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EFFECT OF TREATED WASTEWATER IRRIGATION ON PHYSIOLOGICAL TRAITS AND ANTIOXIDANT DEFENSE ANALYSIS OF TOMATO AND CUCUMBER

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Abstract – Our study is conducted on the behavior of two species, the tomato and the cucumber, irrigated by three types of water from different origins. The first is from spring water, the second is from wastewater from the sewage treatment plant and is biologically treated, and the third is from dam water. The results obtained on seedlings irrigated by treated wastewater show good development compared to seedlings irrigated by spring water and those from the dam. Irrigation with purified water and those from the dam induces oxidative stress in the two species studied during their growth. The response to this xenobiotic is almost similar for both cultures. The analysis of the non-enzymatic parameters showed an overproduction of peroxidation of membrane lipids and glutathione under the effect of the stress generated by the waters of the station and the dam, their levels increase significantly in the two species studied. Analysis of the antioxidant enzymes, catalase, ascorbate peroxidase and guaïacol peroxidase, indicates that irrigation with purified wastewater causes activation of these enzymatic biomarkers in tomato and cucumber.

INTRODUCTION

Algeria suffers as much from insufficient rains as from their poor distribution in time and space. The constraints of the climate, demographic growth and socio-economic transformations are at the origin of a largely deficit water situation. Thus, to meet all water needs, we are forced to use water of marginal quality in agriculture which has experienced great intensification. In this context, the use of purified wastewater has become a priority necessity as a regular free resource, abundant and rich in nutrients.

The reuse of treated wastewater is practiced mainly by farmers, either directly (13%) from wastewater treatment plants or indirectly (87%) from wadis supplying dams (Lehtihet, 2005).

However, for it to be part of a sustainable development framework, the enhancement of the reuse of this wastewater requires a careful and integrated study that takes into account above all environmental aspects. Previous work has shown that the supply of wastewater provides significant amounts of organic matter and major nutrients (N, P, K), secondary nutrients (Ca, Na, Mg, etc.) and trace elements. (Fe, Cu, Co, Ni, Zn,…) allow an improvement of the agronomic parameters of cultivated plants (Lebkiri, 2014).

Tomato (*Lycopersicum esculentum*) and Cucumber (*Cucumis sativus*) are a vital part of human diet, because they are important source of nutrients like proteins, fiber, vitamins, iron and calcium. Wastewater irrigation results in elevated metal uptake by most of the vegetables grown particularly in peri-urban areas thus metals become a vital part of food chain (Farooq *et al.,* 2008). Waste water irrigation may cause growth stage dependent sensitivity in vegetables (Baksh, 2005). It has been estimated that approximately 1/10th of the global population is considered to eat food from plants irrigated with wastewater (Kouser and Samie, 2009).

The present study was executed to evaluate the

effects of irrigation with purified wastewater on crops and the enhancement of the reuse of this water, which can be a source of oxidative stress. It concerns the study and physiological analysis and the antioxidant defense of two species, the tomato and the cucumber, irrigated by the water treated by the Sedrata wastewater treatment plant, wilaya of Souk Ahras, in comparison with that from the Oued El Cheref dam and the spring water.

MATERIALS AND METHODS

Conduct of the experiment

The experiment was laid out in a randomized block design with three waters irrigation variants: treated wastewater irrigation, spring water irrigation and dam water irrigation on two vegetable species, tomato *(Lycopersicum esculentum, cv. Rio grande)* and cucumber (*Cucumis sativus* L., cv. *Super marketer)*] with three replicates.

Seedlings of Tomato and Cucumber with similar size and growth stage were selected and used in this investigation. They were germinated in plastic basins with tap water at 26 °C. After 7 days, 40 seedlings were selected and divided into three groups in plastic containers with Hoagland for 20 days in a greenhouse where temperature (26–28 °C), relative humidity (60 %), and supplementary lighting (14-h photoperiod) were controlled. The irrigation doses are carried out according to the moisture content of the soil and are identical for the three tests. In treatment groups, plants were examined for determinations of chlorophylles content, glutathion, MDA content and antioxidants enzymes activities, catalase ascorbate peroxydase and guaïacol peroxydase.

The physic-chemical parameters (pH, temperature, conductivity) are measured in situ using a multi-parameter WTW device (multi 340i / SET) (Figure 1). The chemical elements were analyzed by volumetric, atomic absorption with flame and colorimetry. The filtered water samples are placed in two 150 mL polyethylene bottles, previously rinsed with filtered water from the sample. The first is for cation analysis (acidified to pH < 2 with nitric acid), the second is for anion analyzes. The samples are stored immediately in a portable cooler with sufficient cold reserve to keep a temperature below 4 ° C until the arrival at the laboratory analysis (Rodier and Legube, 2009). *Biochemical assay*

Chlorophyll Assay

Chlorophyll was extracted in 80% acetone. Absorbance was measured at 663 and 645 nm by a spectrophotometer. Extinction coefficients and equations reported by Lichtenthaler and Wellburn (1983) were used to calculate the amounts of Chl a, Chl b, Chl a+b and carotenoids. Measurements were done in triplicate.

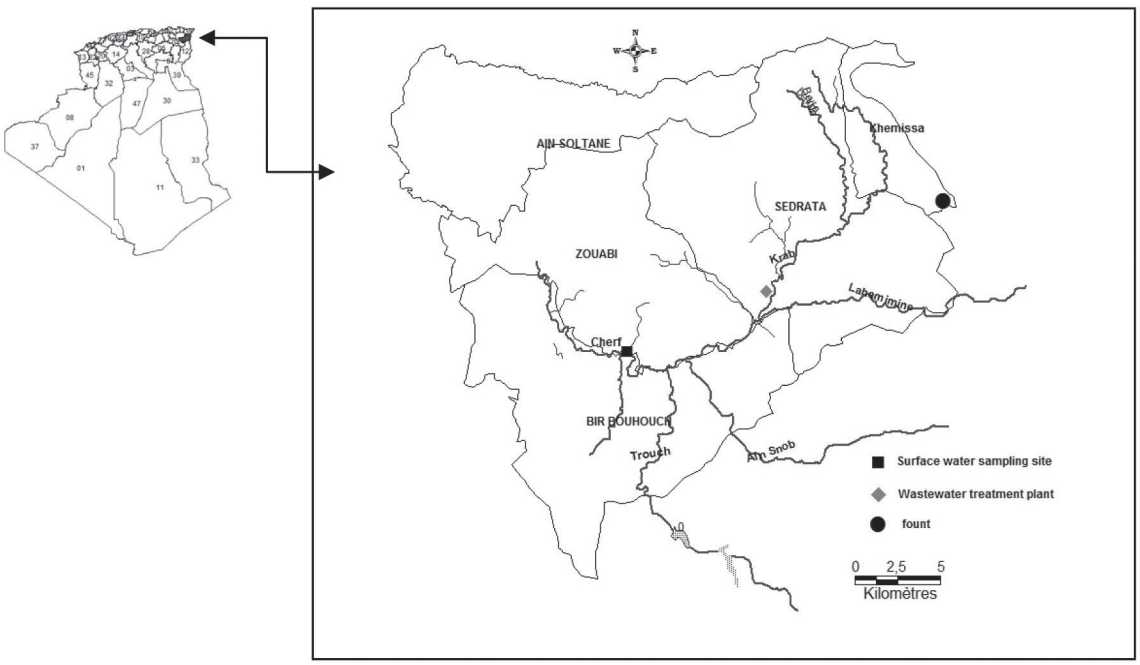

Fig. 1. Irrigation water withdrawal sites.

Determination of Lipid Peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA), a major thiobarbituric acid reactive species (TBARS), and product of lipid peroxidation (Heath and Packer, 1968). Samples (0.2 g) are ground in 3 mL of trichloroacetic acid (0.1%, w/v). The homogenate was centrifuged at 10,000 ×g for 10 min and 1 mL of the supernatant fraction was mixed with 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95 °C for 30 min, chilled on ice, and then centrifuged at 10,000 ×g for 5 min. The absorbance of the supernatant was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

Determination of the reduced glutathion

The rate of glutathione (GSH) is carried out according to the method of Weckberker and Cory (1988), whose principle rests to the colorimetric measure of the acid 2-nitro 5-mercapturic, resulting from the reduction of the acid 5-5' dithio-(a)-2-nitrobenzoic (DTNB) by the groupings thiol (- HS) of the glutathione measured with a wavelength of 412 nm.

Antioxidant enzymes activity analysis

Fresh leaves were homogenized in 50 Mm potassium phosphate buffer pH 7.6, the homogenized samples were centrifuged at 12000 ×g for 20 min and the supernatant was used as crude enzyme extract in CAT, APX and GPX (Loggini *et al.*, 1999).

Catalase (CAT) activity was determined as a decrease in absorbance at 240 nm for 3 min following decomposition of H_2O_2 . The reaction mixture 3 ml contained 50 mM phosphate buffer pH 7.2, 15 mM H_2O_2 and 100 µL of crude enzyme extract (Cakmak and Horst, 1991). The activity was calculated using the extinction coefficient 39400 $M^{-1}cm^{-1}$.

Ascorbate peroxidase (APX) activity was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 3 min in 3 mL of a reaction mixture containing 50 Mm potassium phosphate buffer pH 7.2, 0.5 Mm ascorbic acid, H_2O_2 and 100 µL of crude enzyme extract (Nakano and Asada, 1981). The activity was calculated using the extinction coefficient 2800$M^{-1}cm^{-1}$.

For Guaïacol peroxidase (GPX) activity, the reaction mixture consisted of 50 mM potassium phosphate, 9 mM guaïacol buffer pH 7.2, 50 µ H_2O_2 and 100 µL of crude enzyme extract. The enzyme activity was measured by monitoring the increase in absorbance at 470 nm extinction coefficient of 2470 M^{-1} per cm during polymerization of guaïacol (Hiner *et al.*, 2002).

Statistical analysis

Statistical analyses were carried out with R version 3.6.1 has been released for Windows, by using ggplot 2 and ggpubr packages which can indicate the comparisons made, and employees test p-values; differences among two species were tested by Kruskal-Wallis test (rank-based nonparametric test) followed by pair wise Wilcoxon rank sum tests comparisons. All the statistical analyses were performed at the statistical significance of $p<0.05$.

RESULTS AND DISCUSSION

Water analysis

Irrigation water by its composition can have an influence on the soil and therefore on most crops. The results of the physico-chemical analyzes of the water used for irrigation at the three sites studied are shown in Table 1.

The first reading of the chemical facies of the waters used in irrigation shows clear differences in their composition. Spring water is high salinity water with an EC of 1500 uS / cm and a SAR of 3.40, thus presenting a high risk of salinization and a low risk of sodicity. The irrigation water of the WWTP is also high salinity water with an EC of 1400 uS / cm and a SAR of 11.22; it presents a very high risk of salinization and a high risk of sodicity. The water of

Table 1. Physico-chemical characteristics of the three sites

Site	pH	T°C	Cond uS/cm	Ca^{2+} mg/L	Mg^{2+} mg/L	Na^{+} mg/L	K^{+} mg/L	Cl^{-} mg/L	HCO_3^{-} mg/L	SO_4^{2-} mg/L	SAR
Spring water	7.2	17.5	1500	91	19	25	3,9	71	106	20	3.40
Treated water	7.8	16.8	1400	120	21	94	39	374	121	61	11.2
Dam water	8	12.7	2650	145	27	161	42,9	611	204	14	17.33

the dam is very high salinity water with an EC of 2650 uS / cm and a SAR of 17.33; it presents a very high risk of salinization and a very high risk of sodicity.

Effect of different types of irrigation water on physiology and the antioxidant system

Following a statistical analysis carried out, we recorded significant differences ($p \leq 0.05$) for chlorophyll pigments in tomato and cucumber irrigated by the three types of water. In plants irrigated with spring water, the content of chlorophyll pigments is higher compared to those in plants irrigated by water from the dam and treated water. The deferences are not significant for tomato and significant for cucumber with $P \leq 0.05$ (Figure 2). The decrease in chlorophyll content is one of the primary events in plants subjected to metal stress and results from the inhibition of enzymes responsible for chlorophyll biosynthesis (Mysliwa-Kurdziel and Strzalka, 2002).

On the other hand, a significant increase for carotenoids in seedlings irrigated by water from the dam and those treated, it is very low in those irrigated by spring water (Figure 2). This response is explained by the presence of oxidative stress in plants irrigated by water from the dam and those purified. Carotenoids are known as secondary metabolites which can perform various functions in the plant, including the antioxidant function (De Pascale *et al.*, 2001). It is accepted that during oxidative stress generated by environmental

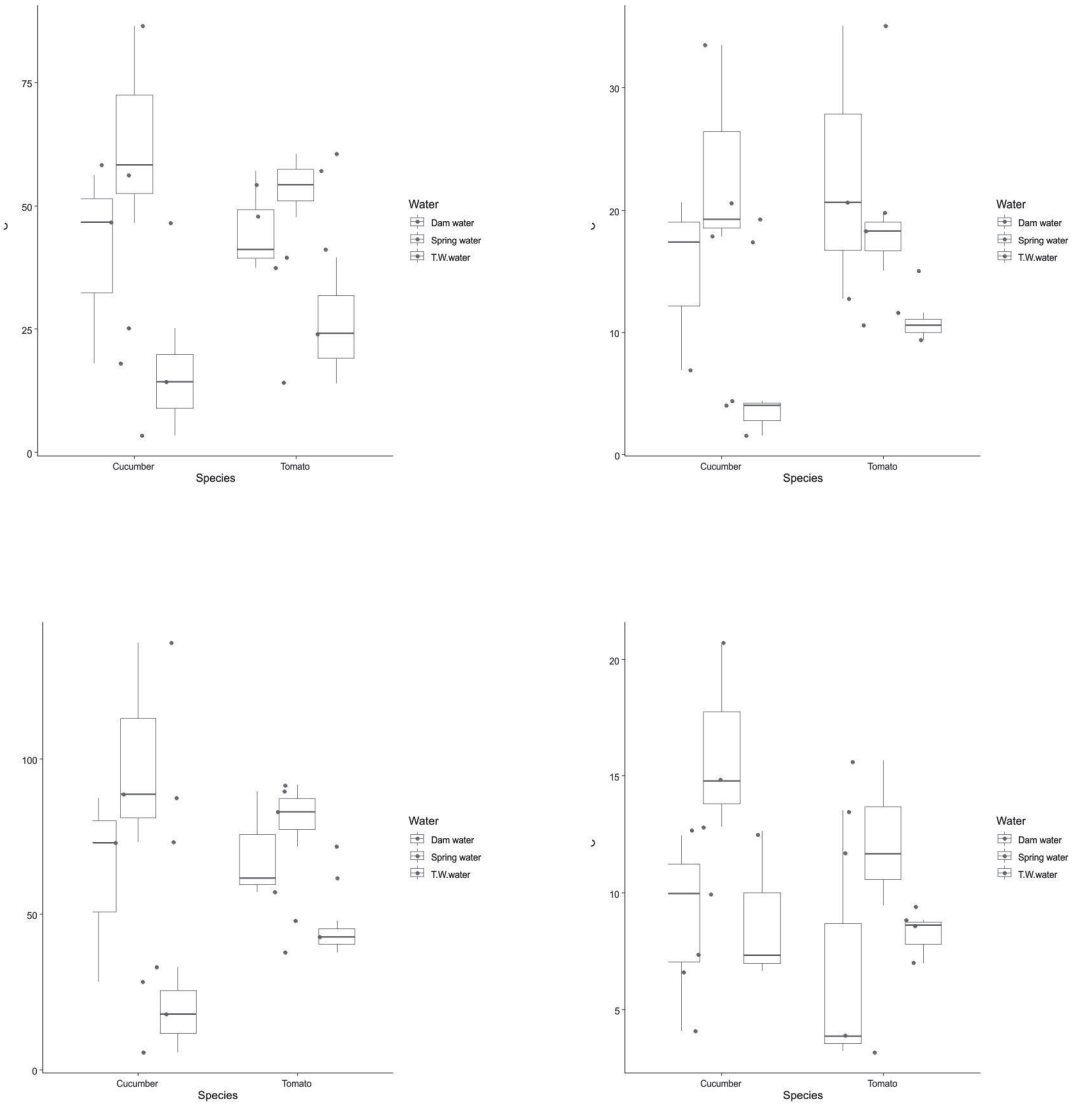

Fig. 2. Variation in photosynthetic pigment content under the effect of irrigation by different types of water

constraints, the increase in the production of reactive oxygen species is accompanied by a stimulation of the production of many non-enzymatic antioxidants such as carotenoids (Allende, 2003).

To confirm the state of stress generated in tomato and cucumber, a non-enzymatic biomarker, Malondialdehyde, which is an expression of lipoperoxidation (Ben Youssef *et al.*, 2002).

The use of this compound as a biomarker of oxidative stress is widespread. l analysis reveals highly significant differences; the highest concentration was recorded in plants irrigated with treated water.

Glutathione (GSH) activity increased in plants of tomato that were irrigated to treated wastewater (Figure 3). Irrigation with dam water relatively affects GSH synthesis in the two species, the GSH level is lower in plants irrigated with spring water. Several studies have demonstrated that the excessive absorption of heavy metals by plants induces the production of reactive oxygen species (ROS) in plant tissues (Singh *et al.*, 2006). These ROS cause imbalances in the antioxidative defenses of plants and induce oxidative stress (Edreva, 2005). Plant antioxidative defenses against ROS may involve antioxidative enzymes and nonenzymatic antioxidants, including ascorbate and glutathione (GSH) in addition to tocopherol, flavonoids, alkaloids and carotenoids (Apel and Hirt, 2004).

Catalase activity (CAT) in plants of cucumber irrigated by the treated water was much higher than in tomato (Figure 4). Ascorbate peroxidase (APX) activity decreased in cucumber plants under the effect of the three types of irrigation water. In tomato plants, the water from the dam positively affects this enzymatic antioxidant (Figure 4). Reductions in antioxidative enzyme activities, such as those observed here, are indicative of very strong oxidative stress conditions, which may affect enzyme biosynthesis and/or the assembly of enzyme subunits (Singh *et al.*, 2006).

In tomato, on the contrary, despite a reduction in GSH concentration, GPX activity increases were observed in plants irrigated by wastewater treated. GPX appears to be capable of using reduced substrates other than GSH, including lipid hydroperoxides, as suggested by Herbette *et al.* (2002). This possibility and the great variety of GPX isoenzymes (Eshdat *et al.*, 1997) may explain, at least in part, the difference in species responses that was observed here. Nevertheless, GPX activity was always higher in tomato, indicating a higher capacity of this species to scavenge the ROS induced. All of the enzymes involved in ROS detoxification show a marked increase in activity. In addition, catalase and APX have complementary roles in the detoxification of hydrogen peroxide, these two enzymes have different cellular localization and target molecules (Barata *et al.*, 2005). APX protects the cell against oxidative damage by eliminating toxic H_2O_2, released in chloroplasts, cytosols, mitochondria and peroxisomes of plant cells (Mittler *et al.*, 2004). Catalase, which is mainly localized in peroxisomes and mitochondria, also participates in the degradation of H_2O_2 generated by the presence of excess xenobiotics in the plant environment (Farrago, 1994). Indeed, catalase is considered to be one of the most sensitive biomarkers of oxidative stress, particularly with

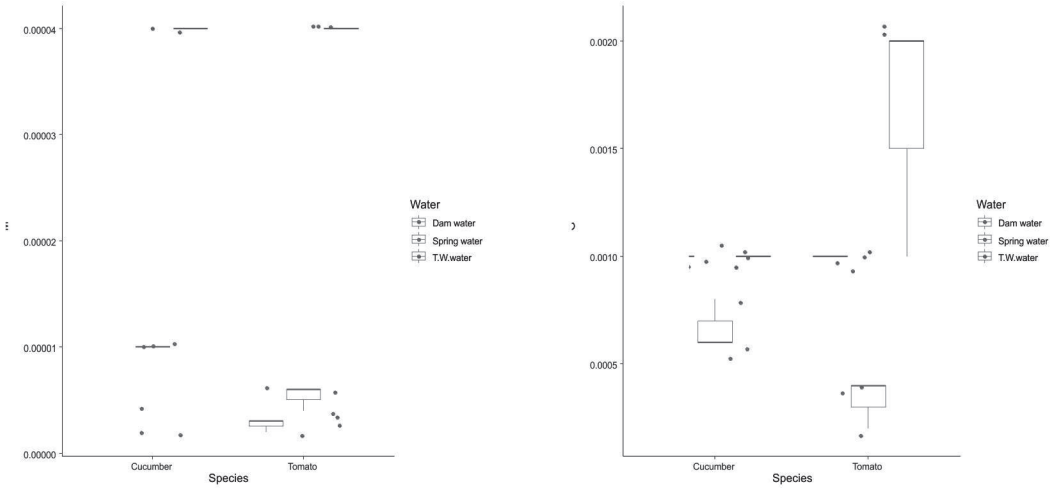

Fig. 3. Variation in MDA and glutathion t content under the effect of irrigation by different types of water

regard to chemical pollutants in the aquatic environment (Regoli *et al.,* 2003).

CONCLUSION

The results obtained indicate that irrigation with treated wastewater causes a disturbance that affects the biochemical processes of the two species studied. Our results show that both species respond favorably to the use of treated wastewater for irrigation due to better assimilation thanks to the nutrient inputs of irrigation water which gives positive results on photosynthetic pigments.

Analysis of antioxidant enzymes indicates that irrigation with treated wastewater causes activation of ascorbate peroxidase, guaïacol peroxidase and catalase enzymes in tomato and cucumber. However, the changes observed in the activities of antioxidant enzymes suggest that the xenobiotic applied induces oxidative stress in both species during their growth. Under the test conditions, induction of antioxidant enzyme activities may be suggested to play a role in xenobiotic tolerance, tomato appears to be relatively the most tolerant species compared to cucumber which becomes sensitive to oxidative stress.

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Conflicts of interest None.

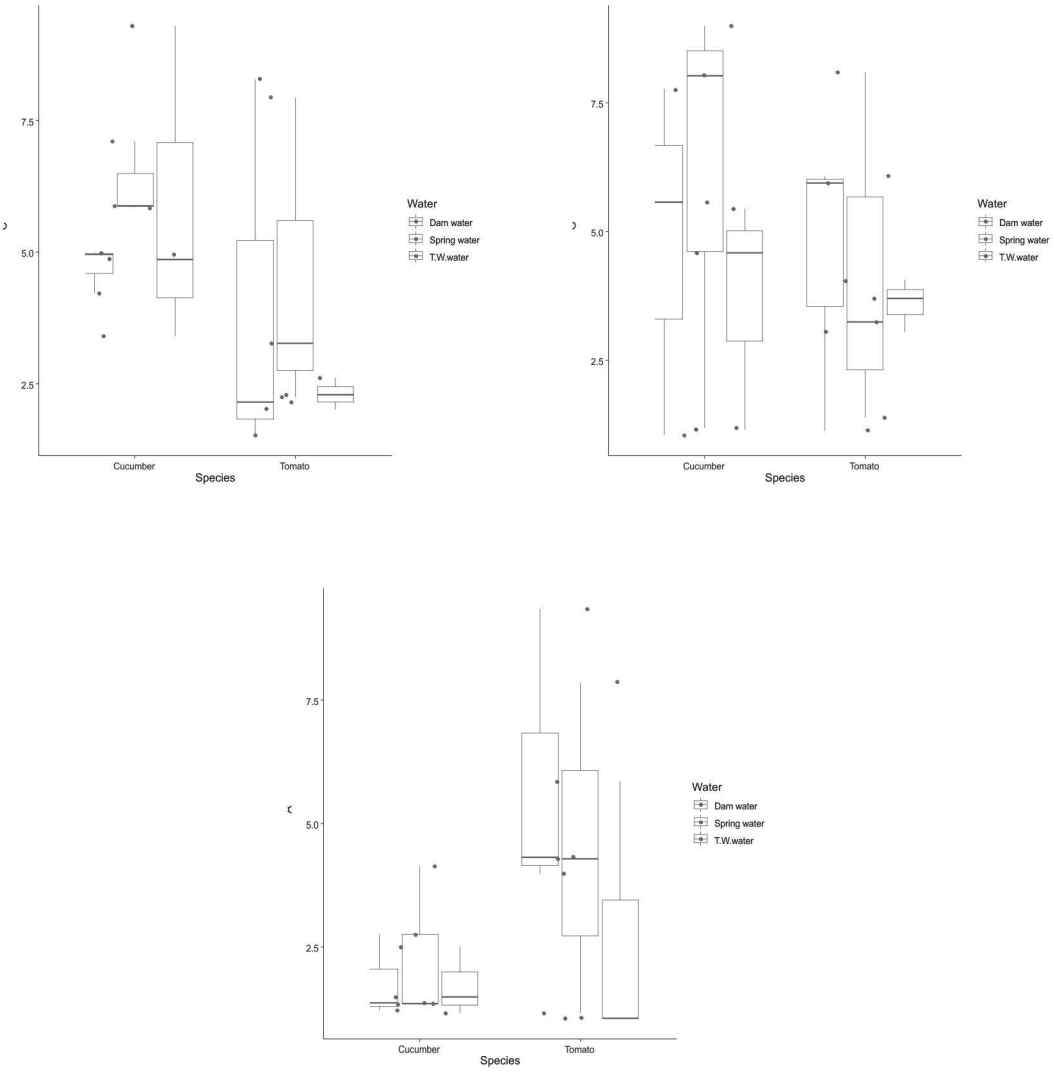

Fig. 4. Variation in antioxydants enzymes content under the effect of irrigation by different types of water

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