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Influence of Bio-fertilization on Physical and Biochemical Parameters of Grapefruit Saplings *Citrus paradisi* Macfad. Under Salt Stress

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Abstract: The experiment was conducted in a partially shaded area (50% shade-net) throughout the 2023 growing season. This experiment aimed to know the role of biofertilization in increasing the ability of grapefruit seedlings to tolerate salt stress. Biofertilizers were applied with four levels (without biofertilizers, mycorrhizal fungi, bacteria, and mycorrhizae + bacteria). 20 g of mycorrhizal fungi were added near the saplings roots. 20 ml of bacteria were injected into the soil and in contact with the root zone. Adding 20 g of mycorrhizal fungi + 20 ml of bacteria to the pots soil at the same time and near the roots of the saplings carried out the mycorrhizae + bacteria treatment. Saplings were watered with three concentrations of saline water (2, 4, and 6 $ds.m^{-1}$ $NaCl_2$). The results indicated that at the 6 $ds.m^{-1}$ level, there was a reduction in physical and biochemical parameters. This decrease is accompanied with an increase in proline, Cl%, and Na%. The integration of mycorrhizal fungus and Azospirillum bacteria showed a significant superiority in root and shoot growth. Biofertilization caused increase the content of carbohydrates, protein, and chlorophyll in plants. Also, obtained result showed increase the content of some macro and micro- elements in plants such as N, P, K, Zn and Fe. While the percentage of Cl and Na and proline content decreased, resulting reduced the damages of salt stress.

Keywords: Abiotic stress, Bacteria, Ion absorption, Mycorrhiza, Proline.

Introduction

Grapefruit (*Citrus paradisi* Macfad.) Is an evergreen fruit tree belonging to genus *Citrus* and Rutaceae; It grows in tropical and subtropical regions, and it's cultivated widespread on a limited scale in central Iraq. Grapefruit requires at least 6-8 hours of direct sunlight per day (Abdel- Shafy & El-Khateeb, 2021). Frost can be harmful to grapefruit trees, so it is important to provide adequate protection during the winter. Grapefruit trees prefer light, well-drained soil with a pH of 5.5- 6.6. It is preferable that the soil has a good water retention capacity and contains organic materials. The optimum temperature for grapefruit trees growth is 22-30° Celsius. The fruits have high nutation value; contain 17.5% carbohydrates, organic acids, metal elements, pectin, fiber, and vitamins (A, B1, and B2) as well as rich content of vitamin C. However, they have low content of protein and fats (Abdel- Shafy & El- Khateeb, 2021; Shah *et al.*, 2024). Grapefruit distinguished by the thickness of its peel and juicy sacs in a circular shape. It is typically not consumed as a fresh fruits; the majority of varieties are used in making jam (Puglisi *et al*., 2017).

Salinity is considered the one of severe problems that affected agriculture, particularly in arid and semi-arid areas. Iraq is the one of Asian and Arab countries that most affected by the salinity problem (Alhaddad *et al.*¸ 2021; Suhim *et al*., 2023; Olayinka *et al.*¸ 2023; Yaquby *et al.*¸ 2024). Fruit trees are generally classified as moderately tolerant to salt, while citrus trees are classified as salt-sensitive plants (Abdulkadhim & Mortada, 2022; Souid *et al*, 2023; Rouari *et al.*¸ 2024). Therefore, to lessen the damage, it is necessary to apply alternative technologies, including several types of anti-salinity, such as biofertilizers to raise the resistance of grapefruit trees against the salinity effect (Abdulkadhim, 2019; Zhou *et al.*¸ 2020).

Biofertilizers are microorganisms that provide plants with their nutritional needs and facilitate nutrients uptake. It also converts elements from an unready form to a form readily absorbed by plants. The mechanism of action of biofertilizers in reducing salt stress is through improving the biological, chemical and physical characteristic of the soil, especially increasing soil airing, as well as improving soil texture and reducing soil acidity, thus improving the availability of nutrients in the root zone, which provides a suitable environment for plant growth against salt stress. Therefore, biofertilizers are a beneficial technique that followed in the sustainable agricultural development. Furthermore, Biofertilizers fix nitrogen through symbiotic and non-symbiotic life cycles and provide the plant with growth regulators, thus promoting healthy crop growth. Additionally, they protect plants from pathogens, reduce environmental pollution and diminish the yield cost. (García-Fraile *et al*, 2015; Kumar *et al.*¸ 2022).

Grapefruit is a salt-sensitive plant (Abdulkadhim, 2019; Abbas & Abdulkadhim, 2024). Thus, Current study was implements to study the role of biofertilizers in improving Grapefruit saplings' tolerance to salinity by assessing their physical and biochemical parameters.

Materials & Methods

Grapefruit saplings and treatments

Grapefruit saplings were obtained from a citrus production nursery is located in Karbala Governorate, Hindiyah District on 16 February/2023. Saplings were chosen as one-year-old and homogeneously as possible. They were planted in 8 kg plastic pots full of with substrate of sand and peat moss at 1:2 ratio. All plants obtained equal service operations such as irrigation, weed removal, and fungicide. 18 weeks is the duration of the experiment, started from 16 February to 1 July/2023.

Experiment factors

First factor: Irrigation with four levels of saline water (2¸ 4 and 6¸ ds.M^{-1}) using $N\alpha Cl_2$ salt. The saline water was prepared by dissolving sodium chloride salt ($N\alpha Cl_2$) in distilled water according to the proportions required in the study. The pots were irrigated with equal quantities of saline irrigation water each time according to the plant's need until the end of the experiment.

Second factor: Biofertilization with (without biofertilizers, mycorrhizal fungi (*Glomus mosseae genus*), bacteria (*Azospirillum brasilense genus*), and both of mycorrhizal and bacterial inoculum). Biofertilizers were obtained from the Agricultural Research Department of the

Ministry of Science and Technology. Mycorrhizal fungi were carried on the peat moss carrier. The bacterial culture was a liquid preparation containing *Azospirillum brasilense* bacteria. 20 g of mycorrhizal fungal inoculum consisting of *Glomus mosseae* fungi was added to the saplings treated with fungal inoculum and near the roots of the saplings. 20 ml of bacteria (*Azospirillum brasilense*) was added to the saplings treated with bacterial inoculum by injection into the soil and in touch with the root zone. Adding 20 g of mycorrhizal fungi + 20 ml of bacteria to the pots soil at the same time and near the roots of the saplings carried out the mycorrhizae + bacteria treatment. Mycorrhiza Fungi and bacteria were added only once at the beginning of the experiment.

Measured parameters: Measurements of all treatments were taken compared to the control group at the end of the study on 1 July/ 2023.

Physical Parameters

- Stem Diameter (mm).
- Plants Height (cm).
- Total Leaf Area (cm^2 plant $^{-1}$).
- Branches Number (branches plant^{-1}).
- Shoots Dry Weight (g plant $^{-1}$).
- Main Root Length (cm).
- Roots Dry Weight (g plant $^{-1}$).

Biochemical Parameters:

- Nitrogen (%): estimated using the Microkjeldahl device (Novozamsky *et al*., 1974)
- Phosphorus (%): estimated using Ascorbic acid and Ammonium molybdate. Measurement was captured by a spectrophotometer at 620 nm (John, 1970)
- Sodium (Na) and Potassium (K) (%): determined in the digested sample by a Flame Photometer. (Horneck & Hanson, 1998)
- Zinc and Iron (mg Kg^{-1} dry weight): determined by the Absorption Atomic Spectrophotometer according to (Allan, 1961). Proline (mg.g^{-1}dry weight): estimated by following (Bates *et al*., 1973). Chloride content of leaves (%): estimated in a digested sample diluted by calibration with (0.05) standard silver nitrate using potassium chromate index (Kalra & Maynard, 1991). Total Carbohydrates (mg kg^{-1} dry weight): estimated as stated by (Ryan *et al*., 2002). Protein (%): estimated according to the dry weight rule (Ryan *et al*., 2002) according to the equation:

$$protein\% = \% N \times 6.25$$

Chlorophyll (mg.g $^{-1}$ fresh weight): Estimated according to (Ranganna, 1999).

Experiment design and static analysis

The experiment included 180 Grapefruit saplings grafted onto *Citrus aurantium* rootstock and placed in a lath house. A two-factor factorial experiment was implemented utilizing a randomized complete block design (Three saline levels and four biofertilizer treatments) and three blocks. The experiment contained 12 experimental units, with 5 saplings per each unit. The data were tested utilizing the statistical program (SPSS 25.00) software. Statistical differences between the averages were tested using Duncan's multiple range analysis (DMRT) at probability level of 0.05.

Results & Discussion

Physical parameters

Physical parameters (Table 1) show significant decrease with the increase in the water salinity. The salinity level at 6 ds m^{-1}

recorded the least average of stem diameter, plant height, total leaf area, branches number, shoots dry weight, main root length, and roots dry weight. These averages were 8.0 mm, 69.1 cm, 1204.0 $cm^2.plant^{-1}$, 4.7 branches $plant^{-1}$, 29.2 g. $plant^{-1}$, 18.9 cm, and 16.4 $g.plant^{-1}$, respectively. However, biofertilizers (Mycorrhiza, Azospirillum) mitigated physical parameters and reduced the harmful influences of irrigation with saline water. The combination of Mycorrhiza and Azospirillum was more effective in improving physical parameters including stem diameter, plants height, total leaf area, branches number, shoots dry weight, main root length, and roots dry weight. These parameters recorded 9.5 mm, 77.3 cm, 1880.0 $cm^2.plant^{-1}$, 6.3 branches $plant^{-1}$, 38.3 $g.plant^{-1}$, 22.5 cm, and 20.6 $g.plant^{-1}$, respectively.

The interaction between salinity and biofertilizers (2 $ds.m^{-1}$ with Mycorrhiza+ Azospirillum) increased physical parameters (stem diameter, plants height, total leaf area, branches number, shoots dry weight, main root length, roots dry weight). These averages were 10.0 mm, 84.2 cm, 2211.0 $cm^2.plant^{-1}$, 7.8 $branches.plant^{-1}$, 44.3 g. $plant^{-1}$, 24.2 cm, and 22.7 $g.plant^{-1}$, compared to the interaction 6 $ds.m^{-1}$ with 0 biofertilizers, respectively.

The decrease in physical indicators at 6 $ds.m^{-1}$ salt level could be associated to high-water effort of the soil solution. Increasing of water effort of soil solution cause inability of the plant to absorb water and reduces the turgor effort of plant cell which affects the closing and opening of stomata. It also affects the expansion and elongation of plant cell resulting reduce the shoot and root growth indicators (Ahmad *et al*., 2014). The accumulation of toxic ions, such as chloride and sodium give rise to reduce photosynthesis activity, which in turn, leads to a lack of effectiveness of growth-stimulating hormones such as cytokinins and gibberellins. The accumulation of chloride and sodium also activates growth-restricting hormones in the abscission zone, such as ethylene and abscisic acid. Moreover, it accelerates leaf aging and shedding, thus reducing the total leaf area (see table 1). Nitrogen is contribute in the chlorophyll synthesis (A & B). Moreover, Cl^- ions compete with NO^{-3} ions, which causes to reducing in N % in the plant's leaves, leading to reduce the chlorophyll content in the leaves and leaf area. (Baccari *et al*., 2020). Many researchers found that the decrease in chlorophyll coincided with increasing salinity of irrigation water (Balal *et al*., 2012; Josefa *et al*., 2013; Suhim *et al.,* 2023).

Biofertilization has a positive role in reducing the risk impact of salinity and increasing vegetative and root growth. The mechanism of action of biofertilizers in reducing the impact of salt stress is via improving the biological, chemical, and physical characteristic of the soil, especially water retention, as well as improving the soil texture and reducing the pH soil. Thus, it augmentation the availability of nutrients in the soil solution, which assists to increased plant development and its content of nutrients. Azospirillum bacteria and Mycorrhizal fungi contribute to the secretion of some compounds that regulate the physiological processes of plants. This improves the plants' ability to uptake water and nutrients reflect positively on physical parameters (Martinez-Medina *et al*., 2011).

Table (1): Influence of biofertilization on physical parameters of grapefruit saplings' leaves under salt stress.

Biofe.	salts ds m^{-1}	P.H. (cm)	S. D. (mm)	T.L.A. (cm^2 $Pl.^{-1}$)	B. N. (b. $pl.^{-1}$)	S.D.W. (g $pl.^{-1}$)	M.R.L. (Cm)	R.D.W. (g $plant^{-1}$)
0 biofe.	2	79.2a	8.0a	1846.0a	6.0a	38.5a	21.3a	18.5a
	4	75.8a	7.8a	1411.0a	5.0a	31.2a	20.5a	16.2a
	6	66.9a	7.2a	913.0a	4.2a	26.4a	17.6a	14.2a
Myc.	2	80.0a	9.0a	2131.0a	6.5a	40.4a	23.3a	21.4a
	4	75.9a	8.8a	1640.0a	5.7a	33.4a	22.4a	18.7a
	6	68.6a	8.3a	1179.0a	4.7a	28.6a	19.8a	17.2a
Az.	2	81.1a	9.0a	2138.0a	6.0a	41.6a	23.2a	20.5a
	4	76.8a	8.0a	1771.0a	5.3a	34.2a	21.0a	17.6a
	6	70.1a	7.7a	1253.0a	4.2a	29.5a	18.1a	15.7a
Myc.+ *Az.*	2	84.2a	10.0a	2211.0a	7.8a	44.3a	24.2a	22.7a
	4	76.6a	9.7a	1985.0a	7.0a	38.3a	23.3a	20.5a
	6	71.0a	9.0a	1471.0a	5.7a	32.3a	20.1a	18.6a
p-value		N. S.	N. S.	N. S.	N. S.	N. S.	N. S.	N. S.
Biof.	0 biof.	73.9a	7.6a	1390.0a	5.1a	32.0a	19.8a	16.3a
	Myc.	74.8a	8.7b	1650.0b	5.6a	34.1b	21.8b	19.1b
	Az.	76.0a	8.2bc	1720.6c	5.2a	35.1c	20.7bc	17.9c
	Myc.+ *Az.*	77.3a	9.5d	1880.0d	6.3b	38.3d	22.5d	20.6d
p-value		N.S	0.9	67.52	0.72	1.872	1.349	0.563
salts ds m^{-1}	2	81.1a	9.0a	2056.5a	6.6a	41.2a	23.0a	20.8a
	4	76.3b	8.6a	1701.8b	5.8b	34.3b	21.8b	18.3b
	6	69.1c	8.0b	1204.0c	4.7c	29.2c	18.9c	16.4
p-value		3.567	0.9	67.52	0.72	1.872	1.349	0.563

Note: Values in a column followed by the same letter are not statistically different at $p<0.05$. NS: not significant. Myc.: Mycorrhiza, Az.: Azospirillium, Biof. : Biofertilizers, PH.: Plant height, SD.: stem diameter, TLA. : Total leaf area, BN: Branches number, SDW. : Shoots dry weight, MRL. : Main root length, RDW. : Roots dry weight.

Azospirillum bacteria and mycorrhizal fungi contribute to stimulating the plant to increase the formation of plant hormones such as auxins, gibberellins and cytokinins, which stimulate cell division and elongation (Bottini *et al.*, 2004). The cytokine receives magnesium, potassium, iron, which contributes to the formation of chlorophyll molecules (Volkov & Beilby, 2017). Mycorrhizae also provide phosphorus in the rhizosphere, which the plant absorbs (Wu & Zou, 2010). Phosphorus involves in the synthesis of phospholipids, amino acids, proteins, and carbohydrates and is important in chlorophyll and plastids formation (Kumar *et al.*, 2022). Mycorrhizae fungi form a strong, large, deep root system and improve the surface area of the root system. It also secretes vesicular dendritic structures called hyphae, which contributes to increasing the absorption of nutrients (Azcon-Aguilar & Barae, 1997). Furthermore, Azospirillum bacteria fix atmospheric nitrogen, which is important in the production of chlorophyll, proteins, and amino acids (Ye *et al.*, 2020).

Biochemical parameters

The data in tables (2 and 3) showed a notable negative impact of irrigation with water salinity on biochemical parameters. High salinity (6 dSm^{-1}) decreased N, P, K, Fe, Zn, carbohydrates, protein, and chlorophyll content while increasing proline, Cl^-, and Na^+ levels.. Recorded averages were 2.18%, 0.41%, 1.60%, 39.7 mg.Kg $^{-1}$ dw, 27.9 mg.Kg $^{-1}$ dw, 3.9 mg Kg $^{-1}$ dw, 10.5%, 59.1 mg g $^{-1}$ fw. At the same level, an increase was observed in proline content and, Cl, and Na portion (5.7 mg.g^{-1} dw, 2.2%, 0.389%) respectively. Irrigated plants with 2 d.s. m^{-1} scored the highest precentage of N, P, K, Fe, Zn, and carbohydrates, protein, and chlorophyll content. Recorded averages were 2.77%, 0.55%, 2.40%, 45.7 mg.Kg $^{-1}$ dw, 33.4 mg.Kg $^{-1}$ dw, 5.1 mg.Kg $^{-1}$ dw, 12.2%, 71.5 mg.g $^{-1}$ fw. On the other hand, 2 dS.m^{-1} irrigated plants showed a reducing in proline content and proportion of Cl, and Na (3.7 mg.g^{-1} dry weight, 1.2%, 0.211%) respectively. Obtained results are consistent with previous studies (Abd *et al*., 2020; Hmiz & Ithbayyib, 2021; Mahdi *et al*., 2022; Abdulkadhim & Mortada, 2022). They found that the salinity of irrigation water caused a reduction in the content of nutrients, carbohydrates, chlorophyll and protein while increasing the levels of proline, chloride and sodium in plants.

The component of biofertilizers (Mycorrhiza + Azospirillum) significantly increased all biochemical traits indicators (N, P, K, Fe, Zn, carbohydrates, protein, and chlorophyll) in leaves. Recorded averages were 2.74%, 0.56%, 2.366%, 44.4 mg.kg^{-1} dw, 33.3 mg.kg^{-1} dw, 5.3 mg.kg^{-1} dw, 12.9%, and 70.1 mg.g^{-1} fw, while the control scored the lowest values 2.06%, 0.38% and 1.60%, and 40.2 mg/kg dry weight respectively. Mycorrhiza + Azospirillum caused a significant decrease in proline, Cl%, and Na%, 4.1, 1.3, 0.260, while the control scored the lowest values 5.6, 2.1 and 0.341, respectively. The interaction between salinity and biofertilizers (2 ds m^{-1} with Mycorrhiza+ Azospirillum) increased all biochemical parameters (N, P, K, Fe, Zn, carbohydrates, protein, and chlorophyll). These averages were 3.07%, 0.62%, 2.76%, 35.8 mg.kg^{-1} DW, 6.0 mg.kg^{-1} DW,14.2 %, and 76.3 mg.g^{-1} fw. While proline, Cl%, and Na% decreased significantly (3.1, 0.8, 0.172), compared to the interaction 6 ds m^{-1} with 0 biofertilizers, respectively.

The decrease in biochemical parameters is due to the increase in the osmotic potential of the soil solution, which leads to difficulty in uptake water and nutrients including nitrogen, phosphorus, potassium, zinc and iron (Volkov & Beilby, 2017). Direct negative effects of salinity occur through competition of Cl^- against NO^{-3}, which leads to decreased nitrate absorption. Indirect negative effects of salinity occur by changing the permeability properties of plasma membranes through affecting of membrane proteins. Cl^- ions present in saline irrigation water compete with HPO_4^{-2}. This in turn reduces the movement of phosphorus through the root system to the shoots, causing a decrease in P% in the leaves. Na^+ ions present in saline water compete with $^{K+}$ ions on absorption sites in roots, results in decreased K^+ absorption (Obead & Jerry, 2019). The salinity increases soil alkalinity, causing the formation of Fe and Zn complex compounds and preventing their absorption by roots.

Plants exposed to abiotic stresses cause increase the formation of some nitrogenous compounds such as proline as an adaptive method. Proline regulates osmotic pressure by protecting the enzymes from the risks of oxidative stress (Boguszewska & Zagdańska, 2012). Plants also use proline as a nitrogen resource (Hayat *et al*., 2012). Furthermore, the accumulation of $NaCl_2$ in the root zone leads to increase the absorption of Cl% and Na% by the roots (Abdulkadhim & Mortada, 2022).

Table (2): Influence of biofertilization on biochemical parameters and ion uptake in grapefruit saplings' leaves under salt stress.

Biof.	salts ds m $^{-1}$	N %	P %	K %	Fe (mg. kg^{-1} dw)	Proline (mg g^{-1} dw)	Cl %	Na %
0 biof.	2	2.13a	0.42a	1.73a	43.4a	4.6a	1.5a	0.247a
	4	2.28a	0.40b	1.97b	39.6a	5.8a	2.3a	0.352b
	6	1.78b	0.31c	1.12c	37.5a	6.5c	2.6a	0.424c
Myc.	2	3.01c	0.61d	2.46d	45.6a	4.0ab	1.0a	0.201ab
	4	3.05c	0.54e	2.43d	42.4a	5.1e	1.7a	0.291abc
	6	2.39c	0.42e	2.43d	39.4a	5.8f	2.0a	0.386d
Az.	2	2.89d	0.53f	2.66e	46.4a	3.4a	1.3a	0.223abcd
	4	2.94d	0.61g	2.57e	43.5a	4.6a	1.9a	0.337e
	6	2.35d	0.43e	1.78ab	40.2a	5.3b	2.3a	0.406f
Myc.+ *Az.*	2	3.07e	0.62g	2.76f	47.4a	3.1ab	0.8a	0.172abc
	4	2.95e	0.60gh	2.57g	44.2a	4.2ab	1.4a	0.269abc
	6	2.21ab	0.48i	1.77a	41.5a	5.0f	1.8a	0.340d
p-value		0.166	0.023	0.061	N.S	0.113	N.S	0.012
Biof.	0 biof.	2.06a	0.38a	1.60a	40.2a	5.6a	2.1a	0.341a
	Myc.	2.82b	0.53b	2.45b	42.5b	5.0b	1.6b	0.293b
	Az.	2.73b	0.52b	2.336c	43.4c	4.4c	1.8c	0.322c
	Myc.+ *Az.*	2.74b	0.56c	2.366d	44.4d	4.1c	1.3d	0.260d
p-value		0.096	0.014	0.035	0.244	0.057	0.11	0.006
Salts dsm $^{-1}$	2	2.77a	0.55a	2.40a	45.7a	3.7a	1.2a	0.211a
	4	2.81b	0.54b	2.39a	42.4b	4.9b	1.8b	0.312b
	6	2.18c	0.41c	1.60b	39.7c	5.7c	2.2c	0.389c
p-value		0.083	0.012	0.031	0.244	0.057	0.11	0.006

Note: Values in a column followed by the same letter are not statistically different at $p<0.05$. NS: not significant. Myc.: Mycorrhiza, Az.: *Azospirillium,* Biof. : Biofertilizers, Pro. : Proline, d.w: dry weight.

Table (3): Influence of biofertilization on biochemical parameters and ion uptake in grapefruit saplings' leaves under salt stress.

Biofertilizers	salts ds m $^{-1}$	Zn (mg kg $^{-1}$ dry weight)	Carb. (mg kg $^{-1}$ dry weight)	Protein (%)	Chl. (mg g $^{-1}$ fresh weight)
0 biof.	2	31.4a	4.5a	10.3a	65.4a
	4	28.4b	3.9b	10.2a	58.4a
	6	24.3c	3.2c	9.4b	52.5a
Myc.	2	32.1a	4.8a	11.6c	70.0a
	4	29.8d	4.0dbc	10.7a	62.6a
	6	26.6bc	3.5ce	10.3a	57.4a
Az.	2	34.2e	5.2f	12.7d	74.1a
	4	31.9f	4.4abc	11.4d	67.1a
	6	29.3bc	4.1abc	10.7a	62.5a
Myc.+ *Az.*	2	35.8abcd	6.0abcde	14.2e	76.3a
	4	32.9ab	5.2g	12.9d	69.8a
	6	31.3ab	4.8abc	11.8b	64.2a
p-value		0.326	0.326	0.339	N.S
Biof.	0 biof.	28.0a	3.8a	9.9a	58.8a
	Myc.	29.5b	4.1b	10.9b	63.3b
	Az.	31.8c	4.5c	11.6c	67.9c
	Myc.+ *Az.*	33.3d	5.3d	12.9d	70.1d
p-value		0.163	0.163	0.169	1.553
Salts ds m $^{-1}$	2	33.4a	5.1a	12.2a	71.5a
	4	30.7b	4.4b	11.3b	64.5b
	6	27.9c	3.9c	10.5c	59.1c
p-value		0.163	0.163	0.169	1.553

Note: Values in a column followed by the same letter are not statistically different at $p<0.05$. NS: not significant. Myc.: Mycorrhiza, Az.: *Azospirillium,* Biof. : Biofertilizers, Carb. : Carbohydrates, Chl.; chlorophyll.

Biofertilizers (Mycorrhiza+Azospirillum) in table (2) caused an improved the content of nutrient in the leaves (N, P, K, Fe, and Zn). Mycorrhizal fungi grow by the proliferation of vesicular dendritic structures called hyphae. These hyphae cause the absorption of P ions located away from the rhizosphere. Moreover, it plays a role in releasing P, Fe, Mn, and Zn from their insoluble forms, and stimulating K^+ absorption (Wu & Zou, 2010; Wu, 2011).

Beneficial bacterial genera are distinguished by their efficiency in secreting some important enzymes such as proteases, phosphatases, and dehydrogenases. Moreover, it secretes hormones such as gibberellins, auxins and cytokinins, which improve the absorption of nutrient by plant roots (Bottani *et al.*, 2004; Spaepen *et al.*, 2008). Beneficial microorganisms are characterized by their efficiency to enhance the biological and chemical properties of the soil. They also fix atmospheric nitrogen, which is important in the synthesis of proteins and amino acids. These changes reflect positively on the physiological processes within plant cells and its resistance to salt stress. (Ye *et al.*, 2020). These results are consistent with (Haran & Thaher, 2019; Fadhil & Abdulkadhim, 2020; Suhim *et al.*, 2023; Abdulkadhim & Hussein, 2023; Hashem & Abdulkadhim, 2024). They found that biofertilizers contribute to increasing the content of nutrients, carbohydrates, protein and chlorophyll while reducing the levels of proline, Cl and Na in citrus and other plants.

Conclusion

Irrigation with 2 ds m^{-1} saline water supported good shoot and root growth in grapefruit saplings without nutrient deficiency symptoms Higher salinity levels (4 and 6 $dS \cdot m^{-1}$) negatively impacted biochemical parameters and ion uptake, leading to increased Na^+, Cl^-, and proline accumulation due to osmotic stress. Biofertilization (Mycorrhiza + Azospirillum) significantly improved growth, biochemical parameters, and ion uptake, while reducing proline, Cl^-, and Na^+ accumulation, demonstrating its effectiveness in mitigating salinity stress.

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Contributions of authors

S.J.A.: I constructed the idea, executed the study, captured and analyzed the data, and wrote the manuscript.

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Conflicts of interest

There are no conflicts of interest associated with this manuscript.

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تأثير التسميد الحيوي في المؤشرات الفيزيائية والكيميائية الحيوية لشتلات الجريب فروت *Citrus paradisi* Macfad. تحت الاجهاد الملحي

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المستخلص: أجريت التجربة في الظلة النباتية (50٪ ظلة شبكية) طوال موسم النمو 2023. هدفت هذه التجربة إلى دراسة دور التسميد الحيوي في زيادة تحمل شتلات الجريب فروت للإجهاد الملحي. تم تطبيق الأسمدة الحيوية بأربعة مستويات (بدون أسمدة حيوية، فطريات المايكورايزا، بكتيريا، والمايكورايزا + بكتيريا). تمت إضافة 20 جم من فطريات المايكورايزا بالقرب من جذور الشتلات. تم حقن 20 مل من البكتيريا في التربة وعلى اتصال بمنطقة الجذر. تم تنفيذ معاملة فطريات المايكورايزا + البكتيريا بإضافة 20 جم من فطريات المايكورايزا + 20 مل من البكتيريا إلى تربة الأصيص في نفس الوقت وبالقرب من جذور الشتلات. تم ري الشتلات بثلاثة مستويات من الماء المالح (2، 4، 6 ديسيسمينز. م$^{-1}$ كلوريد الصوديوم). أشارت النتائج إلى أنه عند مستوى 6 ديسيسمينز. م$^{-1}$ حدث انخفاض في المؤشرات الفيزيائية والكيميائية، ويصاحب هذا الانخفاض زيادة في نسبة البرولين وكلوريد الصوديوم، كما أظهرت معاملة فطريات المايكورايزا +بكتيريا *Azospirillium brasilsnee* تفوقاً معنوياً في نمو المجموع الخضري والجذري، كما أدى التسميد الحيوي إلى زيادة محتوى الكربوهيدرات والبروتين والكلوروفيل في النباتات، كما أظهرت النتائج المتحصل عليها زيادة محتوى بعض العناصر الكبرى والصغرى في النباتات مثل النيتروجين والفوسفور والبوتاسيوم والحديد والزنك، فضلاً عن انخفاض نسبة الكلوريد والصوديوم والبرولين مما أدى إلى تقليل أضرار الإجهاد الملحي.

الكلمات المفتاحية: الإجهاد اللاحيوي، البكتيريا، امتصاص الايونات، المايكورايزا، البرولين.