



B- Alleviating the harmful impact of salinity stress with different levels by using some organic techniques on Chemical characteristics of Wonderful cv. Pomegranate Transplants.

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ABSTRACT

Water and soil salinity were the biggest obstacles to achieving the world's supply - demand ratio, so with the growing population there is no choice but to use salty soil and water in agriculture, and the need for environmentally friendly technologies has emerged to overcome the adverse effects. Therefore, the effect of the halotolerant plant growth-promoting rhizobacteria (HT-PGPR) with organic fertilizers and spirulina algae on the biochemical characters as chlorophyll content, NPK %, total carbohydrates, phenols, proline and antioxidant enzymes activities of pomegranate transplants (Wonderful cv.) under different irrigation water salinity levels (6000, 7000 and 8000 ppm) were investigated. Pot experiment was conducted in Horticulture Research Institute (HRI) nursery, Agriculture research center (ARC), Giza governorate, Egypt, under the greenhouse conditions using compost, halotolerant PGPR (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megaterium* var. *phosphaticum* and *Bacillus circulans*) in addition to, *Spirulina platensis* L. algae in 2019 and 2020. Results indicated that, the organic and biofertilizers reduced soil pH and EC and increased the soil enzymatic activity under saline water condition. The leaf contents of total chlorophyll, carbohydrates, phenols, proline of transplants were increased, in addition to a significant increase in the nutritional elements NPK % in dry leaves were recorded when transplants were treated using PGPR inoculations, excluding sodium and chlorine. The highest efficiency in alleviating irrigation water salinity stress on pomegranate transplants was obtained from treatment contents compost + biofertilizers + spirulina algae with 6000 ppm salinity level compared to the chemical fertilizer treatment. The results of the study indicated that organic fertilizers with halotolerant PGPR inoculations can alleviate the deleterious effects of salt stress conditions and improve the tolerance and healthy growth of pomegranate transplant under salt water irrigation.

Keywords: Saline water, organic and biofertilizers, Wonderful pomegranate, proline and antioxidants enzymes.

INTRODUCTION

Soil and water salinity is considered one of the most important stress factors that limit agricultural production in the world, and the problem is constantly rising as about 20% of cultivated lands and about half of irrigated lands are affected by salinity in the world especially in arid and semi-arid climates (Binici et al., 2022).

Pomegranate (*Punica granatum* L.) belong to the *Punicacea* family, which is familiar in tropical and subtropical areas (Khattab et al., 2011). The newly American cultivar "Wonderful" has proven a successful cultivation in Egypt and widespread in Middle East countries, it is characterized as a moderate tolerant variety to irrigation with saline water (Sun et al., 2018). Recently, pomegranate cultivation has expanded in arid and semiarid areas with limited good

irrigation sources in addition to the wide spreading accumulation of salts that cause soil salinization (Al-Qasimi and Al-Salhi, 2018).

Compost is a natural source of fertilizers, it contains important plant nutrients such as N, P, K, Mg, S, Ca and micronutrients, and improves the soil chemical, physical and biological characteristics by increasing organic matter content, soil microbial population, soil enzymes activities, lowering the soil bulk density and increasing the soil porosity (El-Shaieny et al., 2022).

PGPR including *Azotobacter*, *Azospirillum*, *Bacillus* that live in the rhizosphere acts as biostimulation, biocontrol and biofertilization, which improves plant growth under different environmental stress conditions by stimulating plant growth



hormones, antioxidant, enhances nutritional capacity of the plants, improving the absorption of nutrients, enhancing nitrogen fixation, producing hormones (auxin, cytokinins and gibberellins) and dissolving insoluble nutrients as phosphorus, potassium, zinc and silicon, also they are the cheap and available sources to relieve of different biotic and abiotic stresses (Numan, 2018; Samy and El-zohiri, 2021).

Soils with high salinity become infertile due to poor nutrient content, microbial activity, biomass and organic matter. Halotolerant bacteria are a type of microorganisms that can live and survive around the root of plants creating a rhizosphere microbiome under high salt levels by maintaining osmotic balance. Halotolerant bacteria can be the key to using biofertilizer technology under salt stress; salt tolerant isolates that have the potential to be used as biofertilizer inoculants are one solution trying to use saline land in agriculture, and to boost the plant productivity under salt stress (Khumairah, et al., 2022).

Application of halotolerant plant growth promoting rhizobacteria (HT-PGPR) has the potential to alleviate salt stress in plants, including modification of root systems, production of exopolysaccharides and siderophores, modulation of phytohormones, synthesis of osmolytes and mineral uptake

(Arora et al., 2018). Several species of halotolerant bacteria such as *Azotobacter*, *Azospirillum* and *Bacillus* have been reported to ameliorate salt stress in crops, when used as bioinoculants to increase soil organic matter, improve soil structure and water holding capacity (Saghafi et al., 2019). So, using HT-PGPR in the form of bioinoculants is an eco-friendly and sustainable method of improve the productivity of saline agroecosystems (Arora et al., 2020).

Spirulina platensis is a biofertilizer rich in mineral nutrient, which stimulate establishment and elongation of roots and promote plant growth (Yassen et al., 2019). Cyanobacteria algae can live in highly salinity soil so they can be used to reclaim saline soils. In addition, it can play an important role in improving plant nutrients supply, increasing soil fertility and porosity, producing growth promoting hormones, reducing damage resulted by salt stress, increasing both water holding capacity and biomass of soil after its death and decomposition, Also, increasing nitrogen by nitrogen fixation and soil phosphate by secreting organic acids into the soil (Burjus, et al., 2020).

This study aims to evaluate the effect of using organic, biofertilizers and spirulina algae to mitigate salinity stress for Wonderful cv. pomegranate transplants.

MATERIALS AND METHODS

- Study area

The present investigation was implemented during two successive growing seasons 2019 and 2020 respectively, on (one-year-old) Wonderful cultivar transplants under the greenhouse conditions located in the nursery of Horticulture Research Institute HRI, Agriculture Research Center ARC, Giza, Egypt. All the transplants were chosen to be healthy and symmetrical in vigor with height about 30-35 cm. and girth about 2- 2.5 cm. for the both seasons. (Koshkhooi, 2006).

- Experiment preparation

The selected 126 transplants of pomegranate Wonderful cv. were divided as seven treatments, 3 replicates (each replicate

contains 6 transplants). On the first of March, the transplants were planted individually into plastic pots (25 cm diameter) filled with 4 Kg of clay and sandy soil (2:1 v/v) with electrical conductivity (EC) 1.83 ds/m, field capacity (FC) 22.8 %. The treatments were arranged in a split-plot design with three replications, where the salinity levels (i.e. three levels) were occupied in the main plots, while the fertilization treatments (seven treatments) were randomly distributed in the sub-plots. Plants were irrigated twice a week using 500 ml / transplant with artificial salt water (Hoagland solution) adding 6, 7 and 8 g sodium chloride at three salinity levels (6000, 7000 and 8000 ppm.) until September



(end of the experiment). Moisture content was maintained at field capacity % for all pots by weighing the pots every three days to minimize the harmful effect of saline irrigation water on both soil and biochemical components of transplants by treating them with various organic and biofertilizers

- Materials used

Mineral fertilizers: The recommended quantities of NPK fertilizers were added

according to the recommendations of the Egyptian Ministry of Agriculture and Land reclamation.

Compost: obtained from Horticulture Research Institute nursery, ARC. 100 g compost/pot was mixed with the soil before planting for the specified treatments (Hamdy et al., 2016). Table (1) Physical and chemical analysis of compost (Page et al., 1982)

Table (1): Physical and Chemical properties of the compost used

Character		Value
Physical properties		
Color		Dark brown to gray
Bulk density	kg m ⁻³	716
Moisture content	%	27.40
Chemical properties		
pH		7.70
EC	(ds/m)	2.43
Organic matter	%	31.75
Organic carbon	%	18.42
Ash	%	68.25
Total nitrogen	%	1.28
C: N ratio	%	14: 1
Total phosphorus	%	0.85
Total potassium	%	3.80
Available N NH ₄	ppm	100
Available N NO ₃	ppm	250
Nematode (worm)	--	Not detected
Total coliform	(cfu /g)	Not detected
Weed seed	--	Not detected

Biofertilizers: halotolerance isolates were previously isolated from El Moghra region saline soil, Minia governorate, Egypt.

Two halotolerant N- fixer's bacteria *Azotobacter chroococcum* and *Azospirillum lipoferum* also, phosphorus and potassium solubilizing bacteria as, *Bacillus. megaterium* var. *phosphaticum* and *Bacillus circulans* were activated on their specific media which adjusted to 6000, 7000 and 8000 ppm by 6, 7 and 8 g NaCl/ L. media as, , modified Ashby s medium for *Azotobacter* spp., N- deficient semi solid malate medium for *Azospirillum* spp., Pikovskaya medium for *B. megaterium* and modified Aleksandrov medium for *B. circulans*. (Dobereiner and Day, 1976; Abdel- Malek and Ishac, 1968; Pikovskaya, 1948 and Parmar and Sindhu, 2013) respectively.

Isolates were identified according to standard microbiological methods as

described in Bergy s Manual of Systematic Microbiology (Tan et al., 2009), then cultures mixed together in equal amounts for use as biofertilizers (10⁸/ ml), and added to the soil with irrigation water, once/ 15 day as 30 ml/ pot (Afifi et al., 2014).

Cyanobacteria algae: (*Spirulina platensis*), kindly provided from Agric. Res. Microbiol. Dept. Soil, Water and Environ. Res. Inst. ARC. Egypt. *Spirulina* algae was grown in Zarrouk medium (Zarrouk, 1966), incubated 30 days in growth chamber, shaking at 150 rpm, illumination (2000 Lux) and a temperature of 32° C ± 2° C., well mixed to have a homogenized suspension then filtered and used as a soil drench with irrigation water as 30 ml/ pot (Makhlouf and Helmy, 2022). Chemical analysis of *Spirulina platensis*/ 100 g is shown in Table (2) according (Barron et al., 2008).



Table 2. Nutritional value of *Spirulina platensis* composition per 100 g.

Chemical compositions (100g dry weight)		Micronutrients (mg/100g dry matter)	
Moisture	8.65 %	Sodium (Na)	698.80
Crude protein	48.69 %	Magnesium (Mg)	4.01
Total Carbohydrates	24.68 %	Iron (Fe)	11.03
Total amino acids	55.70 g.	Manganese (Mn)	3.80
Sugars	13.80 %	Calcium (Ca)	31.90
Fat	10.12 g.	Copper (Cu)	3.10
Crude fiber	3.17 g.	Zinc (Zn)	2.00
Ash	10.32 g	Chloride (Cl)	46.80
Antioxidants		Macronutrients %	
Total antioxidants	630.50 (mg/5 b. dry weight)	Total nitrogen	6.77 %
Proline	4.28 (mg/100 g. dry weight)	Total phosphorus	0.70 %
Total Flavonoids	0.790 %	Total potassium	1.77 %
Total phenolic	1.65 %		

Both two experiments included seven treatments with three replicates as follows:

- (T₁) Saline water (Control at 6000, 7000 and 8000 ppm)
- (T₂) Recommended dose of mineral NPK fertilizer as control
- (T₃) Compost (100 g compost /pot)
- (T₄) Compost + Biofertilizers (100 g compost /pot + 30 ml/ pot)
- (T₅) Spirulina algae (30 ml. / pot)
- (T₆) Spirulina algae + Biofertilizers (15 ml. / pot + 15 ml. / pot)
- (T₇) Compost + Biofertilizers + Spirulina algae (100 g compost /pot + 30 ml/ pot).

The influence of the treatments on some chemical compounds of transplants were determined:

- The studied characteristics:

- a. Soil determinations:
 - Soil PH and EC were recorded in the end of the exp. /seasons according to (Page et al., 1982).
 - Biological activities of the PGPRs used
 - Soil enzymatic activity: Nitrogenase activity (μ mole C₂H₄/g soil/h) in the rhizosphere according to (Somasegaran and Hoben, 1994), Dehydrogenases activity (μ g TPF/soil) according to (Skujins, 1976) while, total phosphatase activity was determined according to (Tabatabai, 1982) were

measured at 60, 120 and 150 days in both seasons.

b. Biochemical compounds

- Total chlorophyll content (SPAD): By using nondestructive chlorophyll-meter (Minolta SPAD₅₀₂) in the fresh leaves at the end of both seasons (September) according to (Castelli et al., 2008).

- Total N.P.K % in plant dry weight: leaves of the 5-7th nodes were taken at the middle of both seasons to determine, N % described by (Piper, 1950), P, K, Na and Cl % were determined according to (Chapman and Pratt, 1961).

- Total phenol and carbohydrates content in plant dry weight were determined according to (A.O.A.C., 1990).

- Proline content (%) in plant dry weight was determined according to (Bates et al., 1973).

- Antioxidant enzymes: Peroxidase activity was determined according to (Kochba et al., 1977) and catalase activity was determined according to (Aebi, 1974)

Statistical analysis: the results were statistical analyzed by the analysis of variance (ANOVA) according to Snedecor and Cochran (1990). Comparisons between treatments were held using the new L.S.D. values at 5 % level.

RESULTS AND DISCUSSION

1- Effect of organic and biofertilizers treatments on some soil properties under different salinity levels:

Soil pH and EC

Fig. (1) Showed that the average soil pH and electrical conductivity EC increased with increasing water salinity level during the two seasons. The highest values were recorded at 8000 ppm. While, pH and EC values were decreased when organic and biofertilizers were applied in



both seasons. The lowest values were recorded with T₇ (compost + biofertilizers + Spirulina) in both seasons compare to the treatment mineral fertilizer.

Concerning the interaction effect between the different salinity concentrations and different organic treatments, its clarifying that, organic and biofertilizers cause a noticeable reduction in the results of both pH and EC soil at all concentrations. T₇ gave the lowest results being (7.55, 7.62 and 7) pH, and (5.5, 6.6 and 7.64) EC, in the first season, while (7.6, 7.65 and 7.75) pH and (5.43, 6.67 and 7.8) EC in the second season compare to mineral fertilizers treatment (T₂) which record the highest values during the two seasons.

Soil pH is the most important factor that reflect the general changes in soil chemical properties. The results were in harmony with those obtained by (El -Maaz and Ismail, 2016) found that, organic fertilizer reduced soil pH and EC values due to the activity of microorganisms in decomposing organic matter and releasing organic acids, dehydrogenase enzyme and production of moles of H₂ in the rhizosphere that has positive effect on increasing hydrogen moles that react in root zone to form hydrocarbon acid that leads to a decrease in soil pH. Also, reduces soil salinity and improves soil structure by increasing drainable pores and aggregates thus improving irrigation water filtration. Recently, (Taha et al., 2023) confirmed that Spirulina algae has many advantages in increasing soil fertility by increasing K⁺ and N accumulation, which led to the accumulation of several elements because of its high binding ability and synthesis salt stress proteins.

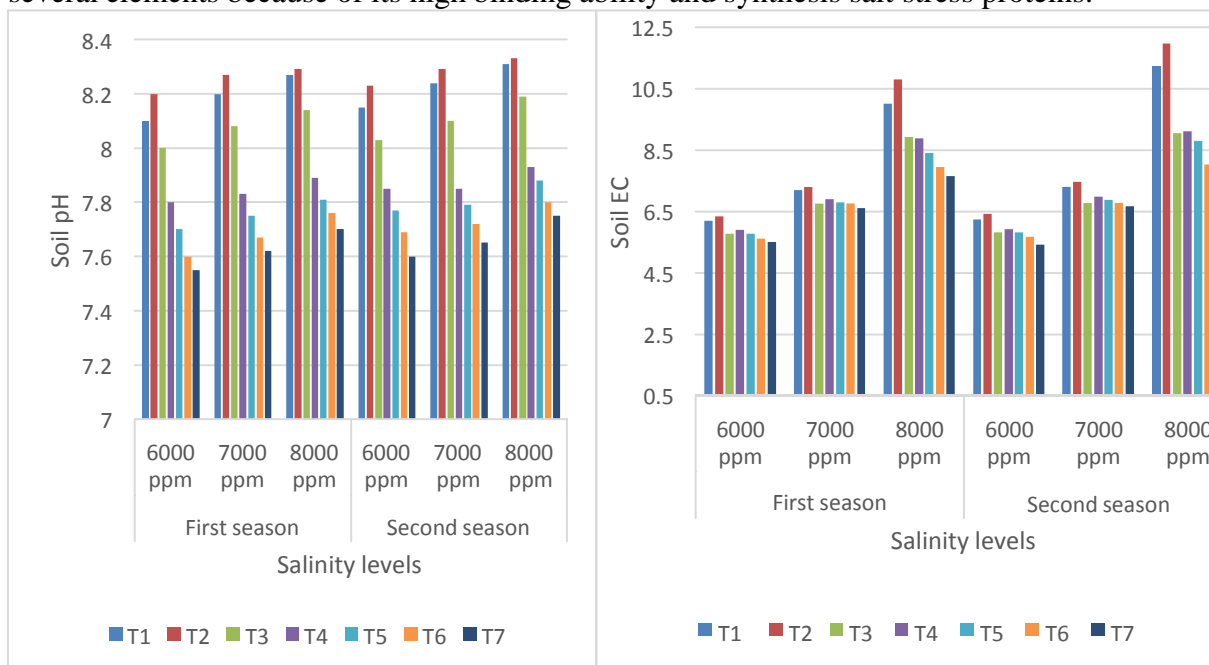


Fig. (1). Effect of using organic and biofertilizers treatments on soil pH and EC soil under different levels of water salinity (T₁) Saline water, (T₂) Mineral NPK fertilizers (T₃) Compost, (T₄) Compost + Biofertilizers, (T₅) Spirulina, (T₆) Biofertilizers + Spirulina and (T₇) Compost + Biofertilizers + Spirulina

- Biological activities of the PGPRs used

Azotobacter chroococcum, *Azospirillum lipoferum*, *Bacillus circulans* and *Bacillus megaterium* were used throughout the current study. The used strains were tested biochemically to clear their capabilities to fixing atmospheric nitrogen, dissolving phosphorus and potassium, excretion of phytohormones and exopolysaccharides (Table 3) according to Heber et al. (1992).

Soil microbes cooperate with each other as well as with plant roots in many ways for

maintaining the ecological balance in soil, Soil fertility is closely related to the balance of microorganisms and plants (Kumar et al., 2021c). There are diverse mechanisms of Salt-tolerant bacterial strains which support the plant growth under salinity stress through N₂ fixation and phosphate, potassium, micronutrient mineralization from the soil (such as Zn, P, Fe, K and other essential mineral nutrients), exopolysaccharides secretion, phytohormone production, proline, siderophore, vitamins, enzymes, antibiotics and



support the restriction of Na⁺ uptake by the roots in salinity conditions (Shilev, 2020). Also, biofertilizers act as phytostimulators or plant growth regulators can produce phytohormones as cytokinin, gibberellins, abscisic acid, and indole-3-acetic acids IAA under environmental stress conditions as salinity to protect the plants

Table (3). Biological activities of the used strains

Strain	Auxin (IAA) (mg/L)	Gibberellic acid (GA) (mg/L)	Proline %	Exopolysaccharide
<i>Azospirillum spp</i>	69.8	130.8	2.3	1.2
<i>Azotobacter spp</i>	68.1	127.7	2.1	1.1
<i>B. megaterium</i>	59.4	123.3	1.5	0.46
<i>B. circulans</i>	58.4	121.6	1.6	--
<i>Spirulina</i>	72.6	150.4	3.7	--

- Soil enzymes activity:

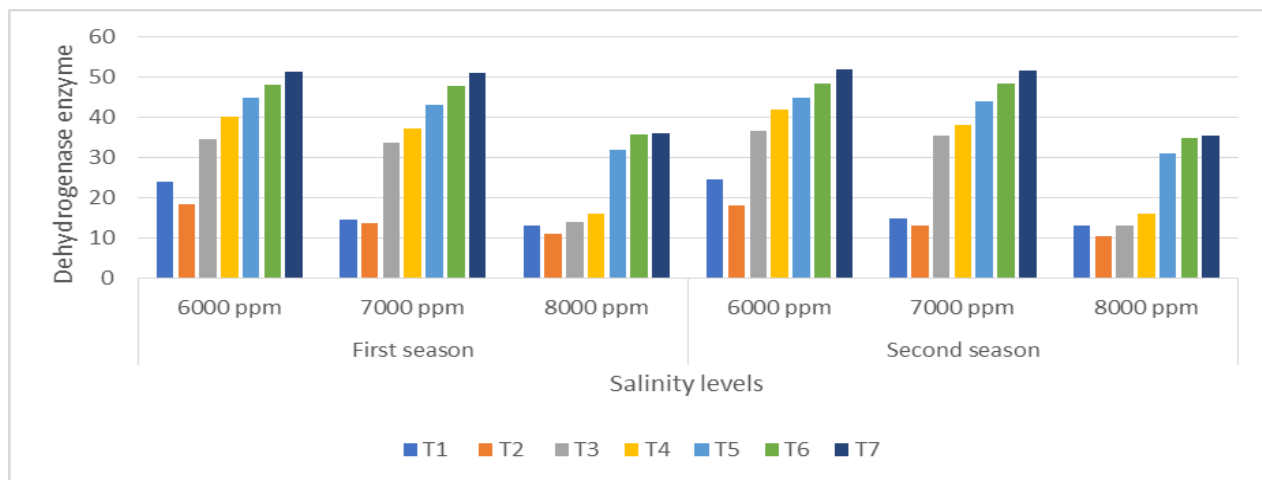
Dehydrogenase (DHA) is a guide for respiration rate and total microbial activity in soil. While, the activities of phosphatase (PA) and nitrogenase (NA) are guides for mineralization processes of organo-phosphorus substrates and as an indication of N₂-fixer’s activity, respectively. Fig. 2 shows that, higher salt concentration leads to decrease the enzymes activity in the soil, this may be due to a decrease in the number of bacteria populations. While, the use of organic and biofertilizers under salinity stress increased microbial enzymes activities as DHA, PA and NA in the rhizosphere compare to the control (mineral fertilizer). Where, the activity of these enzymes, PGPR plays a vital role in promoting plant growth.

Results are harmony with Abdel-Latef et al. (2021) they recorded that plant inoculation

by modifying the phytohormone level within plants (Lopes et al., 2021) and increasing plant growth, root length and the formation of root hairs, which increases water absorption from soil. Also, reduction of ethylene levels in plants through produce ACC (1-aminocyclopropane-1-carboxylate) deaminase (Bhat et al., 2022).

with PGPR as *Azotobacter chroococcum* could mitigate the negative impact of salinity by increasing soil enzymes activity that will serve as indicator for the microbial activity in the rhizosphere. In addition to, the combined biofertilizers with *Azospirillum sp.* and *Bacillus sp.* increased the enzymes activities at all plant growth stages. So, plant growth-promoting rhizobacteria can enhance plants tolerance to various abiotic stresses including salinity (Kumari et al., 2017).

The application of *Spirulina platensis* algae could be stimulating the antioxidant enzyme activities, increasing the manufacturing of photoprotective substances like scytonemin (mycosporine-like amino acids) and enhancing the synthesis of various proteins, as well as boosted DNA damage repair (Salem and Ismail, 2021).



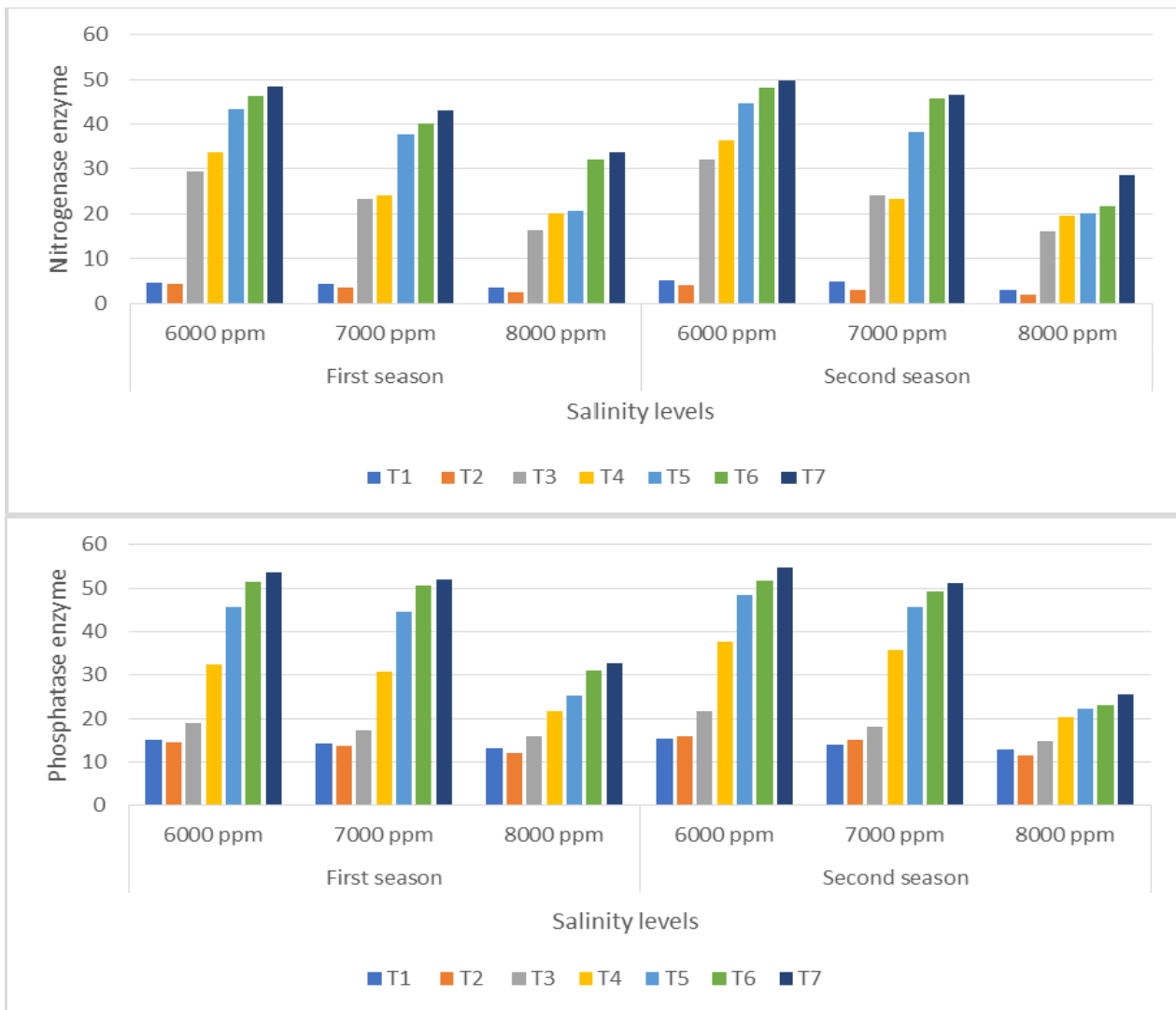


Fig. (2). Effect of using organic and biofertilizers treatments on soil enzymes activity under different levels of water salinity. (T₁) Saline water, (T₂) Mineral NPK fertilizers (T₃) Compost, (T₄) Compost + Biofertilizers, (T₅) Spirulina, (T₆) Biofertilizers + Spirulina and (T₇) Compost + Biofertilizers + Spirulina

2- Effect of organic and biofertilizers treatments on biochemical properties of Wonderful cv. pomegranate transplants under different salinity levels

- Total chlorophyll

Salinity decreased the total chlorophyll content, as Fig. 3 showed that total chlorophyll in wonderful cv. transplant leaves were decreased with increasing salinity, the lowest values were recorded with the saline water 8000 ppm, While, adding organic and biofertilizers increased the total chlorophyll content in leaves. Concerning the interaction, data showed that T₇ (compost + biofertilizers + Spirulina) gave the highest values in the first and second seasons with 6000, 7000 and 8000 ppm respectively. Followed by T₆ (Biofertilizers + Spirulina) then other treatments which

received the organic and biofertilizers compared to mineral fertilizers treatment.

Photosynthetic pigment is an essential physiological feature related to photosynthetic ability under abiotic stresses. Similar results obtained from (Enebe and Babalola, 2018) proved that, enhancement in photosynthetic pigments was recorded in plants that inoculated with PGPR under different saline conditions, these increases may be due to the efficacy of bacterial inoculation in nullifying the harmful effect of salinity stress by improving the activities of electron transporters associated with photosynthesis, as well as, biosynthesis of pigment-related proteins and enzymes.

Spirulina algae is responsible for reducing chlorophyll degradation and delaying leaf aging due to its great importance as a plant growth promoter, as it contains higher levels of



bioactive components such as cytokinins content, auxin, gibberellin, organic matter, microelements, vitamins and fatty acids also, enhancing the efficiency of plant absorption of several nutrients and thus act as a growth

regulators by promoting the permeability of the cell membrane associated with the concentration of leaf chlorophyll and increasing the photosynthetic rate (Mohy El. Din, 2020).

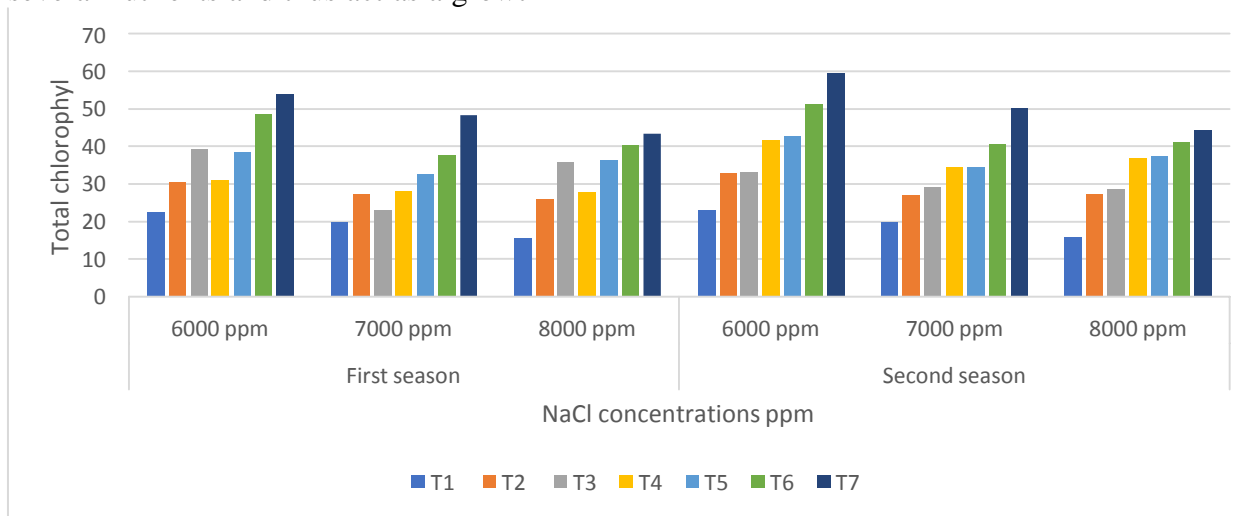


Fig. (3). Effect of using organic and biofertilizers treatments on total chlorophyll content in dry leaves of Wonderful cv. Pomegranate transplants grown under different levels of water salinity.

(T₁) Saline water, (T₂) Mineral NPK fertilizers (T₃) Compost, (T₄) Compost + Biofertilizers, (T₅) Spirulina, (T₆) Biofertilizers + Spirulina and (T₇) Compost + Biofertilizers + Spirulina

- Total N, P and K (%) in dry leaves.

Table 4 showed a gradual decrease in total N, P and K % in dry leaves with increasing salinity levels in irrigation water. The lowest results were recorded when irrigating transplants with 8000 ppm followed by 7000 ppm in both seasons compare to control T₁ (Saline water). While, T₇ (compost + biofertilizers + Spirulina) recorded the

higher results, followed by T₆ (biofertilizers + Spirulina), compare to T₂ (mineral fertilizers). As the interaction of irrigation salinity levels x organic, biofertilizers, T₇ recorded the highest results especially at 6000 ppm followed by 7000 then 8000 ppm compare to the control T₂ (mineral fertilizers) that recorded the lowest results in the two seasons.

Table (4). Effect of organic and biofertilizers on total N. P. K. % in dry leaves of Wonderful cv. Pomegranate transplants grown under different levels of water salinity

Treatments	Total Nitrogen %				Total Phosphorus %				Total Potassium %			
	First season				First season				First season			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
T ₁	1.10	1.03	0.90	1.01	0.10	0.08	0.04	0.07	1.03	0.90	0.73	0.89
T ₂	2.20	1.54	1.08	1.61	0.20	0.20	0.14	0.18	1.84	1.72	1.20	1.59
T ₃	1.50	1.30	1.18	1.33	0.16	0.15	0.14	0.15	2.11	1.70	1.28	1.70
T ₄	1.70	1.50	1.20	1.47	0.20	0.18	0.16	0.18	2.26	1.76	1.38	1.80
T ₅	2.05	1.08	1.31	1.48	0.26	0.25	0.17	0.23	2.31	1.82	1.55	1.89
T ₆	2.10	1.95	1.45	1.83	0.30	0.28	0.18	0.25	2.45	2.05	1.70	2.07
T ₇	2.16	2.00	1.53	1.90	0.33	0.30	0.20	0.28	2.54	2.20	1.90	2.21
Mean (A)	1.83	1.49	1.24		0.22	0.21	0.15		2.08	1.74	1.39	
LSD 0.05	(A)=0.05, (B)=0.03, (AxB)=0.09				(A)=0.02, (B)=0.01, (AxB)=0.04				(A)=0.02, (B)=0.01, (AxB)=0.04			
Treatments	Second season				Second season				Second season			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
	T ₁	1.20	1.03	0.92	1.05	0.11	0.08	0.04	0.08	0.94	0.91	0.70
T ₂	2.40	1.60	1.08	1.69	0.24	0.22	0.13	0.20	1.80	1.60	1.10	1.50
T ₃	1.80	1.50	1.20	1.50	0.22	0.21	0.15	0.19	2.27	1.64	1.40	1.77
T ₄	1.90	1.75	1.15	1.60	0.25	0.23	0.17	0.22	2.12	1.50	1.20	1.61
T ₅	2.08	1.80	1.32	1.73	0.28	0.26	0.19	0.24	2.27	1.64	1.40	1.77
T ₆	2.17	2.00	1.46	1.88	0.32	0.29	0.20	0.27	2.33	1.74	1.54	1.87
T ₇	2.26	2.08	1.55	1.96	0.35	0.31	0.23	0.30	2.50	2.08	1.63	2.07
Mean (A)	1.97	1.68	1.24		0.25	0.23	0.16		2.08	1.67	1.33	
LSD 0.05	(A)=0.04, (B)=0.02, (AxB)=0.06				(A)=0.02, (B)=0.01, (AxB)=0.03				(A)=0.05, (B)=0.03, (AxB)=0.08			

(T₁) Saline water, (T₂) Mineral NPK fertilizer (T₃) Compost, (T₄) Compost + Biofertilizer, (T₅) Spirulina, (T₆) Biofertilizer + Spirulina and (T₇) Compost + Biofertilizer + Spirulina



- Total Na (%) and total Cl (%) in dry leaves:

Table 5 indicated that, the lowest values of Na and Cl % were found in transplants irrigated with 6000 followed by 7000, while 8000 ppm gave the highest values in both seasons. Whereas, applied organic and biofertilizers decreased the effect of salinity on plant, T₇ (compost + biofertilizers + Spirulina) recorded the lowest content of total sodium and chloride % followed by T₆ (biofertilizers + Spirulina) compare to T₂ (mineral fertilizers). With respect of the interaction, data showed that, T₇ recorded the lowest values at 6000 ppm followed by 7000, then 8000 ppm., then T₆ in the same way, in contrary the highest results were recorded in T₂ (mineral fertilizers) in both experimental seasons.

So, many researchers said that, PGPR have a vital role in improving plant salt stress tolerance through several mechanisms; as producing antioxidants and growth regulators which reflecting in enhancement of plant nutrient content, increasing growth parameters and improve plant health under salt stress (Abbas et al., 2019). Salinity in the irrigation water reduces the leaf contents of the essential nutrient elements N, P, K while increasing Na and Cl content. So, organic and biofertilizers application increasing N, P and K uptake, and decreasing Na and Cl contents in plant leaves. these results agreed with Aini et al. (2021) which investigated that, Na uptake in the plants

treated with biofertilizers is lower than that in plants treated without biofertilizers, bacteria can produce exopolysaccharide compounds that can bind sodium in plant tissue and thus reduce Na levels and alleviate salinity stress of plants. Additionally, application of biofertilizers under salinity stress leads to increase N, P and K uptake rates, stimulate the plant nutrient uptake by increasing nutrients movement in the rhizosphere, releases organic acids and enzymes that improve various nutrient cycles in soil and maintain beneficial microbial population in the rhizosphere. (Gao et al., 2022).

Additionally, application of biofertilizers under salinity stress leads to increase N, P and K uptake rates, stimulate the plant nutrient uptake by increasing nutrients movement in the rhizosphere, releases organic acids and enzymes that improve various nutrient cycles in soil and maintain beneficial microbial population in the rhizosphere. (Gao et al., 2022).

While, Cyanobacteria algae has the ability to fixing the atmospheric N₂ and the transformation into an accessible form of ammonia, mobilize insoluble inorganic phosphate forms, decrease Na⁺ levels and its strength in bioaccumulation of several micro elements which related to its high binding ability and synthesis salt stress proteins for many cultivated plants (Taha et al., 2023).

Table (5). Effect of organic and biofertilizers on total Na and Cl % in dry leaves of Wonderful cv. Pomegranate transplants grown under different levels of water salinity

Treatments	Total sodium %				Total chloride %			
	First season				First season			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
T ₁	1.35	1.60	1.75	1.57	1.88	1.91	1.97	1.92
T ₂	1.45	1.75	1.90	1.70	1.90	1.95	2.00	1.95
T ₃	0.90	1.12	1.27	1.10	1.30	1.50	1.70	1.50
T ₄	0.70	1.00	1.15	0.95	1.24	1.40	1.67	1.44
T ₅	0.65	0.80	1.05	0.83	1.11	1.32	1.59	1.34
T ₆	0.30	0.45	0.77	0.51	0.98	1.21	1.55	1.25
T ₇	0.15	0.28	0.67	2.19	0.80	1.03	1.25	1.03
Mean (A)	0.78	0.99	1.23		1.32	1.47	1.68	
LSD 0.05	(A)=0.04, (B)=0.03, (AxB) =0.09				(A)=0.05, (B)=0.03, (AxB) =0.09			
Treatments	Second season				Second season			
	Second season				Second season			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
T ₁	1.47	1.70	1.80	1.66	1.90	2.00	2.12	2.01
T ₂	1.55	1.80	1.95	1.77	1.98	2.06	2.16	2.07
T ₃	0.80	1.09	1.33	1.07	1.27	1.40	1.73	1.47
T ₄	0.60	0.97	1.27	0.95	1.18	1.37	1.71	1.42
T ₅	0.50	0.74	1.09	0.78	1.06	1.30	1.70	1.35
T ₆	0.28	0.32	0.88	0.49	0.90	1.25	1.60	1.25
T ₇	0.12	0.18	0.70	0.33	0.66	1.10	1.27	1.01
Mean (A)	0.76	0.97	1.29		1.28	1.50	1.76	
LSD 0.05	(A)=0.03, (B)=0.02, (AxB) =0.05				(A)=0.05, (B)=0.03, (AxB) =0.09			

(T₁) Saline water, (T₂) Mineral NPK fertilizer (T₃) Compost, (T₄) Compost + Biofertilizer, (T₅) Spirulina, (T₆) Biofertilizer + Spirulina and (T₇) Compost + Biofertilizer + Spirulina



- Total carbohydrates, total phenols and proline content % in dry leaves:

Table (6) shows that the contents of carbohydrate, phenols and proline % were increased with increasing salinity levels, the lowest results were recorded when irrigating the transplants with 6000 followed by 7000 and then 8000 ppm in both seasons compare to the controls T₁ (Saline water). Results decreased when using organic and biofertilizers. So, transplants received compost + biofertilizers + spirulina algae recorded the lowest results compare to T₂ (mineral fertilizers). Whereas, organic and biofertilizers under salinity levels shows the lowest results especially at 6000 followed by 7000, while 8000 ppm came in the last position. The highest results were recorded in the mineral fertilizers in the two seasons.

However, the response of proline content to biofertilizers under salinity stress were investigated, Abdallah et al. (2020) proved that there's a great relationship between the regulatory role of proline and the activity or the function of superoxide dismutase, catalase, and peroxidase enzymes in plant cells as they strongly participate in metabolic response development and could be resist to the environmental factors and provides vital protection in stressed plant cells.

While, (Malash et al., 2023) concluded that, the use of biofertilizers can reduce the severity of salinity stress and enhance salinity mitigation, this may result in reduce proline accumulation.

Table (6). Effect of organic and biofertilizers on total Carbohydrates, Phenols and Proline % in dry leaves of Wonderful cv. Pomegranate transplants grown under different levels of water salinity.

Treatments	Total Carbohydrates %				Total Phenols %				Total Proline %			
	First season				First season				First season			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
T ₁	5.50	6.56	8.11	6.72	9.70	10.00	11.5	10.40	14.30	16.00	20.00	16.77
T ₂	5.80	7.50	8.60	7.30	10.00	10.75	11.90	10.88	14.44	16.50	20.60	17.18
T ₃	4.33	6.00	6.85	5.73	8.62	8.85	10.75	9.41	12.32	14.40	16.50	14.41
T ₄	4.30	5.89	6.60	5.60	8.70	8.90	10.80	9.47	12.28	14.30	16.30	14.29
T ₅	4.10	4.90	6.00	5.00	8.20	8.46	10.20	8.95	11.50	14.00	16.00	13.83
T ₆	3.60	4.30	5.77	4.56	7.44	8.89	9.69	8.67	11.00	13.51	15.50	13.35
T ₇	3.30	4.00	5.60	4.30	6.60	7.19	9.50	7.76	10.50	13.00	15.00	12.83
Mean (A)	4.42	5.59	6.79		8.47	9.01	10.62		12.34	14.53	17.13	
LSD 0.05	(A)=0.2, (B)=0.1, (AxB) =0.3				(A)=0.17, (B)=0.11, (AxB) =0.29				(A)=0.23, (B)=0.19, (AxB) =0.50			
	Second season				Second season				Second season			
T ₁	5.55	6.64	8.13	6.77	10.03	11.06	12.3	11.13	15.00	16.70	20.60	17.43
T ₂	5.83	7.57	8.68	7.36	10.50	11.9	12.63	11.68	15.69	17.00	20.90	17.86
T ₃	4.24	5.85	6.81	5.63	8.00	8.73	10.64	9.12	12.20	14.36	16.48	14.35
T ₄	4.05	5.70	6.53	5.43	8.20	8.80	10.72	9.24	12.10	14.20	16.35	14.22
T ₅	3.80	4.70	5.88	4.79	7.80	8.40	10.27	8.82	11.00	14.04	16.20	13.75
T ₆	3.50	4.00	5.71	4.40	6.77	8.00	9.64	8.14	10.60	13.56	15.70	13.29
T ₇	3.20	3.80	5.66	4.22	5.90	7.00	9.44	7.45	10.00	13.10	14.90	12.67
Mean (A)	4.31	5.47	6.77		8.17	9.13	10.81		12.37	14.71	17.30	
LSD 0.05	(A)=0.1, (B)=0.05, (AxB) =0.12				(A)=0.12, (B)=0.08, (AxB) =0.21				(A)=0.39, (B)=0.25, (AxB) =0.67			

(T₁) Saline water, (T₂) Mineral NPK fertilizers (T₃) Compost, (T₄) Compost + Biofertilizers, (T₅) Spirulina, (T₆) Biofertilizers + Spirulina and (T₇) Compost + Biofertilizers + Spirulina

- Antioxidant enzymes activity (Catalase and Peroxidase activity):

Fig. 4 indicated that, the lowest results of the enzyme activities were recorded with 6000 ppm and increased gradually in 7000 and then 8000 ppm compare to control T₁ (Saline water), while, organic and biofertilizers increasing the values of enzyme activities compare to T₂ (mineral fertilizers). Regarding to the interaction of irrigation salinity levels with organic and biofertilizers, figures showed that, T₇ recorded the high

activity values in all salinity levels especially at 6000 followed by 7000 then 8000 ppm. Compare to control in both seasons.

The antioxidant enzymes systems are considered the first defense line against oxidative damage through scavenging the active oxygen species (O⁻, OH⁻ and H₂O₂) and provides vital protection in stressed plant cells, in addition, there's a great relationship between the regulatory role of proline and the activity or the function of catalase and peroxidase enzymes in plant cells as they



strongly participate in metabolic response development and could be resist to the environmental factors (Abdallah et al., 2020). While, Hoque et al. (2022) suggested that, under salt stress conditions, plants produce different types of antioxidants as plant defensive enzymes to relieve the stress as peroxidase, catalase. While, PGPR promotes plant growth and salt tolerance by improving

plant physiological response and increasing levels of defensive enzymes that are essential components of salt tolerance in plants. In addition Fortt et al. (2022) emphasized that providing the protection to the plants under salt stress is related to the application of PGPR inoculations which have the ability to produce indole acetic acid (IAA) and antioxidant enzymes.

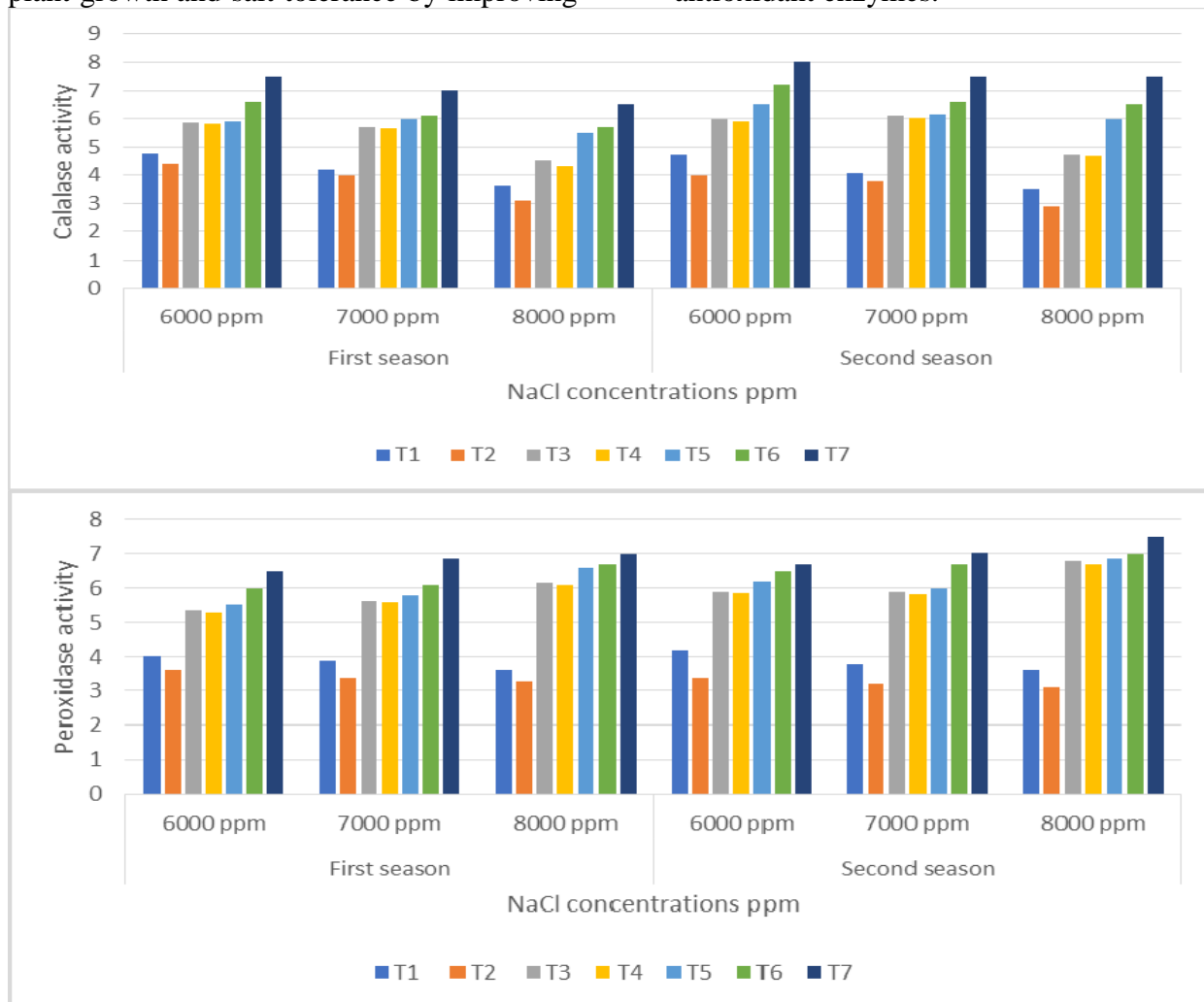


Fig. (4). Effect of using organic and biofertilizers treatments on Peroxidase and catalase content of Wonderful cv. Pomegranate transplants grown under different levels of water salinity.

(T₁) Saline water, (T₂) Mineral NPK fertilizers (T₃) Compost, (T₄) Compost + Biofertilizers, (T₅) Spirulina, (T₆) Biofertilizers + Spirulina and (T₇) Compost + Biofertilizers + Spirulina

Conclusion

In conclusion, the application of halotolerant microbial inoculants (biofertilizers), compost and Spirulina algae are a promising technology for future sustainable farming in the saline regions. It is considered an effective program to mitigate

the effect of salinization for Wonderful cv. transplants till 6000 ppm by improving the soil nutrients and biological properties, increasing the number of beneficial bacteria in the soil, improving soil fertility which enhances the plant growth and quality even under saline condition.



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ب- تخفيف التأثير الضار للاجهاد الملحي بمستويات مختلفة باستخدام بعض التقنيات العضوية على الخصائص الكيميائية لشتلات الرمان صنف الوندرفل

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ملوحة مياه الري والتربة تعد من أكبر العقبات لتحقيق التوازن بين نسبة العرض والطلب ومع تزايد عدد السكان على مستوى العالم أصبح لا يوجد خيار بديل لاستخدام التربة والمياه في الانتاج الزراعي، ولهذا يلزم وجود تقنيات جديدة وصديقة للبيئة للتغلب على التأثيرات الضارة للملوحة وانتاج شتلات صحية آمنة بيئياً.

لذلك أجريت تجربة أصص تحت ظروف الصوبة الزراعية بمعهد بحوث البساتين - مركز البحوث الزراعية - محافظة الجيزة خلال موسمين زراعيين متتاليين ٢٠١٩ و ٢٠٢٠. وتهدف الدراسة لإنتاج شتلات الرمان (صنف الوندرفل) باستخدام التسميد العضوي مثل الكمبوست والتسميد الحيوي (لقاح بكتيري) يتكون من سلالات بكتيرية معززة لنمو النبات و متحملة للملوحة *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megaterium* var. *phosphaticum* and *Bacillus circulans* وكذلك طحلب الإسبيرولينا، والري باستخدام مياه ملحية ذات مستويات ملوحة مختلفة (٦٠٠٠ - ٧٠٠٠ جزء في المليون) ودراسة تأثيرها على المكونات الكيميائية للشتلات مثل محتوى الكلوروفيل في الأوراق، ومحتوى النبات من العناصر المعدنية مثل النيتروجين والفوسفور والبوتاسيوم والصوديوم والكلوريد % وأيضا محتوى النبات من الكربوهيدرات والفينولات والبرولين والإنزيمات المضادة للأكسدة، وكذلك تقدير درجة حموضة وملوحة التربة والنشاط الإنزيمي بالتربة.

وأشارت النتائج إلى أن إضافة التسميد العضوي والحيوي أدى الى انخفاض pH و EC التربة وزيادة نشاط انزيمات التربة تحت ظروف المياه المالحة وزيادة محتوى الورقة من الكلوروفيل الكلى , الكربوهيدرات , الفينولات والبرولين في الشتلات , بالإضافة إلى زيادة معنوية في العناصر الغذائية NPK% في الأوراق الجافة عند معاملة الشتلات باستخدام مخلوط اللقاح الحيوي PGPR باستثناء الصوديوم Na والكلور Cl. وتم الحصول على أعلى كفاءة في تخفيف إجهاد ملوحة مياه الري على شتلات الرمان من المعاملة ٧ والتي تم تسميدها بالكمبوست + مخلوط اللقاح الحيوي + طحلب الأسبيرولينا عند مستوى الملوحة ٦٠٠٠ جزء في المليون مقارنة بمعاملة الكنترول (التسميد الكيميائي).

مما يشير إلى أن إضافة تسميد عضوي وحيوي متكامل أدى إلى التقليل من التأثير الضار لملوحة مياه الري وانتاج شتلات رمان صحية و متحملة للملوحة .