Growth and yield of maize (*Zea mays* L.) treated with bio-fertilizer andirrigated with saline water*

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Abstract

A field experiment was carried out during the growing seasons of 2021 at agricultural research station of the college of Agriculture - University of Basrah, to study the effect of biofertilizers for *Azotobacter* and *Mycorrhizae* (locally prepared and ready-made) on the growth and yield of maize irrigated with various levels of salinity (2, 4 and 8 dS m^{-1}) and nine treatments of fertilization: B0 (control: without bio or mineral fertilizer), B1 (local isolate of *Azotobacter chroococcum:* LA), B2 (local isolate of Mycorrhizae: *Glomus* spp: LM), B3 (LA + LM), B4 (LA + LM + 50% of the fertilizer recommendation :FR), , B5 (ready-made *Azotobacter*: RA + ready-made Mycorrhiza: RM + 50% FR), B6 (LA+ LM+75% FR), B7 (RA+ RM +75% FR) and M (100% FR).

The experiment was designed as a factorial experiment using the randomized complete block design (R.C.B.D.) with three replications, by arranging split-plot, where the salinity levels were placed in the main plots and the fertilizer levels in the sub-plots. The results showed that the high salinity of the irrigation water led to a decrease in most of the plant growth indicators as well as a decrease in the concentrations of NPK nutrients. Regarding the effect of fertilization, the results indicated that the addition of bio-fertilizer led to a significant increase in the concentration of NPK in maize leaves, also increase the height, leaf area, dry weight, grain yield, and biological yield. On the other hand, biofertilizer significantly reduced the harmful effects caused by the high level of salinity of irrigation water and caused a significant reduction of sodium and increased the ratio of potassium to sodium in theleaves compared to control and 100% FR. The best results were achieved when using the bio-fertilizer (*A. chroococcum* + *Glomus spp.*) with 75% FR.

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Introduction

Salinity of soil and irrigation water is a global problem that affects many lands of arid and semi-arid regions, and it can affect all stages of plant development and growth, which leads to a significant reduction in crop yields (Qadir *et al*.,2014). The FAO has diagnosed that about 20% of the irrigated land is affected by salinity, which causes an osmotic effect that makes the roots more difficult to absorb water, and toxicity to the plant through the accumulation of high concentrations of ions that compete with other ions for absorption sites in the plant (Munns and Tester 2008). In addition, many biochemical processes are affected, including protein synthesis, photosynthesis and metabolism (Parida and Das 2005, Al-Hasany, et al,2020). The effect of salinity varies according to the stage of growth, plant type, humidity, temperature, light, management practices and soil fertility level. Many strategies have been applied to reduce the harmful effects of the high level of salinity, including the biological methods that have been adopted in recent years as an inexpensive and practical way to reduce the salt stress in the soil. They are effective, environmentally friendly microbial strains that are added as biological inoculants (bacteria or fungi or both) to the plant growth medium in order to increase the availability and absorption of nutrients and water, raise the efficiency of mineral fertilizers and reducing the harmful effect on plantsand environment.

The Arbuscular Mycorrhiza fungi AMF naturally present in saline soils and coexist with most plants (Abdel-Fattah *et al*., 2012), and the nitrogen-fixing bacteria *Azotobacter* have proven their efficiency in stimulating plant growth and resistance to environmental conditions through a variety of mechanisms, directly or indirectly, by increasing the availability of plant nutrients in the soil, such as fixing atmospheric nitrogen, increasing the solubility of unavailable phosphorous, and producing plant hormones such as (IAA) Indole Acetic acid, , cytokinines, gibberellins and others, as their use has become promising biotechniques to mitigate the effects of stress caused by salinity and a significant reduction in chemical fertilizer levels in addition to the plant's tolerance of some plant pathogens (Kumar *et al.*, 2021, Noaema,et al, 2020)

The combined inoculation (*Azotobacter* + mycorrhizal fungi) achieved a significant positive effect in all phases of plant growth and enhanced vegetative and root growth compared to non-inoculant plants (Behl *et al.,* 2007, Al-Hasany, et al,2021).

Maize (*Zea Mays* L.) is one of the important food and industrial grain crops, comes third after wheat and rice in terms of cultivated area, economic importance and global production. The results of Shuppar and Kamal (2021) study confirmed that inoculation of maize seeds with biofertilizers, either single or combined (*Azotobacter* + Mycorrhizal fungi), achieved a significant increase in the crop growth indicators compared to plants not inoculated with biofertilizer. This study aims to identify the role of biofertilizer in increasing the tolerance of maize plants irrigated with saline water and its effect on improving some growth characteristics and yield of inoculated plants.

Material and Methods

Soil samples and roots of different plants were collected from the rhizosphere of several regions of Basrah Governorate, Placed in sterile bags. All information was recorded, transferred to the laboratory and kept in the refrigerator at a temperature of 4° C until experiments were carried out.

Isolation and identification of *Azotobacter*

Azotobacter was isolated, phenotypic, cultured, microscopic and biochemical tests were performed on it, and it was diagnosed as *Azotobacter chroococcum* as a local isolate (Black 1965). The isolate was tested for atmospheric nitrogen fixation . The isolate has been preserved as Slant, kept in the refrigerator at 4°C until use. The isolates were activated monthly.

Isolation and identification of the fungus Mycorrhiza

Mycorrhizal fungi were isolated from the selected samples by wet sieving and decanting method described by Gerdemann and Nicolson (1963) and their spores were obtained. The numbers of spores were calculated after being isolated in petri dishes and the roots were dyed with Acid Fuchsin dye to ascertain the infection rate according to (Phillips and Hyman 1970). It contained fungal spores, infected roots and soil. It was multiplied by the cultivation of barley. The soil and roots were examined to ensure the presence of spores and the occurrence of infection. The roots were cut into small pieces and mixed with the soil, kept in acool and dry place as a local biological inoculum until the field experiment was carried out. As for the tested ready fungal inoculant, it was obtained from the Agricultural Research Department - Ministry of Science and Technology - Baghdad. It contains infected roots, spores and dry soil of *Glomus spp*.

Preparing the seeds for planting

Maize seeds was fumigated with arabic gum suspension. The bacterial inoculum was carried on sterilized peatmoss at a rate of 5 kilograms per liter of inoculum. Mix it well for half an hour in the shade away from sunlight before adding it to the field experiment for half an hour.

field operations

Before planting, soil samples were taken randomly from different locations of the field at a depth of 0-30 cm., some chemical, physical and biological analyzes were performed (Table 1) according to the methods mentioned .

Table 1: Some chemical, physical and biological properties of the soil before planting

Azotobacter	Total fungus	Total bacteria	Organic matter	total solid carbonate	CEC	ECe	pH)1:1(
Cfu g^{-1} soil			g kg^{-1} soil	g kg^{-1} soil	Cmol (+) kg^{-1}soil	dS m^{-1}	—
0.97×10^3	2.2×10^4	6.1×10^7	6.80	320	10.43	6.90	7.80

clay	Silt	sand	texture	Mg^+	Ca^+	K^+	Na^+	K	P	N
g Kg^{-1} soil			-	dissolved ions mmol l^{-1}				mg kg^{-1} soil		
134	300	566	Sandy loam	9.90	14.20	2.69	40.65	175	10	56

The land was divided into three blocks, each block divided into three equal main plots, which divided into nine experimental unit with dimensions of 3 x 3 m^2 . Before planting, the bacterial inoculant (a mixture of peat moss with bacterial pollen) was added at a rate of 10 g per hole. The mycorrhizal inoculum consisting of (soil + spores + infected roots) was added previously to the treatments in the form of a pad, as 10 g of the inoculant was placed at a depth of 5 cm under the seeds.

As for the fertilizer recommendation (100%), nitrogen fertilizer was added in the form of urea at a rate of 200 kg ha^{-1} (Huthily *et al*., 2020) in two batches (at planting and after a month of germination), phosphate in the form of concentrated superphosphate at a rate of 100 kg P ha^{-1} in one batch, and potassium in the form of potassium sulfate (200 kg ha^{-1}).

The plants were first irrigated with river water, and after the appearance of the first two true leaves, irrigation was started according to the irrigation water salinity factor (2, 4 and 8 dS m^{-1}).

Table 2: Some characteristics of the irrigation water used in the study

treatments	**E.C.**	**pH**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**K^+**	**Cl^-**	**HCO3 =**	**$SO_4^=$**	Water type
	dS m^{-1}		**Dissolved ions (meq l^{-1})**							
S1	**2**	**7.60**	**4.90**	**4.10**	**8.64**	**0.41**	**10.00**	**1.35**	**2.70**	**C3**

S2	**4**	**7.52**	**5.35**	**9.95**	**16.94**	**0.85**	**25.20**	**1.45**	**4.12**	**C4**
S3	**8**	**7.41**	**10.30**	**22.11**	**28.20**	**1.84**	**52.24**	**1.95**	**8.35**	**C6**

The data were analyzed statistically (analysis of variance) using the statistical program SPSS version 23, and the means were compared using the least significant difference test at a probability level of 0.05.

Results and discussion: -

The concentration of nitrogen, phosphorous and potassium in leaves

The results of the statistical analysis and the data in Table 3 indicated that there was a significant effect of the combined biofertilization treatments (A. *chroococcum* bacteria + *G. spp*. fungus supported by 75% of FR) on the concentration of N, P and K in maize leaves. The treatment of B6 and B7 gave the highest means for N, P and K, which were (2.62, 2.49%), (0.31, 0.29%) and (3.97, 3.74%), respectively, recorded an increase by (15.4, 9.7%), (19.2, 11.5%) and (24.1, 16.9%), respectively, compared to the treatment M, which gave an average of 2.27%, 0.26% and 3.20% for N, P and K respectively, which did not differ significantly from the treatments of B4 and B5. The B0 treatment recorded the lowest value for N, P, and K concentration, which amounted to 1.45%, 0.16% and 1.42%, respectively. The increase in NPK concentration by using B6 and B7 treatments may be due to the biological synergy between the organisms used (bacteria and fungi) which increase the availability of nitrogen, phosphorous and potassium and encourage the plant roots to absorb them and other nutrients. Several studies have shown the possibility of *Azotobacter* to increase nitrogen fixation in the soil and secrete some growth stimulantsand reduce the pH of soil, in addition to increasing the absorption area of the roots by Mycorrhizae, which has nonpathological and beneficial relationship with many plants. It improved the process of dissolving phosphorus and increased nutrients absorption (Hayat *et al*., 2010). Biofertilizers increase the effectiveness and efficiency of mineral fertilizers, so they are considered a complement to mineral fertilizers and reduce their amounts added to the soil.

Table 3: Effect of biofertilizer and irrigation water salinity on the concentration of nitrogen, phosphorous, potassium and sodium in maize leaves.

Nutrient	Water salinity	Fertilizers treatments									The mean
		B0	M	B1	B2	B3	B4	B5	B6	B7	
N	**2**	1.78	2.93	2.25	2.00	2.58	2.88	2.72	3.36	3.27	**2.64**
	4	1.42	2.27	2.10	1.76	2.25	2.36	2.30	2.77	2.63	**2.21**
	8	1.14	1.60	1.23	1.18	1.37	1.64	1.47	1.73	1.57	**1.44**
The mean		**1.45**	**2.27**	**1.86**	**1.65**	**2.07**	**2.29**	**2.16**	**2.62**	**2.49**	
P	**2**	0.18	0.30	0.19	0.21	0.22	0.31	0.30	0.35	0.33	**0.27**
	4	0.17	0.26	0.18	0.20	0.21	0.28	0.27	0.32	0.30	**0.24**
	8	0.13	0.23	0.14	0.16	0.18	0.23	0.22	0.27	0.24	**0.20**
The mean		**0.16**	**0.26**	**0.17**	**0.19**	**0.20**	**0.27**	**0.26**	**0.31**	**0.29**	

K	**2**	1.97	3.74	2.73	3.10	3.60	4.31	4.20	4.92	4.60	**3.69**
	4	1.44	3.21	2.30	2.45	3.00	3.81	3.60	4.24	4.02	**3.12**
	8	0.84	2.64	1.37	1.41	1.72	2.23	2.31	2.74	2.59	**1.98**
The mean		**1.42**	**3.20**	**2.13**	**2.32**	**2.77**	**3.45**	**3.37**	**3.97**	**3.74**	
Na	**2**	2.06	0.95	1.45	1.33	1.16	0.50	0.54	0.40	0.47	**0.98**
	4	2.11	1.01	1.53	1.44	1.24	0.77	0.79	0.66	0.72	**1.14**
	8	2.70	1.42	2.07	1.89	1.80	1.28	1.29	1.03	1.11	**1.62**
The mean		**2.29**	**1.13**	**1.68**	**1.55**	**1.40**	**0.85**	**0.87**	**0.70**	**0.77**	
LSD (P < 0.05)				Fertilizers		Water salinity			interaction		
		N		0.19		0.11			0.33		
		P		0.019		0.011			0.034		
		K		0.34		0.20			0.59		
		Na		0.089		0.051			N.S.		

The data in Table 3 showed that the increase in the level of salinity of irrigation water had a significant effect and led to a decrease in the percent of N, P and K in maize leaves, as it decreased from2.64, 0.27 and 3.69% at irrigation level 2 dS m^{-1} (S_1) to (2.21 and 1.44%), (0.24 and 0.20%) and (3.12 and 1.98%) for levels S_2 and S_3 respectively.

The negative effect of salinity is due to the increase in osmosis and the reduction of water and nutrient absorption and the competition of salt ions, which causes an increase in the accumulation of sodium and chloride and some other ions (Parida and Das, 2005), Also, the toxicity of some ions leads to an inhibition in many biochemical processes, especially the decrease in the net rate of photosynthesis and CO_2 metabolism. This is consistent with what was obtained by Hassan and Al-Ansari (2016), who noted that the increase in soil salinity led to a significant decrease in the level of nitrogen and phosphorous absorbed in maize grown in soil exposed to salinity levels of 3 - 24 dS m^{-1}.

The results also showed a significant effect of the interaction between the biofertilizer application and the salinity levels of irrigation water in the percent of N, P and K. In general, it is noted that there isa positive effect of the bio-fertilizer in reducing the negative effect of salinity in many growth indicators, especially the treatments of the double-bio-fertilizers supplemented. Treatment S_1B_0 gave the highest values for N, P and K concentrations, which were 3.36, 0.35 and 4.92%, respectively, while treatment S_3B_0 gave the lowest values. In general, it is noted that the concentration of N, P and K decreased in maize leaves for all treatments under study, with an increase in the salinity level of the irrigation water.

As for sodium, the results in Table 3 showed that B_6 and B_7 treatments gave the lowest value (0.70 and 0.77% respectively) compared to 2.29, 1.13, 1.68 and 1.55% in B_0, M, B_1 and B_2 respectively. This is in agreement with Mahato and Kafle (2018).

There was a significant increase in the sodium concentration in maize leaves with an increase in the salinity of the irrigation water, as it

increased from 0.98 percent for irrigation treatment S1 to 1.14 and
1.62 percent for S2 and S3, respectively. This result agrees with what was obtained by Fortmeier and Schubert (1995), which also indicated that sodium is the main anion that causes the toxic effect under saline stress conditions for maize crop.

K / Na ratio in maize leaves:

The results of Figure 1 showed that the addition of the double biological inoculant supplemented with mineral fertilizer achieved a significant increase in the ratio of K / Na compared to control. Treatment B6 recorded the highest values of 7.14, followed by treatment B7, which gave 5.91, while the lowest values were recorded at control (0.65). The fertilizer recommendation treatment (M) recorded 2.99. The treatments of bio-fertilizer B4 and B5 recorded higher values than the fertilizer recommendation (M), and the percentages were 5.12 and 4.74, respectively. This is due to the possibility of biological inoculant in increasing the concentration of potassium and decreasing the concentration of sodium (Table 3). The results of (Lahamo *et al.*, 2022) confirmed the ability of organisms to produce plant growth hormones and osmotic protection materials such as the accumulation of sugars and proline and the production of Exopolysaccharides (ESP) that bind sodium cations and restrict their uptake in the root and reduce their competition with potassium and that increased the K/Na ratio compared with non-inoculated plants.

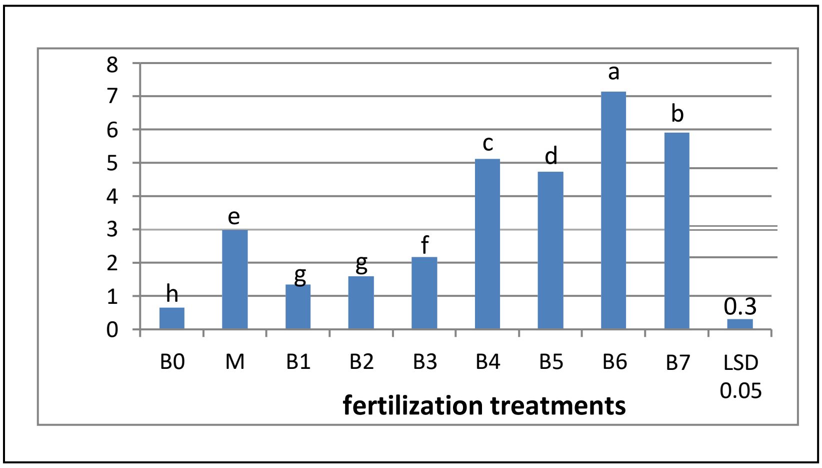

Figure 1: Effect of Fertilization on K/Na Ratio

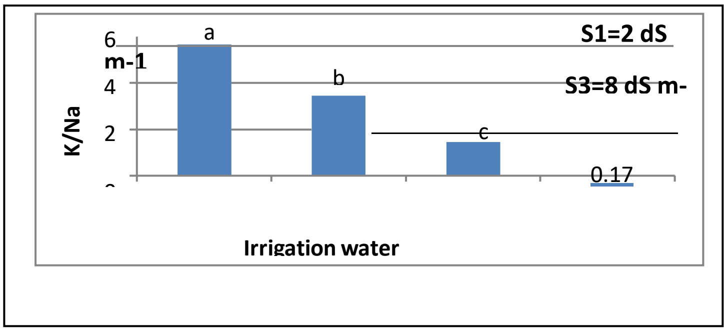

Figure 2: Effect of salinity of irrigation water on the ratio of K / Na

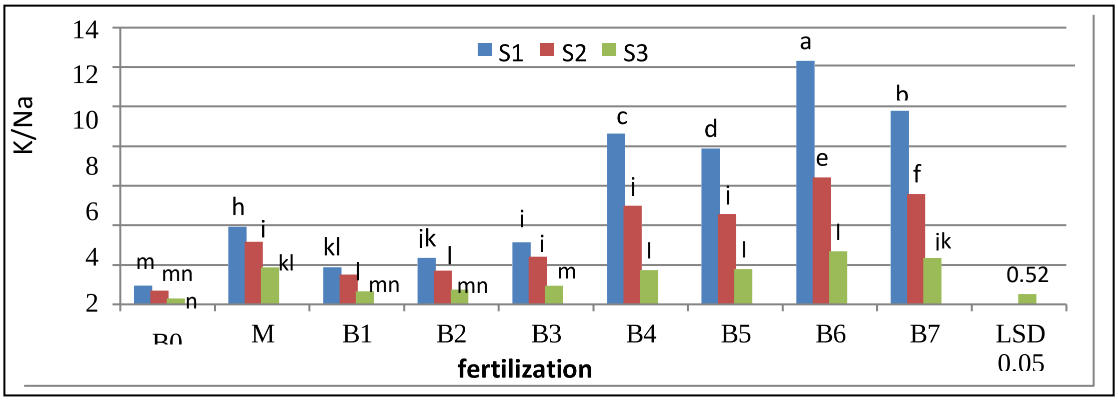

Figure 3: The effect of the interaction between biofertilizer and the level of salinity of irrigation water onthe ratio of K / Na

Figure 2 shows that the increase in the salinity level of the irrigation water led to a significant decrease in the K / Na ratio in maize leaves, as it decreased from 5.65 for irrigation treatment S1 to 3.45 and 1.46 by the effect of irrigation treatments S2 and S3, respectively. These results agree with Azevedo and Tabosa (2000) who indicated that there was competition in absorption between sodium and potassium by increasing the salt stress, which significantly reduced the potassium concentration in both the leaves and roots of the maize crop. In contrast, the sodium concentration increased.

As for Figure 3, there is a significant effect of the interaction between the fertilization treatments and the levels of salinity of the irrigation water. The highest ratio of K/Na was 12.31 with the effect of treatment B6 and irrigation with water with salinity of 2 dS m^{-1} (S1B6), while the lowest ratio was 0.313 for the interaction (S3B0). The reason here is attributed to the synergistic effect of bacteria associated with mycorrhizal fungi (AMF) in the rhizosphere, which both strengthened biological protection against the effects of different abiotic stresses on the plant, which enhanced the absorption of nutrients in a balanced manner. And the increase in the ratio of potassium to sodium is one of the important indicators of the salt tolerance of the plant (Santoyo *et al.*, 2021). Some researchers also attributed it to the ability of the mycorrhizal fungi to reduce the concentration of sodium ion in the plant in general and in the vegetative group in particular, which increased the ratio of K/ Na in plant tissues. This is due to its production of many substances that act as osmotic regulators, including glutamate, glycine, proline, proline and polysaccharides (Minakshi *et al.*, 2011),

Plant height (cm) and leaf area(cm^2):

The results of Table 4 showed that all biological and mineral fertilization treatments were significantly superior compared to control. B6 and B7 treatments outperformed all other treatments. They gave the highest plant height of 164.9 and 155.0 cm with an increase by 81.6 and 70.7 % compared to the control treatment (B0) which gave 90.8 cm. They also recorded the highest leaf area reached to 5853 and 5677 cm^2, respectively, compared with 3001 cm^2 in the control treatment (B0). There is no significant differences were shown between treatment (M) and treatment B7 in their effect on plant height, also, the combined biofertilizer (B3) outperformed the bacterial and fungal single biofertilizer treatments (B1 and B2). This may be attributed to the fact that the addition of biological inoculants led to an increase in the concentration of nutrients N, P and K and reduced the amount of Na in the leaves (Table 2) in quantities that encourage the activity and action of enzymes, which improved the process of photosynthesis and increased the plant's ability to assimilate nutrients and convert them into carbohydrates that helped increase plant growth.

There was a significant decrease in plant height and leaf area with an increase in the level of salinity of irrigation water, as the averages decreased from 149.3 cm and 5368 cm^2 for irrigation treatment S1 to (136.7 and 104.2) cm and (5064 and 4204) cm2 for irrigation treatments S2 and S3, respectively. Increasing the salinity of irrigation water reduces the absorption of water and nutrients and causes an imbalance in the hormonal balance, enzymes and metabolism, which negatively affects plant growth(Emdad and Fardad 2000). The effect of the interaction between fertilization and salinity was significant in the leaf area, and the B6S1 factorial treatment recorded the highest value of 6432 cm2 superior to all other interactions.

Table 4: Effect of biofertilizer and irrigation water salinity on plant height (cm) and leaf area (cm^2) of maize

grain yield and biological yield (tons ha^{-1}) :

Plant character	Water salinity	Fertilizers treatments									The mean
		B0	M	B1	B2	B3	B4	B5	B6	B7	
plant height	**2**	100.0	170.3	120.3	116.7	141.3	165.4	156.0	194.0	180.0	149.3
	4	92.0	158.0	110.4	102.3	132.4	150.7	140.5	179.0	165.1	136.7
	8	80.4	113.0	95.2	96.0	99.1	109.0	103.5	121.7	120.0	104.2
The mean		90.8	147.1	108.6	105.0	124.3	141.7	133.3	164.9	155.0	
leaf area	**2**	3234	5870	4954	4888	5191	5832	5810	6432	6104	5368
	4	3059	5492	4684	4591	5109	5344	5327	6060	5907	5064
	8	2710	4601	3928	3877	4094	4321	4216	5067	5021	4204
The mean		3001	3521	4522	4452	4798	5166	5118	5853	5677	
LSD (P < 0.05)				Fertilizers		Water salinity		interaction			
		plant height		13.7		7.9		N.S.			
		leaf area		185		107		320			

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The results of the statistical analysis and the data in Table 5 showed that the use of biological and mineral fertilizers contributed to a significant increase in grain yield and biological yield, as the local and ready-made joint bio-fertilizer treatment (Azotobacter + Mycorrhizal fungi) +75% of the fertilizer recommendation (B6 and B7) outperformed all treatments.

It gave averages of 5.29 and 5.05 ton ha^{-1} for grain yield and (14.92 and 13.85) ton ha^{-1} for biological yield, respectively. The fertilizer recommendation (M) did not differ significantly from the two treatments (B4 and B5), and the grain yield averages (4.09, 3.94, and 3.89) tons ha^{-1} and the biological yield (11.97, 11.45 and 11.03) tons ha^{-1}, respectively,

The combined bio-fertilizer (B3) treatment was also superior to the single bacterial (B1) and fungal (B2) bio-fertilizer treatments, all of which were superior to the control treatment B0 which gave the lowest value for grain yield (1.89 tons ha^{-1}), and for the yield Bio (6.84 tons ha^{-1}),This is due to the synergistic effect between organisms (nitrogen-fixing bacteria and mycorrhizae) and their important role in improving some growth characteristics (Table 3), which was positively reflected in improving and increasing yield components (data not published here), and this in turn was also positively reflected in increasing the total seed yield and biological yield. These results are consistent with what was found by Shuppar and Kamal (2021).

The results in Table 5 also showed that the increase in the level of salinity of irrigation water led to a significant decrease in the average total grain yield and biological yield, as the grain yield decreased from 4.51 tons ha^{-1} for irrigation treatment S1 to 3.84 and 2.71 tons ha^{-1} for treatments S2 and S3 with a decrease of 15% and 40%, respectively

The biological yield also decreased from 12.87 tons ha^{-1} for irrigation S1 to 11.63 and 8.06 tons ha^{-1} for treatments S2 and S3, with a significant decrease of 9.6% and 37.4%, respectively. This is due to the exposure of the maize plant to salt stress, especially the increase in sodium in the plant tissues, which caused a decrease in the rate of absorption of plant nutrients, moisture content and photosynthesis, and consequently, a decrease in plant cell division and inhibition of plant growth which negatively affected the height of the plant and its leaf area, and then on the components of the yield and yield.

The interaction between bio-fertilizer and irrigation water salinity levels had a significant effect on the trait of total grain yield and biological yield, as the S1B6 treatment outperformed all treatments and gave a value of 6.69 tons ha^{-1} for total yield and 17.55 tons ha^{-1} of biological yield. Whereas, the treatment of S3B0 achieved the lowest values of 1.28 tons ha^{-1} and 5.85 tons ha^{-1} for the studied traits, respectively,

Table 5: Effect of biofertilizer and irrigation water salinity on Grain yield (ton ha^{-1}) and biological yield (ton ha^{-1}) of maize

Plant character	Water salinity	Fertilizers treatments									The mean
		B0	M	B1	B2	B3	B4	B5	B6	B7	
Grainyield	**2**	2.55	4.81	3.58	3.42	4.09	4.47	4.45	6.69	6.34	4.51
	4	1.83	4.45	2.99	2.63	3.64	4.26	4.15	5.41	5.17	3.84
	8	1.28	3.02	1.93	1.72	2.88	3.10	3.07	3.76	3.64	2.71
The mean		1.89	4.09	2.83	2.59	3.54	3.94	3.89	5.29	5.05	
Biological yield	**2**	7.77	14.76	9.83	10.11	11.59	13.81	13.49	17.55	16.93	12.87
	4	6.89	13.45	9.05	9.55	11.25	12.30	11.78	15.97	14.41	11.63
	8	5.85	7.69	6.87	7.20	7.42	8.25	7.80	11.25	10.21	8.06
The mean		6.84	11.97	8.58	8.95	10.09	11.45	11.03	14.92	13.85	
LSD (P < 0.05)			Fertilizers	Water salinity	interaction						
	Grain yield		0.37	0.21	0.64						
	Biological yield		0.86	0.50	1.48						

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