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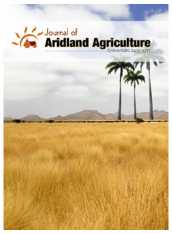


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# Effect of treated wastewater irrigation on plant growth and biochemical features of two wheat cultivars under the elevated level of $CO_2$ and UV-B radiation

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## ABSTRACT

Climate change is a serious problem affecting worldwide agricultural production and encourages researchers to investigate plant responses and grow crops under changed growing conditions. In arid and semiarid regions, treated wastewater is a common alternative source of water for irrigation. The proposed study examined the impact of irrigation with treated wastewater and the effects on the growth of wheat crops of environmental stress factors, including UV radiation and carbon dioxide. The experiment was conducted in a transparent Open Top Chambers facility and the treatments were administered in the hot UAE climate for ninety days. In order to understand the physiological mechanisms of plant adaptation under the conditions given, physiological and biochemical characteristics such as anti-oxidant enzymes have been assessed. The results revealed that the elevated $CO_2$ level increased the growth parameters, whereas when compared to control, the UVB treatment affected plant growth. In the seedling process, established under regulated development, the differential response of antioxidant activity, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) activities were observed among intrinsic biochemical activity in the selected Wheat varieties. Our findings show that wheat varieties are suitable as industrial crops for the production of antioxidants under irrigation with treated wastewater because the quantity and quality of their yield have not been affected. This practice will contribute to a clean environment and the stress on freshwater will be reduced by its reuse.

**KEYWORDS:** Climate change, wastewater, Antioxidant, wheat crop, UV-B

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## INTRODUCTION

Climate change is a serious problem affecting worldwide agricultural production and is a challenge for researchers to examine plant responses and to breed crops under changing growing conditions. Plants that resist or tolerate environmental stress depend on the productivity of agricultural and ecological systems (Zhang *et al.*, 2018). Variables of climate change, including precipitation (amount and distribution), temperature, radiation and concentrations of atmospheric $CO_2$, are expected to change the patterns of agricultural production worldwide. For crop production, abiotic stresses are the most important, affecting about 96.5 percent of arable land worldwide. Either abiotic stresses are often interrelated, individually or in combination, they cause morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity, and ultimately yield. The improvement of abiotic stress tolerance crops is of paramount importance for the global future of agriculture. Stress-tolerant varieties must therefore be obtained in order to deal with this upcoming food security issue.

Wastewater treatment plants (WWTPs) are in the second position after landfills regarding Greenhouse gases (GHG) emissions (Bogner *et al.*, 2007) and in the eighth position among the stationary sources for biogenic $CO_2$ emissions (USEPA, 2014). The levels of GHG emissions are not so high, but the assessment of GHG emissions from waste water treatment has become a topic of great interest due to climate change and environmental impact issues. Treated waste water (TWW) is generally classified into primary, secondary and tertiary-treated waste water, depending on the equipment used (Kalavrouziotis *et al.*, 2015). Wastewater can contain some nutrients that are useful for agriculture, but its continuous application increases toxic metals in plants and soil (Rattan *et al.*, 2005). A broad scientific portfolio backs our expertise on wastewater treatment, reuse and applications in agriculture, but ongoing research in this field is exploring ways of optimizing the effectiveness of wastewater in agriculture, with both qualitative and quantitative findings, without risks to the environment and health (Pilatakis *et al.*, 2013).

Increases in atmospheric $CO_2$ concentration, higher temperatures, altered precipitation and transpiration regimes, increased frequency of extreme temperature and precipitation events, and weed, pest and pathogen pressure will respond to plant development, growth, yield, and ultimately output of crop and pasture species (IPCC, 2007). Estimates based on climate change integration and crop yield models have projected further losses in the productivity of major crops, including rice, wheat and maize, which could have a significant effect on food safety (Tigchelaar *et al.*, 2018). Carbon dioxide is a fertilizer for plants, and atmospheric enrichment will increase the productivity of plants.

The increase in crop yield is smaller than the photosynthetic response. In comparison with current atmospheric $CO_2$ concentrations of about 380 ppm, crop yields grow in the range of 10-20 percent for $C_3$ crops and 0-10 percent for $C_4$ crops at 550 ppm $CO_2$ on average over many species and under unstressed conditions (Long *et al.*, 2004). Increased concentrations of atmospheric carbon dioxide ($CO_2$), drought and ultraviolet (UV)-B radiation are the key factors that cause major changes in yield and economic impacts on many plant species, including wheat. In comparison to $CO_2$, the effects of UV radiation on plants have long been recognized as stress-mediated and harmful, so changes in biomass growth and accumulation and primary and/or secondary metabolites are expected to cause (Ballar´e *et al.*, 2011).

Using controlled fumigation techniques, numerous studies that reported UVB and $CO_2$ effects in crop plants have been carried out. Open-top chambers (OTCs) have been a common experimental method for such experiments. Since the 1970s, when they were first in use in the USA to investigate the relationship between crop growth and yield to gaseous air pollutants in situ, open-top chambers have been an accepted design for evaluating air pollutant effects on plants (Heagle *et al.*, 1973). OTC is inexpensive and has a small shading effect, especially lower intake of $CO_2$, since air exchange is reduced by the closed side walls and frustum (Vanaja *et al.*, 2006). Passive OTCs provide researchers with a low-maintenance and cost-effective approach to investigate the influence of year-round warming on plant communities (Arft et al., 1999). The restricted height of passive OTCs (c. 0.4 m) has limited their use to low-status plant populations, early life phases and low productivity plant systems, despite their usefulness (Elmendorf et al., 2012).

In comparison, any significant conditions that change the performance of plant growth rely on the alteration of physiological and metabolic processes by changes in the different enzymatic, biochemical and molecular phenomenons. Importantly, the production and counteraction of reactive oxygen species by antioxidant enzymes regulates plant cell metabolic and oxidative homeostasis (Hussain et al., 2019). Because antioxidant enzymes play a vital role in controlling physiological and metabolic changes, a significant difference between plant species is shown by their differentiating response with their different levels of quantity. Under regulated conditions between two different wheat varieties, the generation of free radical species and their counteraction by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) were investigated. In addition, the analysis of complex environmental pressures on plants offers a clear level of data to evaluate the effect of any constraints during the growth cycle and enhances our understanding of their impact and sustainable development.

Since crop plants such as wheat are considered important subsistence crops in most desert areas of the world, the research on the day-to-day impact of the study increases stress factors on the very important plant. The predicted climate changes, weather extremes and the interaction between the various abiotic stresses would, however, have a profound effect on the adaptation and development of crops. The responses of different growth and biochemical parameters of wheat crop plants to combinations of environmental factors must therefore be evaluated. As some of the outcomes of this research, the identified crop traits tolerant to abiotic stresses will be valuable assets for the growers and breeder for the development of new varieties suited for the UAE growing conditions.

## MATERIALS AND METHODS

### Experimental Conditions

Six transparent plastic tops to allow full sunlight to pass through Open top chambers (OTC) located at the UAE University Al-Foah Experimental Farm Research Facility (24.2006° N, 55.6760° E), College of Food and Agriculture, UAE, were used for this analysis. Each OTC chamber is covered with transparent plastic at the top to allow full sunlight to pass through. During the experiment, the greenhouse air temperature was held at levels of 37°C. The OTCs have a diameter of 4 m and a height of 6 m. Within the OTCs, the airflow rate of 12,000 $m^3$ hr-1 adjusts the air three to four times per minute to keep the microclimate within the chambers close to outside the chambers. Eight fluorescent UV-313 (Q-Panel Company, Cleveland, OH, USA) with UVB radiation (280 and 320 nm) emissions were used to impose current (control) and elevated (U-VB) level UVB radiation treatments. The OTC units are capable of accurately monitoring temperatures and chambers ($CO_2$) at specified set points and at near ambient radiation levels. The fully automated control and monitoring system is the first of its kind in the UAE, which includes a $CO_2$ analyzer, UVB display, PLC and PC SCADA software to maintain the necessary $CO_2$ and UVB levels within the OTCs. A $CO_2$ analyzer tests the actual concentration of carbon dioxide inside the OTC, and UV-B radiation is monitored by a dedicated computer system assisted by inlet valve regulation (Uprety *et al.*, 2006; Vanaja *et al.*, 2006).

**Experimental Plants**

Under natural light conditions, the experiment was carried out. The wheat seedling site for open field growth is located at Al-Foah Experimental Farm, Faculty of Food and Agriculture, University of the UAE. The open field climate in the UAE is very hot and mild in the winters. The soil is sandy loam in texture. The open field was divided in to 3 blocks that possess 3 replications. The planting distance was 50 × 50 cm. Two distinct advanced genotypic lines derived from the 33rd ESWYT (V1) and 20th SAWYT (V2) international trials were used as targets for this analysis and these wheat varieties were collected from the CIMMYT Genetic Resource Centre, El Batan, Mexico. In the three sets composed of one plant each, the two wheat verities were planted (in pots with sand). As recommended for plant cultivation, plants were well supplied with nutrients and water. During the experiment, the greenhouse air temperature was held at levels of 37°C. For 10 days, wheat seeds were germinated, and 13 seedlings were then transplanted into each pot. With a 12 h light and 12 h dark illumination schedule, the temperature was held at 38.4°C. For the experiment, two separate treated waste water (TWW) from Al Wathba, Abu Dhabi and Al SAAD, Al Ain, were used. Standard water from the local well was used as control.

**Morphological Parameters**

The plants were harvested 120 days after UVB and $CO_2$ treatment and the root length was immediately determined, followed by measurements of plant height and head count. From the soil level to the tip of the shoot, plant height was measured and expressed in cm. From the point of the first cotyledonary node to the tip of the longest root, the plant root length was measured and expressed in cm. The total number of fully formed heads was counted and expressed as the number of heads per plant. After 120 days after UVB application, the leaf tissue components were measured in the fresh leaf materials.

**Estimation of Chlorophyll and Carotene**

The Chlorophyll and carotenoid contents of the wheat samples analyzed by the method described by Arnon (Arnon, 1949) with slight modifications. In brief, 0.5g of freshly selected leaf material was ground in a pestle-mortar with 10 ml of 80 percent acetone at 4°C and homogenized. The ground paste was moved to a 15 ml centrifuge tube after centrifugation and residue with 80 percent acetone was re-extracted until the green color vanished. In a tube containing 4.5 ml of 80 percent acetone, 0.5 ml of the supernatant was then transferred. After that, at 4 ° C for 15 minutes, the homogenized contents were centrifuged at 4000 rpm and subjected to colorimetry. The absorption was calculated as a blank against 80% acetone at 645, 663 and 480 nm in a Spectrophotometer (ColeParmer, USA) against 80% acetone.

Total Chlorophyll: 20.2(A645) + 8.02(A663)
Chlorophyll a: 12.7(A663) – 2.69(A645)
Chlorophyll b: 22.9(A645) – 4.68(A663)

The carotenoids content was estimated using the Kirk and Allen (1965) method and expressed in milligrams per gram fresh weight.

Carotenoid: (A480 + (0.114(A663)-(0.638-A645)) × V/1000 × W

**Superoxide Dismutase (SOD)**

For the superoxide dismutase (SOD) assay from protocols used by Hwang *et al.* (1999), crude enzyme extract was prepared. 10 ml of ice-cold 50 mM potassium phosphate buffer (pH 7.8) containing 1mM PMSFF was homogenized with one gram of fresh tissue. The supernatant was made up with 10 ml extraction buffer and used for estimation of the SOD enzyme activity. The reaction mixture contained 0.1M methionine, $2x10^{-5}$ potassium cyanide and $5.6 \times 10^{-5}$ M nitroblue tetrasodium salt (NBT), dissolved in 0.05 M phosphate buffer (pH 7.8). The response was triggered by the addition of 1.3μM of riboflavin and exposure for 15 minutes under two 15W fluorescent lamps (Philips Tornado Energy Saver, Philips Electronics). Illumination began for one hour to initiate the reaction at 30°C. Saved as blank, a non-irradiated mixture without illumination. The reaction stopped by switching off the lamps and covering the blue-coloured photoreduced reaction samples with a piece of black cloth. The photoreduction of the samples' NBT-2HCl content read using a spectrophotometer set at 560nm. One unit (U) of SOD is defined as the amount required inhibiting the photoreduction of NBT-2HCl by 50%, and is expressed as enzymes units per mg ($U.mg^{-1}$) protein.

**Catalase (CAT) Assay**

Using the Chandlee and Scandalios method (Chandlee *et al.*, 1983) with modification, the catalase (CAT) assay was calculated. In 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing 1mM PMSFF, 500 mg of the plant sample was homogenized. The enzyme protein was determined by Bradford (1976) method. The 3.1mL assay contained 100mM potassium phosphate buffer (pH 7.0) and 100μL of enzyme extract. The potassium phosphate buffer designated as the blank. The reaction initiated with the addition of 6mM $H_2O_2$. The decomposition of $H_2O_2$ monitored using a spectrophotometer set at 240nm for every 30 seconds up to three minutes, at an ambient temperature of 25°C. The catalase activity calculated using an extinction coefficient of $39.4M^{-1}.cm^{-1}$. The enzyme activity was expressed in units 1 mM of $H_2O_2$ reduction per minute per mg protein.

**Peroxidase Activity**

Peroxidase activity was measured by the method of Kumar and Khan (1982). Assay mixture of peroxidase contained 0.1M of sodium phosphate buffer at pH 1% guaicol and 30% $H_2O_2$ being tested on 0.5 mL of enzyme extract. The amount of purpurogallin formed was determined by pectrophotometrically at 420 nm for 3 minutes against a blank prepared by adding the extract after the addition of 2.5 N $H_2SO_4$ at zero time. The peroxidase enzyme activity calculated as specific enzyme activity where the one unit of enzyme activity is the amount of enzyme used to reduce hydrogen peroxide in the reaction vessel. One unit defined as the change in the absorbance by 0.1 $min^{-1}$ $mg^{-1}$ protein.

$$\text{Total peroxidase activities} = \frac{\text{Absorbance value x dilution factor x 1000}}{\text{Sample}}$$

### Statistical analysis

The analysis of variance (ANOVA) was performed using SPSS software (v. 21.0) to test the effect of UVB and $CO_2$. The significant difference between mean was determined by Duncan's multiple range test at the $P \leq 0.05$ level. The principal component analysis (PCA) was performed using PROC PRINCOMP procedure of SAS software (SAS 9.4).

## RESULTS AND DISCUSSION

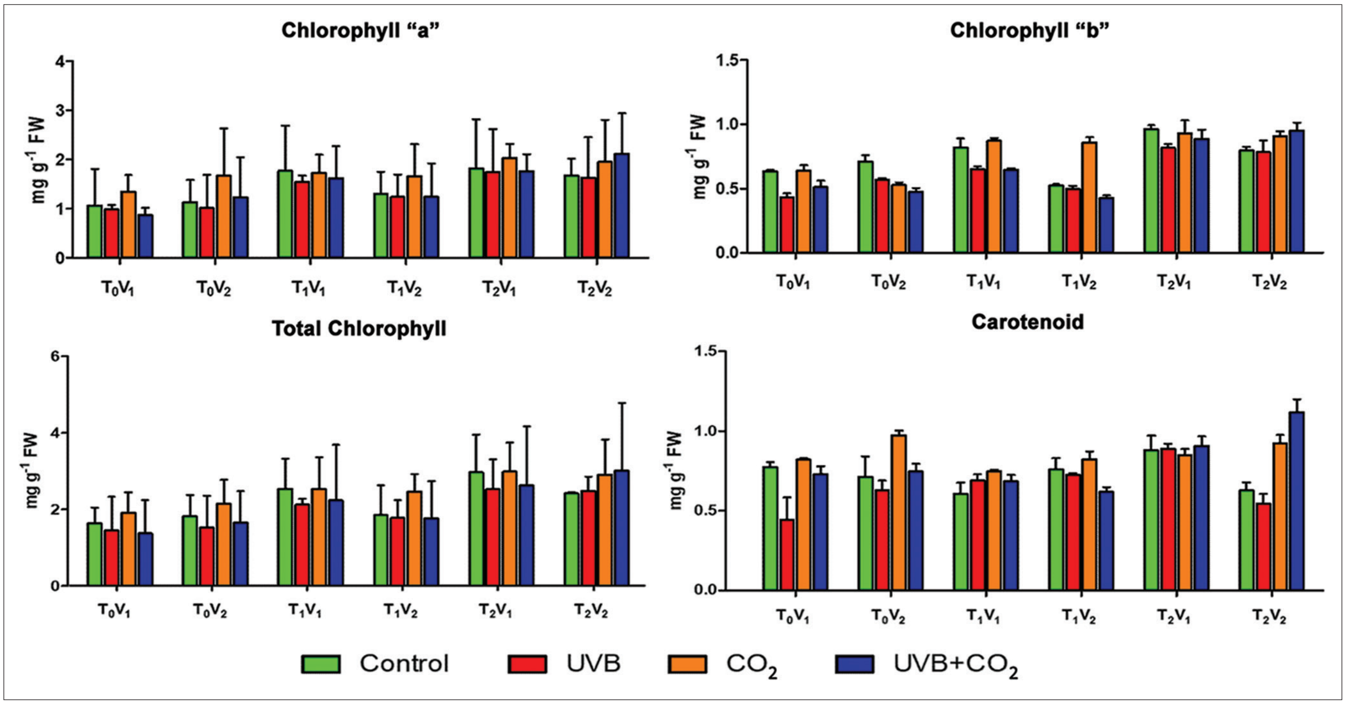
The results showed that, under OTC conditions, high $CO_2$ and UV-B radiation induced major alterations in the photosynthetic and biochemical content of the two wheat varieties tested. After each of the four months of therapy, wheat varieties which were grown under UV-B at elevated $CO_2$ were larger than those under control (UV-B at ambient $CO_2$) (Table 1). In the last two tests, they were also taller than plants grown under UV-B with elevated $CO_2$. However, there was no substantial difference in height between plants grown under UV-B with ambient $CO_2$ and control plants in all measurements. There was no major impact of UV-B radiation and $CO_2$ concentration on the root duration (Table 1). There was also no major association between the number of heads (P>0.05) between UV-B and $CO_2$. The volume and quality of crops can be influenced by UV-B radiation and atmospheric $CO_2$ concentrations (Long *et al.*, 2004). Previous studies have shown that high $CO_2$ increases biomass and seed yield, while UV-B reduces biomass and seed yield (Sullivan, 1997). Our research also found that UV-B radiation reduces wheat plant height and seed yield, particularly at ambient $CO_2$ (Tables 1). In other species, decreased height has been found in plants exposed to UV-B radiation (Dai *et al.*, 1994). The predicted higher doses of incoming UV-B radiation would stimulate a range of higher plant responses (Kakani *et al.*, 2003). Plants are affected by altered gene expression as their DNA is compromised due to an increase in UV-B radiation, mostly proteins/enzymes, stomatal regulation via membrane structure disintegration and altering morphological and physiological characteristics of plants, mainly reproductive sections (Kakani *et al.*, 2004). The findings on the chlorophyll content of the studied wheat varieties showed that after 8 hours of UVB and $CO_2$ treatment, the chlorophyll 'a' and 'b' content in Variety 2 was gradually reduced [Figure 1]. Compared with control crops, the chlorophyll 'a' content of UVB-exposed T0 treated crops V1 and V2 (T0V1 and T0V2) was found to be significantly reduced.

However, in variety one treated with UVB+$CO_2$, as compared to all other groups studied, the content of chlorophyll 'a' was significantly reduced. However, when compared to UVB treated groups, $CO_2$ exposed groups showed a substantial increase in chlorophyll "a". Especially, T0 water treated crops V1 and V2 (T0V1 and T0V2) was found to be significantly decreased chlorophyll "b" content, up to the level of 0.433±0.03 and 0.571±0.01 respectively. Increased levels of chlorophyll 'b' content indicated sensitivity of $CO_2$ to all treatments and varieties. There was no major difference between T0V2 and T1V2, however. In the UVB+CO2 exposed community varieties, the levels of total chlorophyll content in T0 and T1 treatments were substantially reduced. This may be due to the arrest of biosynthesis of UVB pigments (Musil *et al.*, 2002). These chlorophyll a and chlorophyll b content of UVB alone exposed wheat varieties results are supportive in line to the results reported by Juozaitytė *et al.* (2008) in *Pisum sativum* L *crop* varieties. A marked reduction in the rate of photosynthetic pigments such as carotenoids in the wheat varieties studied was noted in the current study. The carotenoid levels were reduced in UVB exposed control water treated varieties such as T0V1 and its levels came up to 0.445±0.14 mg g-1 FW. Varieties treated with different treated waters such as T0 and T1 increased the levels of carotenoids significantly. The results of this study are in accordance with the study of Fedina *et al.* (2003) in which

**Table 1: Effect of UVB and $CO_2$ on morphology and physiology of two Wheat varieties after 8 hours of treatment**

| Parameters | Treatments | Name of the variety | | | | | |
|---|---|---|---|---|---|---|---|
| | | CV1 | CV2 | T1V1 | T1V2 | T2V1 | T2V2 |
| Plant length | Control | 54.7±1.3 | 60.5±3.7 | 45.2±0.8 | 57.2±1.6 | 46±2.3 | 53.7±1.9 |
| | $CO_2$ | 55.8±2.3 | 55.6±3.1 | 53.5±1.2 | 54±1.5 | 43±2.8 | 52±1.2 |
| | UVB | 63.5±2.6 | 58.5±1.5 | 57.5±1.9 | 47±3.7 | 39±2.8 | 49.5±1.8 |
| | $CO_2$+UVB | 55.3±3.0 | 63±1.3 | 49.7±2.1 | 52±3.8 | 46±1.2 | 55±2.6 |
| Root length | Control | 12±0.14 | 14±0.12 | 10.5±0.8 | 15.5±1.4 | 10.5±0.9 | 12.5±1.1 |
| | $CO_2$ | 14±0.16 | 16.5±1.2 | 16.5±1.2 | 16±1.2 | 9±0.9 | 11.5±0.8 |
| | UVB | 11±0.13 | 12±0.6 | 12.5±1.1 | 14±1.2 | 11±0.7 | 17±1.3 |
| | $CO_2$+UVB | 16±0.9 | 15±0.4 | 12±0.9 | 10.5±0.1 | 11±0.09 | 10.5±0.1 |
| No. of head | Control | 2±0.01 | 3±0.02 | 1±0.01 | 4±0.03 | 1±0.01 | 1±0.01 |
| | $CO_2$ | 5±0.01 | 3±0.02 | 5±0.03 | 2±0.01 | 1±0.01 | 1±0.01 |
| | UVB | 4±0.03 | 3±0.03 | 3±0.02 | 1±0.01 | 1±0.01 | 3±0.02 |
| | $CO_2$+UVB | 4±0.03 | 5±0.04 | 2±0.01 | 1±0.01 | 1±0.01 | 2±0.02 |

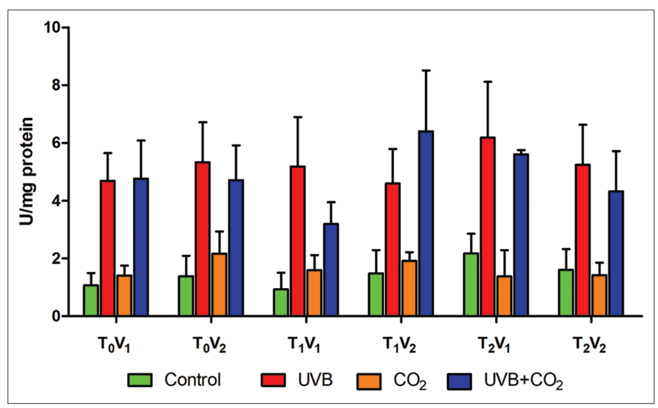
Values are the mean of three replicates;-Significant at $P<0.05$ level




*Data are expressed as mean ± SD for three different samples in each group. Values not sharing a common superscript letter in each figure differ significantly at P < 0.05*

**Figure 1:** Chlorophyll and carotenoid levels in combined effect of UVB and $CO_2$ on photosynthic changes of two Wheat varieties after 8 hours of treatment.

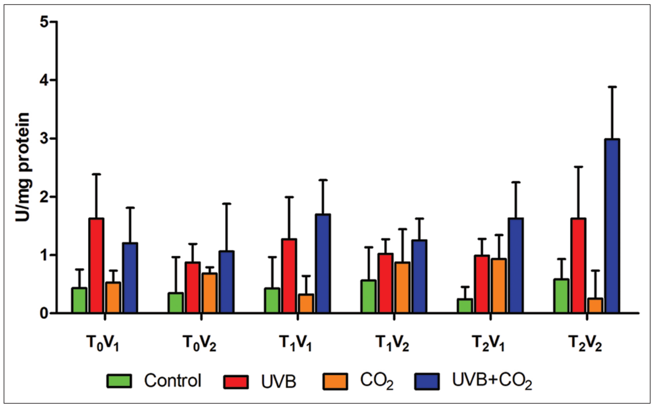
UV-B radiation showed an increase of carotenoid concentrations in Barley seedlings. Figure 2 demonstrates the activity of SOD in various treatments and varieties. Both water-treated classes of UVB-exposed and/or $CO_2$-treated crops have been found to have increased levels of SOD activity. Group crops treated with T2 water displayed much higher levels of SOD operations. However, the maximum of SOD activities was observed in T1V2 crops (6.398±2.11). Since, there was no statistically significant found between T1 and T2 treated groups of UVB + CO2 exposed crops. UV-B radiation increased the activity of SOD in peas, Arabidopsis and rice, but did not affect barley and soybeans. Supplemental UV-B increased SOD activity in mungbeans in a field study and induced differential responses between soybean cultivars (Agrawal *et al*., 2009). While plants generally had lower SOD activities in elevated CO2 than plants grown at ambient CO2 concentrations, the differences in the intermediate nutritional situation were most pronounced and negligible under a high nutrient supply rate (Polley *et al*., 1997).




*Data are expressed as mean ± SD for three different samples in each group. Values not sharing a common superscript letter in each figure differ significantly at P < 0.05*

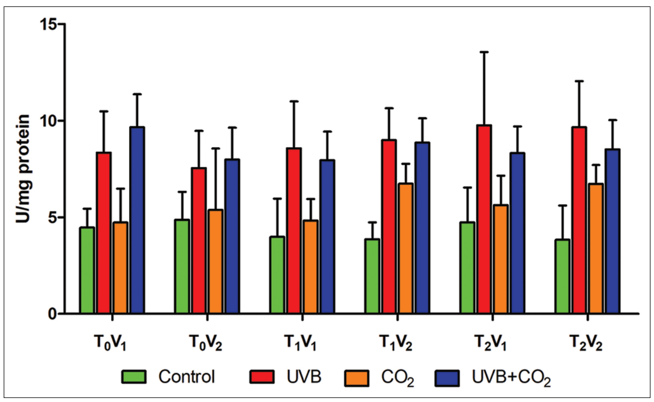
**Figure 2:** SOD level in combined effect of UVB and $CO_2$ treated two Wheat varieties after 8 hours of treatment

The catalase operation of the various water treatment plants and crop varieties was calculated and shown in Figure 3. The level of operation in all water treatments has risen dramatically. In T2 water treatment T2V2, the uttermost increment was found, the values are 5.010±0.50, showing significantly higher than other groups. However, in UVB+$CO_2$ exposure also, when comparing all the groups, $T_2V_2$ showed the significantly higher level of activities of catalase. However, under certain stress conditions, a decline in CAT activity is commonly observed, whereas other enzymes in the active oxygen scavenging system, such as SOD, APX, and GR, are typically caused by stress treatments (Shim *et al*., 2003). In general, the activity of these two enzymes has been significantly reduced compared to the controls produced under ambient $CO_2$ concentrations. Decreases in SOD activity have been observed in both deciduous and coniferous plants grown in growth chambers and OTC. Therefore, both SOD and CAT playing important and effective roles in the enzymatic response of wheat to high levels of UVB-induced ROS. The activity of peroxide concentrations in various water treated wheat crops is shown in Figure 4. Data from both samples indicated an increase in peroxidase activity levels relative to the control group. Since, the levels were not increased much in




*Data are expressed as mean ± SD for three different samples in each group. Values not sharing a common superscript letter in each figure differ significantly at P < 0.05*

**Figure 3:** Catalase activity in the combined effect of UVB and $CO_2$ treated Wheat varieties after 8 hours of treatment




*Data are expressed as mean ± SD for three different samples in each group. Values not sharing a common superscript letter in each figure differ significantly at P < 0.05*

**Figure 4:** Peroxides levels in the combined effect of UVB and $CO_2$ treated two Wheat varieties after 8 hours of treatment

few groups such as $T_0V_1$, which was 4.723±1.76. The maximum levels of peroxidase activities was found in $T_2$ water treated group, $T_2V_1$ (9.755±3.79). When comparing with other water treated groups, $T_2$ shows the significant higher levels, shows its efficiency in influencing the peroxidase activities. The activities of plant peroxidases typically increase in response to different environmental stress factors along with the activities of other antioxidant enzymes such as CAT, SOD and GR (Shigeoka *et al.*, 2002). A common response to oxidative and abiotic stresses is increased peroxidase activity. Peroxidase may also be part of the enzymatic mechanism linked to an increase in ethylene formation in plants such as spinach (Ozturk & Demir, 2003). These peroxidase activities are supportive in agreement to the results reported by Karthishwaran *et al.* (2020) in date palm varieties.

## CONCLUSIONS

Evidence of the chlorophyll content of the studied wheat plants showed that after 8 hours of $CO_2$ and UV-B application, the content of chlorophyll 'a', 'b' and carotenoids in UV-B decreased gradually and increased in the wheat of Al Ain water treated SAWYT (V2). The use of effluent treated water and UVB + increased $CO_2$ for irrigation and optimizing the yield and growth characteristics of wheat varieties is justified by the supportive results of antioxidant and enzyme assays. To better measure the potential impacts of climate change on arid land systems, recognizing the primary dynamics that characterize the interactions of elevated $CO_2$, enhanced UV-B with changes in climate variables, waste water cultivation, and remains a priority. The findings also advance our understanding by elucidating the different physiological and biochemical processes responsible for the characteristics resistant to abiotic stress among the crop varieties. It can therefore be inferred that other biochemical and climate control examinations may be carried out on the wheat varieties to justify their performance. While there is global concern about the negative environmental effects of $CO_2$ and other greenhouse gases, which tend to be a cause of global warming, high $CO_2$ emissions may have a beneficial impact on plants by mitigating the harmful effects of UV-B radiation. However, the genetic basis of the tolerant and intermediate hybrids must be further studied with intensive molecular assisted genetic engineering methods to assist for overcoming extreme abiotic stresses.

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