatment i.e. elevated CO_2 and combination with elevated temperature as compared to *C. album* under ambient conditions at 90 DAT. According to current theory, the effect of increased carbon dioxide levels on photosynthesis is dependent on leaf temperature. The underlying mechanism is based on both enzyme kinetics (decreasing CO_2/O_2) specificity of Rubisco as temperature raises and to a lesser degree, gas solubilities, which also shift in favor of oxygenation in a warmer climate. Both theoretically (Farquhar *et al.*, 1980) and experimentally, this is supported (Nijs *et al.,* 1992).High CO_2 has two well-known effects on plants: an increase in photosynthesis rate and a decrease in stomatal conductance and transpiration (Usuda 2006). Furthermore, several studies have shown that plants grown under elevated CO_2 for longer periods of time have a ‘down regulation' of photosynthesis rate (Koike *et al.,* 2015). CO_2 enrichment increased water-use efficiency in sorghum, according to Conley *et al.,* (2001), who went on to say that greater stimulation of water-use efficiency in weed species than in crops species could give weeds a competitive advantage.

When C3 plants are exposed to high CO_2, their rate of CO_2 assimilation also increases immediately. During leaf growth, photosynthesis of tomato leaves exposed to ambient and elevated CO_2 reached the same maximum value; however, leaves of plants grown at elevated CO_2 grew faster and reached maximum photosynthesis sooner. The increase and decrease in photosynthetic ability in tomato leaves is dependent on the stage of leaf growth. Morrison (1985) observed a decrease in stomatal conductance and transpiration in both C3 and C4 species at elevated CO_2, which corroborated our findings, and the argument was strengthened by a substantial increase in transpiration efficiency (CO_2 assimilated per unit of H_2O transpired). According to Carlson and Bazaz (1980), the CO_2 concentration from 300 to 660 ppm resulted in a variable increase in water use efficiency in different organisms (5 % in sunflower, 54 % in corn, 48 % in soybean). Plants grown under elevated CO_2 increased total dry biomass by 36 and 66%, respectively, while yields (dry mass of tubers) increased by 40 and 85 % in *S. tuberosum* and *S. curtilobum*. Increased photosynthesis and decreased stomatal conductance are two well-documented responses of plants to elevated CO_2 (Unsworth and Hogsett 1996), and our findings are consistent with the above article.

Effect of elevated temperature and elevated CO_2 on activity of enzymes

Effect of elevated temperature and elevated CO_2 individually and in combination was studied on the activity of important enzymes involved in antioxidant defence (catalase, ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase, glutathione reductase and nitrate reductase)in the leaves of *C. album* at 45 and 90 DAT. In *C. album*, a substantial increase in catalase activity was recorded in plants grown at in combination elevated CO_2+temperature and marginally increase in elevated CO_2 and elevated temperature alone as compared that in plants grown in ambient conditions at 45 DAT. In *C. album*, increase in the activity of catalase was also evident at individually both elevated CO_2 and elevated temperature, while a slightly decrease was observed in combination treatment (elevated temperature + elevated CO_2) at 90DAT (Table 4).

Ascorbate peroxidase is another important enzyme of antioxidant defence pathway. In *C. album*, ascorbate peroxidase activity increased significantly in plants grown at elevated temperature as compared to that in plants grown under ambient conditions at 45 DAT. In *C. album*, significant increase in ascorbate peroxidase activity was noticed at elevated CO_2 and in combination with temperature as compared to that in plants grown under ambient conditions at 90 DAT. Guaiacol peroxidase activity increased significantly in plants grown at elevated CO_2 and combination treatment (elevated temperature + elevated CO_2)

as compared that in plants grown in ambient conditions in *C. album* at 45 DAT. Apart from this, in *C. album*, significant increase in guaiacol peroxidase activity was noticed only at combination treatment (elevated temperature + elevated CO_2) as compared that in plants grown in ambient conditions at 90 DAT.

Table 4: Effect of elevated temperature and CO_2 on activity of guaiacol peroxidase, catalase, nitrate reductase, glutathione reductase, superoxide dismutase and ascorbate peroxidase (mg protein/min) of *C. album*

Treatment	Guaiacol peroxidase		Catalase		Nitrate reductase		Glutathione reductase		Superoxide dismutase		Ascorbate peroxidase	
	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT
Temperature												
Ambient	6.24^{b}	11.23^{b}	9.69^{b}	8.45^{b}	2.68^{a}	5.15^{b}	6.04^{b}	6.27^{a}	6.12^{b}	8.79^{b}	16.90^{b}	10.87^{a}
Elevated	7.86^{a}	12.94^{a}	16.90^{a}	9.53^{a}	2.70^{a}	5.72^{a}	7.00^{a}	6.18^{a}	6.76^{a}	9.39^{a}	18.97^{a}	11.70^{a}
Carbon dioxide												
Ambient	5.30^{b}	10.75^{b}	9.59^{b}	8.56^{b}	2.50^{b}	4.80^{b}	6.20^{b}	5.99^{b}	6.33^{a}	8.77^{b}	17.96^{a}	9.79^{b}
Elevated	8.81^{a}	13.43^{a}	16.99^{a}	9.42^{a}	2.89^{a}	6.07^{a}	6.83^{a}	6.46^{a}	6.55^{a}	9.41^{a}	17.91^{a}	12.77^{a}
Temperature and Carbon dioxide												
C: Ambient (AT+AC)	3.91^{c}	9.93^{c}	6.68^{c}	6.54^{c}	2.39^{c}	4.15^{c}	5.32^{b}	5.45^{c}	5.12^{b}	8.30^{c}	16.02^{c}	8.64^{c}
AT + EC	8.57^{a}	12.53^{b}	12.70^{b}	10.36^{a}	2.98^{a}	6.15^{a}	6.76^{a}	7.08^{a}	7.12^{a}	10.48^{a}	17.79^{b}	13.09^{a}
ET + AC	6.69^{b}	11.57^{bc}	12.51^{b}	10.59^{a}	2.60^{b}	5.45^{b}	7.09^{a}	6.53^{ab}	7.53^{a}	9.24^{b}	19.89^{a}	10.95^{b}
ET+ EC	9.04^{a}	14.32^{a}	21.29^{a}	8.47^{b}	2.80^{ab}	5.99^{a}	6.90^{a}	5.83^{bc}	5.98^{b}	8.34^{c}	18.04^{b}	12.44^{ab}

The values with same letter cases are not significantly different at p ≤ 0.05 level.
DAT- days after treatment, AT: Ambient temperature, AC: Ambient CO_2, ET: Elevated Temperature, EC: Elevated CO_2

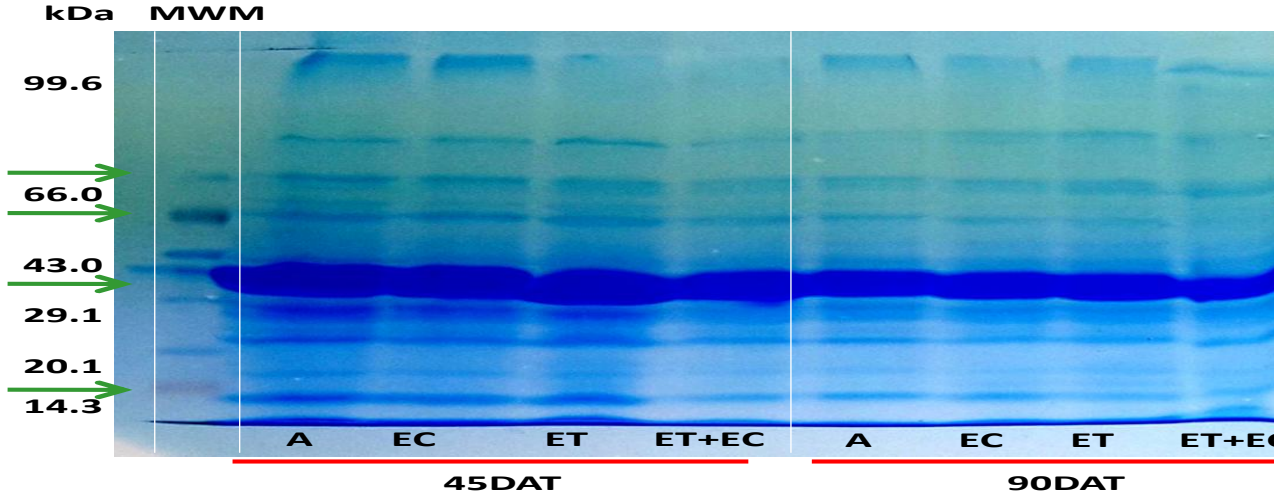
Superoxide dismutase mediates the dismutation of superoxide radicals. Activity of superoxidedismutase increased significantly in plants grown at elevated CO_2 and elevated temperature as compared that in plants grown in ambient conditions in *C. album* at 45 DAT and 90DAT. The activity of glutathione reductase increased significantly in plants grown at elevated CO2 and elevated temperature and combination treatment (elevated temperature + elevated CO_2) as compared that in plants grown in ambient conditions in *C. album* at 45 DAT and On the other hand, in *C. album,* the activity of glutathione reductase increased significantly in plants grown at elevated CO_2 and elevated temperature as compared that in plants grown in ambient conditions at 90DAT.

Nitrate reductase (NR) is a key enzyme in the nitrogen assimilation process (Table 4). The activity of nitrate reductase increased significantly in plants grown at elevated CO_2 and elevated temperature and combination treatment (elevated temperature + elevated CO_2) as compared that in plants grown in ambient conditions in *C. album* at 45 DAT and apart from this, in *C. album,* the activity of nitrate reductase increased significantly in plants grown at elevated CO_2 and combination treatment (elevated temperature + elevated CO_2) as compared that in plants grown in ambient conditions at 90DAT. Plants must adapt to cope with any deviation from their natural environment, and this can be accomplished by biochemical and molecular changes. Plants also developed a complex antioxidant mechanism to combat the harmful effects of reactive oxygen species (ROS), which plays a key role in their ability to withstand harsh environmental conditions (Noctor and Foyer 1998). Overall, antioxidant enzyme isoenzyme trends indicated differential regulation and sometimes induction of new isoforms in response to elevated CO_2; however, regulation and induction of isoenzymes cannot be generalised and appears to be species and treatment specific. Bokhari *et al.,* (2007) discovered 57 protein spots with differential expression patterns in rice at elevated CO_2, with the majority of these proteins belonging to photosynthesis, carbon metabolism, and energy pathways, or being molecular chaperones and APX. Pritchard *et al.,* (2000) found that elevated CO_2 reduced the activity of all antioxidative enzymes, with a focus on high-CO_2-induced reductions in the activities of SOD and CAT in soybeans of all genotypes. Schwanz *et al.,* (1996) discovered that elevated CO_2

reduced the activities of SOD, APX, and CAT in both pendunculate oak and maritime pine; however, elevated CO_2 had little to no effect on the activities of DHAR (dehydroascorbate reductase), MDHAR (monodehydroascorbate reductase), GR, and POX.

Our findings corroborate the above findings, as the activity of most enzymes involved in antioxidant pathways decreased as CO_2 levels rose. McKee *et al.*, (1995) proposed that elevated CO_2 protects mature wheat flag leaves from O_3 stress, and that the protective effect of elevated CO_2 is mediated by a decrease in stomatal conductance, which decreases O_3 influx to the plant. Many studies have shown that high temperatures cause oxidative stress (Larkindale and Knight, 2002). Changes in different components of the antioxidant defence pathway were observed in the current study under elevated temperature alone or in combination with elevated CO_2.Higher rates of nitrate assimilation, according to *Geiger et al.,* (1999), are necessary to promote faster growth under elevated CO_2.

At 45 and 90 DAT, SDS-PAGE was used to profile peptides in the leaves of *C. album* grown at ambient temperature, elevated temperature + elevated CO_2, and elevated temperature + elevated CO_2. At 45 DAT, a total of 14 bands could be seen, while at 90 DAT, 16 bands could be seen in the *C. album*. At 45 DAT, the 7th band from the top appeared only at elevated CO_2, and the 5th and 6th bands from the top appeared only at elevated temperature, indicating that they are both unique to elevated CO_2 and elevated temperature, but not to a combination of elevated temperature and CO_2. Apart from that, the 4th and 5th bands from the top vanished when compared to ambient. Similarly, in *C. album* at 90DAT, the 3rd and 15th bands from the top appeared only at elevated CO_2, the 5thband from the top appeared only at elevated temperature, and the sixteenth band from the top appeared only at elevated temperature + elevated CO^2.The 4th and 5th bands from the top in *C. album* appeared under control conditions but were absent in other treatments, suggesting that this band vanished whenever plants were exposed to elevated CO_2 and elevated temperature at 45 DAT. The 4th from top band only appeared at elevated temperatures and elevated temperatures + elevated CO_2, suggesting that this band is only active at elevated CO_2 and temperature in *C. album* at 90 DAT. (Figure 1).



MWM: molecular weight markers, A: ambient, ET: elevated temperature (ambient + 2 ^{o}C), EC- Elevated CO_2 (550 ppm), ET + EC: elevated temperature + Elevated CO_2

Figure 1: Changes in peptide profile (SDS-PAGE) in *C. album* under elevated temperature and elevated CO_2. Arrows indicate position of differentially expressed peptides at 45 and 90 DAT

Every living organism needs protein as a macromolecule. There hasn't been much research conducted to date that can definitively clarify the degree and essence of the effects of elevated CO_2 on plant protein metabolism. The effect of increased atmospheric CO_2 on the protein concentration of major food crops was studied using meta-analysis techniques. Using 2-dimensional electrophoresis, Bokhari *et al.,* (2007) investigated the proteomic response of

rice seedling leaves to elevated CO_2 levels (2-DE). There were 57 spots that had different speech patterns. MALDI-TOF/TOFMS research showed that the majority of the proteins were involved in photosynthesis, carbon metabolism, and energy pathways. The response of several molecular chaperones and ascorbate peroxidase to increased CO_2 levels was also discovered. The levels of enzymes involved in the Calvin cycle's regeneration process were also reduced in tandem with the reduction in photosynthesis and stomatal conductance. In rice leaves grown in CO_2 regulated chambers, the effects of elevated CO_2 (double that of ambient) on soluble protein content and 2-dimensional electrophoretic pattern were investigated (Fukayama *et al.,* 2009). The polypeptide profiles of soluble protein analysed by 2-DE using the same amount of protein in leaves grown under elevated CO_2 were completely unchanged between ambient and elevated CO_2.

The study found that elevated CO_2 and/or elevated temperature, as well as the combination of elevated CO_2 + temperature, had a major positive impact on growth and development, physiological and biochemical parameters, and protein profiling. *C. album* was discovered to be more resistant to changes in climatic conditions. Under the current climate change system, this attribute can be ascribed to a higher relative growth rate and a higher inherent antioxidant capacity. Plant output is affected by rising CO_2 levels in the atmosphere in a species-specific manner. *C. album* was found to be the most sensitive to increased CO_2, with higher transpiration and stomatal conductance, as well as a stronger antioxidant protection mechanism. *C. album* was found to be the most susceptible to elevated CO_2, with higher transpiration and stomatal conductance as well as a stronger antioxidant protection mechanism. Because of its higher cooling by transpiration and effective scavenging of free-oxygen radicals, *C. album* has a special adaptive ability to cope with newer environments. When CO_2 levels are high, the weed–crop relationship shifts in favors of the weeds, resulting in significant yield losses in associated crops. Not only for agriculture, but also for plants in general, how CO_2-induced changes in plant growth and metabolism would affect the complex interactions with weeds is an understudied region. As a result, more research is needed in this field to establish the best management strategies for the agriculture industry to respond to future environmental change successfully.

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Conflicts of interest

There are no conflicts of interest, and contributions of all of the authors are equal.

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