A-Alleviating the harmful impact of salinity stress with different levels by using some organic techniques on vegetative growth characteristics of Wonderful cv. Pomegranate transplants

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ABSTRACT

One of the major environmental stresses is salinity in soil and/or irrigation water which needs more understanding for its adverse effects on plant growth and soil in addition to limited attempts to improve plant salt tolerance. hence, Pot experiment was carried out during two successive seasons 2019 and 2020 under the greenhouse conditions in the nursery of Horticulture Research Institute, ARC, Giza, Egypt, to investigate the effect of compost, active halotolerant biofertilizers namely (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megaterium* var. *phosphaticum*, *Bacillus circulans*) isolates and (*Spirulina platensis* L.) algae on vegetative growth measurements, aerial parts fresh and dry weights, root measurements, root fresh and dry weights were determined in Wonderful cv. pomegranate transplants grown under different levels of saline water irrigation (6000, 7000 and 8000 ppm.) to mitigate the harmful impact of salinity. The obtained results showed a noticeable decrease in all growth aspects, root measurements, fresh and dry weights of the transplants as response to the high levels of salty water, especially 8000 ppm. While, all the treatments had an observed promotion effects on those aforementioned parameters, Furthermore, the combined applications of organic and different biofertilizers present a mechanism of alleviating the damage impact of salts accumulation than the application of chemical fertilizers even under high concentrations of salinity. Overall, it could be suggested that Wonderful cv. transplants performed well at 6000 ppm when providing an ideal fertilization program with the natural organicand various biofertilizers used.

Keywords: Saline water, organic and biofertilizers, Spirulina algae, Wonderful pomegranate, vegetative growth.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is native to central Asia (Iran) and widely planted in Egypt where it appropriate with the Mediterranean climate and soil conditions (El-Kholy et al., 2019), it was adjusted for a diverse range of soil and geographic conditions, whereas the best regions were tropical and subtropical countries in addition to hot and dry summers area (Verma et al., 2010). The foreign pomegranate "Wonderful" cv. was originally ascribed to the USA and has recently been successfully expanded in the new reclaimed regions in Egypt due to its distinctive vigorous and productive qualities (Salama et al., 2020). So, it's classified as moderately sensitive to salinity (Sun et al., 2018). Therefore, utilizing its possibility against salinity in light of the deficiency of the quantity and quality of water that hinders agricultural expansion with the influence of overpopulation.

Recently, climate change had indirect effect on salt water concentrations and water cycle balance; it's clearly having a significant impact through increasing global temperature (Rezk et al., 2015). Soil or water salinization is one of the most serious environmental problems in agriculture which leads to elevating the potential evapotranspiration from both water and soil subsequently leads to changes in salinity levels (Corwin, 2021). Furthermore, Saline irrigation water with excess of NaCl salt causes osmotic stress which plays a role in the accumulation of low molecular mass compounds such as proline with decreasing the vigor rate (Mastrogiannidou et al., 2016).

One of the organic inputs is compost which a good alternative to chemical fertilizers, it solves several soil problems through enhancing soil structure as well as maintaining the balancing of the organic matter and humus which led to increasing the availability of various nutrients and microbial activity thus will helping in sustaining plant productivity under salt stress (Bello et al., 2021).

A new strategy requirement for the sustainable agriculture is depending on treating the transplants with the biofertilizers. Hence, the use of plant growth-promoting rhizobacteria (PGPR) as an effective tool for agricultural operations has attracted considerable attention (Goswami et al. 2016). The rhizosphere of halophytes is a rich source of osmotic stress tolerant bacteria which could affect positively the growth and productivity of plants under stress. The benefits of these bacteria are to promoting plant growth by improving nutrient uptake, enhancing nitrogen fixation, producing hormones (such as auxin, cytokinin and gibberellin), dissolving insoluble nutrients such as phosphorus, potassium, zinc and silicon (Samy and El- zohiri, 2021). Several species of halotolerant soil bacteria such as *Azotobacter*, *Azospirillum* and *Bacillus* have been reported to ameliorate salt stress in crops (Saghafi et al., 2019). The use of halotolerant -PGPR has recently been applied as a viable solution to issues associated with water and soil salinity in agricultural lands. These halotolerant microorganisms are already adapted to alive and survival in salty environments they can influence host plant survival, root development and growth under salt stress (Kumar et al., 2023).

Spirulina platensis is a photosynthetic blue-green microalga that is considered an essential biofertilizer, as a rich source of mineral nutrient, which stimulate root establishment, root elongation and promote the vegetative growth of plants (Yassen et al., 2019). Cyanobacteria play a vital role in increasing and maintenance soil fertility by the accessibility of several nutrients in soil, thus increasing the growth and productivity for many crops (Sivalingam, 2020).

Therefore, the aim of this investigation is to evaluate the influence of using organic, biofertilizers and *spirulina* algae to alleviate salinity stress on vegetative growth of Wonderful cv. pomegranate transplants.

MATERIALS AND METHODS

- **Plant material, experimental design and site**

A pot experiment was carried out during two successive seasons 2019 and 2020 under the greenhouse conditions in the nursery of Horticulture Research Institute, Agricultural Research Center (ARC), Giza, Egypt. One-year-old of Wonderful cv. transplants were selected, uniform in growth and ranging in length from 30-35 cm. and girth from 2- 2.5 cm for both seasons, respectively **(**Koshkhooi, 2006). At the beginning of March, 126 transplants (7 treatments x 3replicates x 6 transplants/replicate) were planted separately into plastic pots (25 cm diameter) containing about 4 kg a mixture of clay and sand (2:1 v/v), EC was 1.83 ds/m, field capacity (FC) was 22.8 %. The treatments were arranged in a spilt-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 with Hoagland solution (artificial saline water with added of NaCl at three levels of salinity represented as 6000, 7000 and 8000 ppm (6, 7 and 8 g NaCl/ L.). A volume of 500 ml. saline water/ transplant was used to irrigate the transplants twice a week till the end of the experiment in September, to reduce the harmful effect of saline irrigation water on vegetative growth measurements in transplants treated by organic and biofertilizers. The moisture content of all pots was kept approximately at field capacity by weighing the pots every three days.

- **Sources and doses of different applications used:**

Mineral NPK fertilizer: The recommended doses of mineral fertilization were applied according to the recommendations of the Egyptian Ministry of Agriculture and Land reclamation.

Compost: was obtained from the nursery of Horticulture Research Institute, ARC, Giza Egypt. 100 g compost per pot was mixed with the soil for specified treatments before planting **(Hamdy *et al*., 2016).** The physical and chemical characteristics of compost are found on Table (1) according to **(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	**%**	27.4
Chemical properties		
pH		7.7
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	**%**	3.8
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: In this experiment, halotolerance isolates were previously isolated from saline soil located in El Moghra region, Minia, Egypt. The active strains are *Azotobacter chroococcum* and *Azospirillum lipoferum* as nitrogen fixing bacteria, *Bacillus megaterium* var. *phosphaticum* and *Bacillus circulans* as phosphorus and potassium solubilizing bacteria, were activated on their specific media as the nitrogen deficient semi solid malate medium (Dobereiner and Day, 1976) for *Azospirillum* spp., modified Ashby s medium (Abdel- Malek and Ishae, 1968) for *Azotobacter* spp., and phosphate solubilizing bacteria were isolated using Pikovskaya medium (Pikovskaya, 1948) for *Bacillus megaterium* and, modified Aleksandrov medium (Parmar and Sindhu, 2013) for *Bacillus circulans*. The selected isolates were identified according to standard microbiological methods as describedin Bergy s Manual of Systematic Microbiology (Tan et al., 2009), the growth media were adjusted to 6000, 7000 and 8000 ppm, by adding 6, 7 and 8 g NaCl/ L., and mixed together in equal amounts for use as biofertilizers (1ml contains 10^8 cell), they were added to the soil as 30 ml/ pot (4 kg) (Afifi et al., 2014) the biofertilizers were added once each 15 day.

Spirulina algae: Cyanobacteria (*Spirulina platensis*) strain was provided by Agric. Res. Microbiol. Dept. Soils, Water and Environment. Res. Inst. ARC, Giza, Egypt. it was cultured in Zarrouk medium (Zarrouk, 1966), and incubated for 30 days in growth chamber under continuous shaking at 150 rpm, illumination (2000 Lux) and a temperature of 32° C ± 2° C., mixed with a mixer to have a homogenized suspension, then the suspension was filtered until use and added as soil drench with irrigated water as 30 ml/pot (Makhlouf and Helmy, 2022). Chemical analysis of *Spirulina platensis* / 100 g is shown in Table (2) according to (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	55.7 g.	Manganese (Mn)	3.8
Sugars	13.8 %	Calcium (Ca)	31.9
Fat	10.12 g.	Copper (Cu)	3.1
Crude fiber	3.17 g.	Zinc (Zn)	2.0
Ash	10.32 g	Chloride (Cl)	46.8
Antioxidants		**Macronutrients %**	
Total antioxidants	630.5 (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 %		

Seven recovery treatments were investigated as follow:

(T_1) Saline water (Control at 6000, 7000 and 8000 ppm)

(T_2) Recommended dose of mineral NPK fertilizer as control

(T_3) Compost (100 g compost/pot).

(T_4) Compost + Biofertlizers (100 g compost/pot + 30 ml/pot).

(T_5) Spirulina algae (30 ml./pot).

(T_6) Spirulina algae + Biofertlizers (15 ml./ pot + 15 ml/pot)

(T_7) Compost + Biofertlizers + Spirulina algae (100 g compost /pot + 30 ml/ pot)

The response of pomegranate transplants to the different treatments were determined by the following parameters:

1- **Vegetative growth measurements**: at the end of the experiment the transplants were divided into roots, stems and leaves to determine the growth parameters
   a. Stem length (cm).
   b. Stem girth (mm) were measured by a vernier caliper.
   c. Number of new sprouted shoots.
   d. Leaves number and Leaf area (cm^2): the fourth one from the top was measured according to the equation of as follows:

Leaf area (cm^2) = 0.41 (Leaf length × width) + 1.83 (Ahmed and Morsy, 1999)

2- **Root measurements:** Root length (cm) and number were counted and presented at the end of the both seasons

3- **Aerial parts and roots fresh and dry weights**: Fresh and dry weights of aerial parts (shoots and leaves) and roots were washed carefully, oven dried at 70 ^{O}C and measured.

4- **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 irrigation water salinity levels and different treatments on vegetative growth characteristics of Wonderful cv. transplants

- Stem length (cm), girth (mm) and number of new sprouted shoots.

Data presented in Table (3) clarify that both averages stem length (cm), girth (mm) and number of new sprouted shoots are an important indicators to assess the growth characteristics changes under various saline water stress. Hence, all treatments are significantly influenced by the three salinity levels, since the concentration 6000 ppm exceeded statistically the other two salinity levels 7000 and 8000 ppm treatments during both seasons which caused a noticed reduction in this respect. Similarly, treating the transplants with compost + biofertilizers + Spirulina algae succeeded in maintaining the normal values of stem length, girth and number of new sprouted shoots in both seasons than the control.

As for the interaction effect of salinity levels with different treatments combinations it's clear that fertilizing wonderful cv. transplants with compost + biofertilizers + Spirulina algae under the irrigation with the saline level 6000 ppm will gave the best results during season 2020 where they achieved 122.87 cm, 0.70 mm and 9.80 for stem length, stem girth and number of new sprouted shoots respectively compared with NPK treatment.

Table (3): Effect of irrigation water salinity levels and different treatments on stem length (cm), girth (mm) and number of new sprouted shoots of Wonderful cv. transplants.

Treatments	Stem Length (cm)							
	2019				2020			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean(B)
1- Saline water	61.23	53.87	36.93	**50.68**	62.53	55.30	37.90	**51.91**
2- Mineral NPK (control)	96.03	88.70	58.23	**80.99**	98.83	90.40	59.13	**82.79**
3- Compost	91.37	83.17	52.27	**75.60**	94.80	85.53	52.53	**77.62**
4- Compost + Biofertilizers	101.67	90.63	60.97	**84.42**	104.23	92.77	64.03	**87.01**
5- Spirulina algae	105.47	94.13	68.30	**89.30**	108.03	95.90	69.53	**91.15**
6- Spirulina algae + Biofertilizers	115.00	102.27	82.73	**100.00**	116.13	104.03	84.40	**101.52**
7- Compost + Biofertilizers + Spirulina algae	121.97	111.20	91.67	**108.28**	122.87	112.33	93.93	**109.71**
Mean (A)	**98.96**	**89.14**	**64.44**		**101.06**	**90.89**	**65.92**	
	LSD (A)=0.96 LSD (B)=0.63 LSD (AxB) =1.67				**LSD (A)=1.00 LSD (B)=0.66 LSD (AxB) =1.74**			
Treatments	**Stem girth (mm)**							
1- Saline water	0.31	0.22	0.16	**0.23**	0.32	0.23	0.18	**0.24**
2- Mineral NPK (control)	0.48	0.42	0.23	**0.38**	0.51	0.44	0.24	**0.40**
3- Compost	0.47	0.36	0.20	**0.34**	0.49	0.39	0.22	**0.40**
4- Compost + Biofertilizers	0.53	0.48	0.22	**0.41**	0.55	0.50	0.24	**0.43**
5- Spirulina algae	0.55	0.51	0.25	**0.44**	0.58	0.53	0.26	**0.46**
6- Spirulina algae + Biofertilizers	0.60	0.54	0.28	**0.47**	0.63	0.55	0.29	**0.49**
7- Compost + Biofertilizers + Spirulina algae	0.66	0.60	0.31	**0.52**	0.70	0.62	0.33	**0.55**
Mean (A)	**0.51**	**0.45**	**0.24**		**0.54**	**0.47**	**0.25**	
	LSD (A)=0.24 LSD (B)=0.16 LSD (AxB) =0.42				**LSD (A)=0.01 LSD (B)=0.008 LSD (AxB) =0.019**			
Treatments	**Number of new sprouted shoots**							
1- Saline water	1.97	1.47	1.07	**1.50**	2.17	1.63	1.23	**1.68**
2- Mineral NPK (control)	5.27	4.30	2.40	**3.99**	5.93	5.03	3.13	**4.70**
3- Compost	4.23	3.27	2.17	**3.22**	4.87	4.10	2.43	**3.80**
4- Compost + Biofertilizers	6.03	5.10	3.60	**4.91**	6.33	5.57	4.00	**5.30**
5- Spirulina algae	6.53	5.60	4.07	**5.40**	6.77	6.27	4.40	**5.81**
6- Spirulina algae + Biofertilizers	7.10	6.10	4.50	**5.90**	8.10	6.97	4.83	**6.60**
7- Compost + Biofertilizers + Spirulina algae	8.83	7.53	5.33	**7.23**	9.80	7.83	5.80	**7.81**
Mean (A)	**5.71**	**4.77**	**3.31**		**6.28**	**5.34**	**3.69**	
	LSD (A)=0.21 LSD (B)=0.13 LSD (AxB) =0.36				**LSD (A)=0.18 LSD (B)=0.12 LSD (AxB) =0.31**			

- Leaves number and Leaf area (cm^2):

It is obvious from Table (4) that all the salinity levels had a pronounced effect in terms of leaves number and leaf area, with the exception for the first level 6000 ppm which was recorded acceptable values during 2019 & 2020 seasons. On the contrary, the excessive use of salinity levels up to 8000 ppm led to adverse impacts in all vegetative parameters.

As for the organic and biofertilizers treatments, the same trend was observed by using compost+ biofertilizers + Spirulina algae which enhanced the values of the previous characteristics comparing with the mineral fertilized transplants in both seasons.

Regarding the interaction between salinity levels and the combined treatments, all the transplants subjected to the triple treatment (compost + biofertilizers + Spirulina algae) with the irrigation at 6000 ppm of saline water had the ability to overcome the deleterious effect of salt stress increment with a significant effect on number of leaves and scored the largest leaf area in both growing seasons as compared with the control which either received saline water alone or mineral fertilization.

The importance of estimate vegetative growth characteristics which considered the major index to evaluate transplant's salt resistance since they show a normally adaptation with the moderate level and with no obvious injuring symptoms in Tunisi cv. pomegranate was noticed by Liu et al. (2018) Also, the above-mentioned results are in line with Cuiyu et al. (2020) where they proved that plant growth parameters increased with low or moderate salinity levels while increasing salinity stress will cause a reduction in all growth parameters in different pomegranate cultivars.

The vital role of the combined applications of the effective microbial inoculation PGPR and compost in enhancing total net growth and lead to reduce the negative effects of salinity stress were attributed to decreasing the osmotic stress affected by salt and promoting ATP synthesis then the plant could have the ability to convert the light energy to the chemical one thus improve the photosynthetic pigments and metabolic processes as a noticeable results were explained by (Olmo et al., 2019) in three pomegranate cultivars.

Table (4): Effect of irrigation water salinity levels and different treatments on leaves number and leaf area (cm^2) of Wonderful cv. transplants.

Treatments	Leaves number							
	2019				2020			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
1- Saline water	63.91	48.00	19.92	**43.94**	64.70	49.51	20.11	**44.77**
2- Mineral NPK (control)	102.55	70.76	34.46	**69.26**	105.06	73.05	36.35	**71.49**
3- Compost	91.91	67.21	31.25	**63.46**	94.86	68.86	32.26	**65.33**
4- Compost + Biofertilizers	110.74	81.29	40.11	**77.38**	113.80	83.37	41.55	**79.57**
5- Spirulina algae	113.81	83.62	44.63	**80.69**	114.45	85.14	46.13	**81.91**
6- Spirulina algae + Biofertilizers	118.54	90.93	52.26	**87.24**	121.70	93.60	53.93	**89.74**
7- Compost + Biofertilizers + Spirulina algae	127.43	99.65	63.90	**96.99**	129.84	104.01	64.28	**99.38**
Mean (A)	**104.91**	**77.35**	**40.93**		**106.34**	**79.65**	**42.09**	
LSD (A)=1.13 LSD. (B)=0.74 LSD (AxB) =1.96					**LSD (A) =1.35 LSD (B)=0.88 LSD (AxB) =2.34**			
Treatments	**leaf area (cm^2)**							
1- Saline water	2.07	1.71	1.05	**1.61**	2.12	1.74	1.13	**1.66**
2- Mineral NPK (control)	3.58	3.30	1.73	**2.87**	3.98	3.82	2.15	**3.32**
3- Compost	3.06	2.88	1.51	**2.48**	3.21	3.28	1.75	**2.75**
4- Compost + Biofertilizers	4.22	3.93	2.10	**3.42**	4.67	4.82	2.51	**4.00**
5- Spirulina algae	5.23	4.72	2.52	**4.16**	6.02	5.80	3.27	**5.03**
6- Spirulina algae +Biofertilizers	6.83	5.48	3.22	**5.18**	7.56	6.38	4.02	**5.99**
7- Compost + Biofertilizers + Spirulina algae	8.11	6.93	4.88	**6.64**	8.94	7.77	5.08	**7.26**
Mean (A)	4.73	4.14	2.43		5.21	4.80	2.84	
LSD (A)=0.10 LSD (B)=0.06 LSD (AxB) =0.17					**LSD (A)=0.13 LSD (B)=0.09 LSD (AxB) =0.23**			

Moreover, the positive action of *Spirulina platensis* algae on vegetative growth characters might be ascribed to its higher with natural components of vitamins and provitamins such as (B1, B2, B6, B12 and E), various amino acids including leucine, isoleucsive and valine, folic acid and different minerals namely P, Fe, Ca and K, fatty acids, chlorophylls, carotenoids and the highest hormonal levels, all these synthesizes of several biofertilizers will surely reflecting on stimulating growth measurements through promoting the permeability of cell membrane thus will led to enhancing the plant efficiency of absorption several nutrients (Hussein, 2017) on date palm and (Hussein and Gad El-Kareem, 2021) on olive trees.

This trend could be supporting the results by **(Hamouda *et al.*, 2022)** they explained the mitigation impact of micro-algae extracts *Spirulina platensis* on salt stressed on wheat seedlings which led to the improvement of the morphological parameters, photosynthetic activity and transpiration rate. As well as, increasing nutrient and water intake hence enhancing fertilizers uptake by the plants. This can be credited to its richness with several valuable stimulators substances and the large source of bioactive components produced by *S. platensis.* Moreover, their ability for nitrogen fixation and the noticeable reduction in lipid peroxidation and the lower production of superoxide radicals.

2- Effect of irrigation water salinity levels and different treatments on root measurements of Wonderful cv. transplants.

- Root length (cm) and number:-

Rooting success was measured by the transplant's strength of continues roots production in terms of length and number under different salinity levels. Results in Table 5 indicated that various salinity irrigation levels had influenced on both root length and number while the suitable level was 6000 ppm which had the ability to give the tallest roots with greater number in both seasons. The opposite result was recorded by increasing the irrigation grade of with saline water till 8000 ppm. While, the medium level 7000 ppm was in between in this respect. The considered various treatments had a great impact on root parameters, this improvement attributed to the fertilization with compost and biofertilizers plus Spirulina algae as compared with NPK (control) in both seasons.

Concerning factors interaction, data revealed that irrigated Wonderful cv. transplants with 6000 ppm saline water in addition to fertilization with compost + biofertilizers + Spirulina algae significantly reduced the damage occurring by increasing salinity levels during 2019 & 2020 seasons. Meanwhile, the opposite result was true in transplants received the highest salinity level 8000 ppm with NPK fertilization or saline water alone.

Table (5): Effect of irrigation water salinity levels and different treatments on root length (cm) and number of Wonderful cv. transplants.

Treatments	Root length (cm)							
	2019				2020			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
1- Saline water	12.34	10.70	7.47	**10.17**	12.85	11.45	7.96	**10.75**
2- Mineral NPK (control)	19.55	17.23	12.53	**16.44**	21.49	19.06	13.31	**17.95**
3- Compost	17.33	13.85	10.48	**13.89**	18.99	17.12	11.73	**15.95**
4- Compost + Biofertilizers	20.63	18.28	13.13	**17.35**	22.70	20.42	15.33	**19.48**
5- Spirulina algae	23.85	20.48	15.20	**19.84**	24.90	22.16	16.43	**21.16**
6- Spirulina algae + Biofertilizers	30.34	23.76	16.80	**23.63**	31.64	26.43	18.01	**25.36**
7- Compost + Biofertilizers + Spirulina algae	36.50	30.75	20.65	**29.30**	37.93	34.39	21.07	**31.13**
Mean (A)	**22.93**	**19.29**	**13.75**		**24.36**	**21.58**	**14.83**	
LSD (A)=0.47 **LSD (B)=0.31** **LSD (AxB) =0.82**					**LSD (A)=0.72** **LSD (B)=0.47** **LSD (AxB) =1.26**			
Treatments	**Root number**							
1- Saline water	14.44	11.30	8.10	**11.28**	14.89	11.61	8.55	**11.68**
2- Mineral NPK (control)	20.63	16.09	12.31	**16.34**	21.93	17.20	13.11	**17.41**
3- Compost	18.37	14.11	10.13	**14.20**	19.28	15.34	10.75	**15.12**
4- Compost + Biofertilizers	22.58	19.40	13.34	**18.44**	23.57	20.66	14.19	**19.47**
5- Spirulina algae	23.58	22.22	14.87	**20.22**	24.30	23.15	15.45	**20.97**
6- Spirulina algae + Biofertilizers	28.37	24.71	17.58	**23.55**	30.01	25.98	18.50	**24.83**
7- Compost + Biofertilizers + Spirulina algae	31.90	27.60	20.62	**26.71**	32.94	29.02	21.42	**27.79**
Mean (A)	**22.48**	**19.35**	**13.85**		**23.85**	**20.42**	**14.57**	
LSD (A)=0.39 **LSD (B)=0.26** **LSD (A x B)=0.68**					**LSD (A)=0.45** **LSD (B)=0.29** **LSD (A x B) =0.79**			

The application of organic fertilizers mixed with microbial inoculation treatments are responsible for root morphogenesis by stimulating the activity of soil enzymes which indeed reduced by increasing Na^+ and Cl^- levels. So, this will have a great impact on water and nutrient transportation as well as the production of gibberellin, cytokinin and auxin which strongly reflected on the quantity and quality of roots formed resulted in an increasing of lateral root length and intensity (Kheyrodin, 2022). Furthermore, the idea of using compost in mitigation the negative impact of the excessive using saline water is due to reducing the availability of interchangeable Na^+ around the root region by improving water infiltration and salt filtering which promote root production (Fouguira et al., 2023).

Comparable results in improving root effectiveness in terms of establishment and elongation were strongly attributed to the safety application of Cyanobacteria "*Spirulina platensis*" which had valuable composition of macro and micronutrients in addition to polyamines which acquired by the decarboxylation of algal L-amino acids and acting as an active film surrounding the root zone against the biotic stress hence resulted in enhancing the overall plant growth (Mógor et al., 2018).

These results are in harmony with (Hamouda et al., 2022) who confirmed that *S. platensis* liquid biofertilizer had a great potential effect against salinity stress due to their higher concentrations of growth regulators which related to boosting root growth of wheat seedlings.

3- Effect of irrigation water salinity levels and different treatments on fresh and dry weights of aerial parts and roots (g) of Wonderful cv. transplants.

- Aerial parts fresh and dry weights (g)

Table 6 displayed that fresh and dry weights of aerial parts had reverse relationship with increasing the salinity levels gradually as the lowest weights were recorded by 8000 ppm while 6000 ppm saline water detected significantly the heaviest weights at the two seasons.

Wonderful cv. transplants received the fully combined treatments as compost + biofertilizers + Spirulina alga scored the heaviest fresh and dry weights among all the other treatments especially the mineral fertilized transplants at the end of both seasons.

Looking to the response of fresh and dry weights of aerial parts to the interaction between the salinity levels, organic and biofertilizers treatments. It's clear that all the transplants fertilized with the ideal treatment containing compost as organic fertilizer with various biofertilizers and Spirulina algae under the irrigation with 6000 ppm gave the heaviest values as compared with the control at the end of the two seasons. On the other hand, the highest of salinity levels, the greatest of its negative impact were noticed in all parameters.

Table (6): Effect of irrigation water salinity levels and different treatments on aerial parts fresh and dry weights (g) of Wonderful cv. transplants.

Treatments	Aerial parts fresh weight (g)							
	2019				2020			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
1- Saline water	20.39	14.49	9.26	**14.71**	20.67	14.60	9.56	**14.94**
2- Mineral NPK (control)	30.00	19.91	14.27	**21.39**	32.05	21.10	16.00	**23.05**
3- Compost	35.57	23.14	17.20	**25.30**	36.96	24.60	18.65	**26.74**
4- Compost + Biofertilizers	38.94	26.26	19.81	**28.34**	40.60	27.65	20.63	**29.63**
5- Spirulina algae	43.22	30.50	22.88	**32.20**	44.32	32.93	23.86	**33.70**
6- Spirulina algae + Biofertilizers	51.36	41.74	28.10	**40.40**	53.63	43.13	26.06	**40.94**
7- Compost + Biofertilizers + Spirulina algae	58.81	50.68	32.64	**47.38**	60.45	51.92	33.13	**48.50**
Mean (A)	**39.76**	**29.53**	**20.59**		**41.24**	**30.85**	**21.13**	
LSD (A)=0.52 LSD (B)=0.34 LSD (AxB) =0.91					**LSD (A)=0.47 LSD (B)=0.31 LSD (AxB) =0.83**			

Treatments	Aerial parts dry weight (g)							
1- Saline water	9.62	8.04	4.90	**7.52**	10.44	9.08	5.47	**8.33**
2- Mineral NPK (control)	14.50	13.27	8.69	**12.15**	16.23	15.04	10.61	**13.96**
3- Compost	16.72	15.24	10.04	**14.00**	18.22	16.90	11.88	**15.67**
4- Compost + Biofertilizers	20.03	17.17	10.63	**15.94**	21.51	18.53	12.07	**17.37**
5- Spirulina algae	21.34	17.72	11.62	**16.89**	22.94	19.17	13.12	**18.41**
6- Spirulina algae + Biofertilizers	27.21	21.04	17.44	**21.90**	29.50	22.39	19.08	**23.66**
7- Compost + Biofertilizers + Spirulina algae	37.13	31.92	21.83	**30.29**	39.89	33.30	22.99	**32.06**
Mean (A)	**20.94**	**17.77**	**12.16**		**22.68**	**19.20**	**13.60**	
LSD (A)=0.54 **LSD (B)=0.36** **LSD (A x B) =0.93**					**LSD (A)=0.42** **LSD (B)=0.27** **LSD (A x B) =0.73**			

- Roots fresh and dry weight (g)

As illustrated in Table 7, the heaviest fresh or dry root weight was attained by the lowest saline level 6000 ppm used in this experiment for the considered transplants. While, this is untrue for the highest levels of saline irrigation in both seasons. Referring to the organic and biofertilization treatments, the seventh treatment (compost + biofertilizers + Spirulina algae) surpassed statistically the other treatments and the control in both seasons. Finally, interaction results proved that the considered transplants received the combined application of compost + biofertilizers + Spirulina algae in presence of the irrigation with the lowest level at 6000 ppm of saline water scored the heaviest weights in both studied seasons. Oppositely, the transplants irrigated with high levels of saline water resulted in adverse influence of all the recorded measurements.

Table (7): Effect of irrigation water salinity levels and different treatments on root fresh and dry weights (g) of Wonderful cv. transplants.

Treatments	Root fresh weight (g)							
	2019				2020			
	6000 ppm	7000 ppm	8000 ppm	Mean (B)	6000 ppm	7000 ppm	8000 ppm	Mean (B)
1- Saline water	12.83	11.65	8.33	**10.94**	12.49	12.16	8.43	**11.03**
2- Mineral NPK (control)	16.70	15.18	10.02	**13.97**	18.14	17.22	11.3	**15.55**
3- Compost	20.82	19.33	12.21	**17.45**	22.69	20.44	14.00	**19.04**
4- Compost + Biofertilizers	18.70	18.15	10.90	**15.92**	20.15	19.10	13.70	**17.65**
5- Spirulina algae	23.50	21.04	13.40	**19.31**	25.04	22.85	15.98	**21.29**
6- Spirulina algae + Biofertilizers	25.03	23.20	15.37	**21.20**	27.20	24.57	17.16	**22.98**
7- Compost + Biofertilizers + Spirulina algae	28.87	24.40	16.87	**23.38**	30.74	26.47	18.60	**25.27**
Mean (A)	**20.93**	**18.99**	**12.44**		**22.35**	**20.40**	**14.17**	
LSD (A)=0.30 **LSD (B)=0.20** **LSD (AxB) =0.53**					**LSD (A)=0.64** **LSD (B)=0.42** **LSD (AxB) =1.11**			
Treatments	**Root dry weights (g)**							
1- Saline water	4.36	1.72	1.97	**2.68**	4.70	1.79	2.12	**2.87**
2- Mineral NPK (control)	7.28	3.61	4.30	**5.06**	8.18	3.93	4.73	**5.61**
3- Compost	8.20	6.40	5.28	**6.63**	9.63	7.39	6.27	**7.76**
4- Compost + Biofertilizers	9.87	7.31	4.05	**7.08**	10.71	8.71	5.89	**8.44**
5-Spirulina algae	10.53	8.15	6.11	**8.26**	11.94	9.06	7.43	**9.48**
6-Spirulina algae + Biofertilizers	13.85	9.23	8.58	**10.55**	14.57	11.35	9.43	**11.78**
7- Compost + Biofertilizers + Spirulina algae	16.53	11.96	9.88	**12.79**	17.99	13.72	10.73	**14.15**
Mean (A)	**10.09**	**6.91**	**5.74**		**11.1**	**7.99**	**6.66**	
LSD (A)=0.27 **LSD (B)=0.18** **LSD (A x B) =0.47**					**LSD (A)=0.34** **LSD (B)=0.22** **LSD (A x B) =0.59**			

The previous results are in parallel with those of Jadidi et al., (2020) they reported that both fresh and dry biomass decreased gradually with the increasing of salinity levels in pomegranate seedlings may be due to the increase in salt absorption will led to ione toxicity problems and obvious osmotic effect on various pomegranate varieties. On the other hand, to avoid the adverse impact of the high salinity levels it should be maximizing the role of organic fertilizer single or mixed with biofertilizers applications which causes a limiting in salinity problems by enhancing root production and vegetative growth fresh and dry weight were ascribed to their influence on lowering the salt concentration around the root area, improving soil enzyme activity and balancing or the good distribution of the nutritional status (Olmo et al., 2019) on three pomegranate cvs. and (Kheyrodin, 2022) on olive trees.

The abundant availability in organic matter, vitamins, vital amino acids, minerals and proteins are correlated with the significant role of cyanobacterial Spirulina strain aside from their strength in inducing endogenous hormone production might have boosting photosynthetic rate, fresh and dry matter of partitioning plant parts (Al Dayel and El Sherif, 2022).

Conclusion

Briefly, for mitigating the deleterious impact of salinization with various levels on Wonderful cv. pomegranate transplants, it could be recommended, the implementation of model fertilization program contains the combined applications of organic; multiple halotolerance PGPRs biofertilizers with Spirulina algae achieved a good performance in all aspects at 6000 ppm.

REFERENCES

Abdel-Malek, Y. and Ishae, Y.Z. (1968). Evaluation of methods used in counting azotobacters. J. Appl. Bacteriol., 31(3): 267-275.

Afifi, M.M.I.; El-Sayed, G.A.M.; El-Gamal, M.A.H. and Massoud, O.N. (2014). Synergistic effect of biofertilizers containing n-fixer, p and k solubilizers and humic substances on *Sorghum bicolor* productivity. Middle East J. Appl. Sci., 4(4): 1065-1074.

Ahmed, F.F and Morsy, M.H. (1999): A new method for measuring leaf area in different fruit species. Minia. J. of Agric. Rec. & Dev., 19: 97 - 105.

Al Dayel, M.F. and El Sherif, F. (2022). *Spirulina platensis* foliar spraying *Curcuma longa* has improved growth, yield, and curcuminoid biosynthesis gene expression, as well as curcuminoid accumulation. Horticulturae. 8, 469.

Barron, L.B.; Torres-Valencia, M.K.; Chamorro Cevallos, G. and Zooiga-Estrada, A. (2008). Spirulina as an antiviral agent, in M.E. Gershwin & Amha belay (ed.) *Spirulina* in human nutrition and health, CRC press, Taylor & Francis, 227 p.

Bello, S.K.; Alayafi, A.H.; Al-Solaimani, S.G. and Abo-Elyusr, K.A.M. (2021). Mitigating soil salinity stress with gypsum and bio-organic amendments: a review. Agron.,11,1735.

Corwin, D.L. (2021). Climate change impacts on soil salinity in agricultural areas. Eur. J Soil Sci., 72:842–862.

Cuiyu, L.; Ming, Y.; Xianbin, H. and Zhaohe, Y. (2020). Effects of NaCl stress on growth and ion homeostasis in pomegranate tissues. Eur. J. Hortic. Sci., 85(1):42–50.

Döbereiner, J. and Day, J. M. (1976). Physiological aspects of N_2 -fixation by a Spirillum from Digitaria roots. Soil Biol. Biochem., 8:45–50.

El-Kholy, R.E.B; Atawia, A.R.; El-Akkad, T.A.M. and El-Gioushy, S.F. (2019). Impact of NPK Bio-Fertilization on some growth pomegranate of Manfaloty and Wonderful pomegranate transplants. Annals of Agric. Sci., Moshtohor, 57(4):1003 – 1012.

Fouguira, S.; Mhaned, A.; Ben Abbou, M.; Ammar, E.; El Haji, M. and Benhra, J. (2023). Effectiveness of compost use in salt-affected soil in an automated greenhouse irrigation system. E3S Web of Conferences, 364, 03002.

Goswami, D.; Thakker, J.N. and Dhandhukia, P.C. (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food & Agriculture, 2: 1127500, 19 p.

Hamdy, A.E.; Khalifa, S.M.; Shawer, S.S. and Mancy, A.G.A (2016). Effect of water stress on the growth, nutritional and biochemical status of two varieties of pomegranate seedling. J. Plant production, Mansoura Univ., 7(12):1321- 1329.

Hamouda, R.A.; Shehawy, M.A.; Mohy El Din, S.M.; Albalwe, F.M.; Albalawi, H.M.R. and Hussein, M.H. (2022). Protective role of *Spirulina platensis* liquid extract against salinity stress effects on *Triticum aestivum* L., Green Processing and Synthesis, 11: 648–658.

Hussien, M.A. (2017). Productive performance of Sewy date palms in relation to spraying *Spirulina Platensis* algae, plant compost tea, salicylic acid and tocopherol. N Y Sci J., 10(7):126-135.

Hussein, E. M. E. and Gad El- Kareem, M. R. (2021). Response of koroneiki olive trees to foliar application of *Spirulina Platensis* algae and salicylic acid. S VU-International Journal of Agricultural Sciences, 3(3): 245-254.

Jadidi, E.; Tatari M.; Ghasemnezhad, M. and Salemi, H.R. (2020). The salinity tolerance of pomegranate cultivars: Effects of salt stress on root and leaf mineral content. Adv. Hort. Sci., 34(3): 325-335.

kheyrodin, H. (2022). Investigation of the effect of biofertilizer application on nutrient uptake and reduction of salinity stress and yield components in pharmaceutical olive tree. Archives of Nutrition and Public Health, 4(1): 7p.

Koshkhooi, M. (2006). Plants propagation (Fundamentals and Methods). Shiraz University Press, Shiraz, 1467. (In Persian).

Kumar, V.; Raghuvanshi, N.; Pandey, A. K.; Kumar, A.; Thoday-Kennedy, E. and Kant, S. (2023). Role of halotolerant plant growth-promoting rhizobacteria in mitigating salinity stress: recent advances and possibilities. Agriculture, 13(168): 22 p.

Liu, C.; Yan, M.; Huang, X. and Yuan, Z. (2018). Effects of salt stress on growthand physiological characteristics of pomegranate (*punica granatum* L.) cuttings. pak. j. bot., 50(2):457-464.

Makhlouf, B.S.I. and Helmy, S.A.M. (2022). Impact of compost tea and *Spirulina platensis* algae on sugar beet grown under different levels of inorganic nitrogen fertilizer. Pak. J. Biol. Sci., 25(9):781- 795.

Mastrogiannidou, E.; Chatzissavvidis, C.; Antonopoulou, C.; Tsabardoukas, V.; Giannakoula, A. and Therios, I. (2016) Response of pomegranate cv. Wonderful plants to salinity. J. Soil Sci. Plant Nutr., 16(3):621-636.

Mógor, Á.F.; Ördög, V.; Lima, G.P.P.; Molnár, Z. and Mógor, G. (2018). Biostimulant properties of cyanobacterial hydrolysate related to polyamines. J. Appl. Phycol., 30:453–460.

Olmo, A.; Garcia-sanchez, F.; Simon, I.; Lidon, V.; Alfosea-Simon, M.; Camara-Zapata, J.M.; Martinez-Nicolas, J.J. and Simon-Grao, S. (2019). Characterization of the ecophysiological responses of three pomegranate cultivars to salinity. Photosynthetica, 57(4):1015-1024.

Page, A.L.; Miller, R.H. and Keeney, D.R. (1982). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy. In Soil Science Society of America, Vol. 1159.

Parmar, P. and Sindhu, S.S. (2013). Potassium solubilisation by rhizosphere bacteria: Influence of nutritional and environmental conditions. Journal of Microbiology Research, 3(1): 25-31.

Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with vital activity by some microbial species. Mikrobiologiya,, 17: 362-370.

Rezk, H.; Tyukhov, I. and Raupov, A. (2015). Experimental implementation of meteorological data and photovoltaic solar radiation monitoring system, Int. Trans. Electr. Energ. Syst., 25:3573–3585.

Saghafi, D.; Ghorbanpour, M.; Ajirloo, H.S. and Lajayer, B.A. (2019). Enhancement of growth and salt tolerance in *Brassica napus* L. seedlings by halotolerant Rhizobium strains containing ACC-deaminase activity. Plant Physiol. Rep., 24(2):225–235.

Salama, A.S.M; Shaddad, A.M. G. E. and Diab,S.M. (2020). Effect of nitrogen and humic acid on vegetative growth, yield and fruit quality of wonderful pomegranate trees. Int. J. Adv. Res. 8 (6), 1147-1161.

Samy, M.M. and El- zohiri, S.S.M. (2021). Improving seed productivity of garlic by using nitrogen, potassium fertilization and some growth regulators. J. of Plant Production, Mansoura Univ., 12(7):805-812.

Sivalingam, K.M. (2020). Isolation, identification and evaluation of *Spirulina platensis* for its effect on seed germination of groundnut (*Arachis hypogaea* L.), Wolaita Sodo, Southern Ethiopia. J. Algal Biomass Utln., 11(2):34-42.

Snedecor, G.W. and Cochran W.G., (1990). Statistical Methods.7th Ed., Iowa State University Press, Ames Iowa, USA. 593 p.

Sun, Y.; Niu, G.; Masabni, J.G. and Ganjegunte, G. (2018). Relative salt tolerance of 22 pomegranate (*Punica granatum*) cultivars. Hortscience, 53(10):1513–1519.

Tan, G.H.; Nordin, M.S.; Kert, T.L.; Napsiah, A.B. and Jeffrey, L.S.H. (2009). Isolation of beneficial microbes from biofertilizer products. J Trop Agric and Fd. Sc., 37(1):103–109.

Verma, N; Mohanty, A and Lal, A (2010). Pomegranate genetic resources and germplasm conservation: a review. 4: 120-125.

Yassen, A.A.; Essa, E.M. and Zaghloul, S.M. (2019). The Role of vermicompost and foliar spray of *Spirulina Platensis* extract on vegetative growth, yield and nutrition status of lettuce plant under sandy soil. Research Journal of Agriculture and Biological Sciences, 14(1):1-7.

Zarrouk, C. (1966). Contribution a l’ etude d’ une cyanobacterie: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setchell et Gardner) Geitler. PhD thesis, University of Paris, France.

أ- تخفيف التأثير الضار للاجهاد الملحى بمستويات مختلفة بإستخدام بعض التقنيات العضوية على خصائص النمو الخضري لشتلات الرمان صنف الوندرفل

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واحده من الضغوط البيئية الرئيسية هي ملوحة التربة و/ أو مياه الري والتي تحتاج إلى مزيد من الفهم لآثارها الضارة على نمو النبات والتربة بالإضافة إلى المحاولات المحدودة لتحسين تحمل النبات للملوحة. ومن ثم، تم إجراء تجربة الأصص خلال موسمين متتاليين ٢٠١٩ و ٢٠٢٠ تحت ظروف الصوبه في مشتل معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر، لدراسة تأثير السماد العضوي والمخصبات الحيوية النشطة المتحملة للملوحه وهي عزلات

(*Azotobacter chroococcum* ، *Azospirillum lipoferum* ،*Bacillus Megaterium* var. *phosphaticum*, *Bacillus circulance*).

و طحلب (*Spirulina platensis* L.) على قياسات النمو الخضرى , الاوزان الطازجه والجافه للاجزاء الهوائيه , قياسات الجذور و الاوزان الطازجه والجافه للجذور على شتلات الرمان صنف " الوندرفل" والمنزرعه تحت مستويات مختلفة من الري بالمياه المالحة (٦٠٠٠، ٧٠٠٠، ٨٠٠٠ جزء في المليون) للتخفيف من التأثير الضار للملوحة. أظهرت النتائج المتحصل عليها انخفاضا ملحوظا في جميع جوانب النمو وقياسات الجذور والأوزان الرطبة والجافة للشتلات استجابة لارتفاع مستويات المياه المالحة وخاصة ٨٠٠٠ جزء في المليون. في حين أن جميع المعاملات كان لها تأثير ملحوظ على تلك العوامل المذكورة أعلاه، علاوة على ذلك، فإن التطبيقات المشتركة للأسمدة العضوية والحيوية المختلفة تمثل آلية لتخفيف تأثير الضرر الناجم عن تراكم الأملاح عن استخدام الأسمدة الكيماوية حتى في ظل تركيزات عالية من الملوحة. عموما، يمكن الاقتراح بان أداء شتلات الرمان صنف "الوندرفل" كان جيدا عند ٦٠٠٠ جزء في المليون مع توفير برنامج تسميد مثالي باستخدام الأسمدة العضوية الطبيعية والعديد من المخصبات الحيويه المستخدمه.