The efficiency of Azotobacter and Mycorrhizal fungi locally isolated and its effect on some chemical and biological soil properties of maize (*Zea mays* L.) irrigated with saline water

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Abstract

A field experiment was carried out during the growing season 2021 at agricultural research station of the college of Agriculture - University of Basrah , to evaluate the efficiency of biofertilizers of *Azotobacter and Mycorrhizae* (locally isolated) and compare it with ready-made biofertilizer on some chemical and biological soil properties 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 (locally isolated of *Azotobacter chroococcum*:LA), B2 (locally isolated of Mycorrhizae: *Glomus* spp.:LM), B3 (LA + LM), B4 (LA + LM and 50% of the fertilizer recommendation:FR , B5 (ready- made *Azotobacter*:RA + ready-made Mycorrhiza:RM and 50% FR), B6 (LA+ LM and75% 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 added in the main plots and the fertilizer levels in the sub-plots. The results showed that addition of bio-fertilization led to a significant effect in both reducing soil salinity (4.55 dS m^{-1}) and a pH of (7.59), also increase the number of azotobacter ($70.39x10^4$ cfu g^{-1} soil) , colonization of mycorrhizae (60.81%) and the residual of NPK nutrients in the soil (82.97, 22.18, 284.73 mg kg^{-1}) respectively compared to control . The best results showed when using the bio-fertilizer (*A. chroococcum+Glomus* spp.)locally isolated with 75% FR. while the high salinity of irrigation water led to decrease in most of the all the above-mentioned parameters .

Key words: **Biofertilizers, salinity, Mycorrhiza, Azotobacter .**
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Introduction

Arid and semi-arid regions suffered from the scarcity of irrigation water sources for the agricultural process, impels them to irrigate with medium or high salinity water which is followed by the conversion of the land into saline soils unsuitable for the growth and productivity of field crops then reduces plants capable to coexist under conditions of salt stress)Zorb *et al.,* 2019). Irrigation with saline water has an effect on the rate of biological activity in the rhizosphere which is often known as osmotic and ionic stress (Shrivastava and Kumar 2015(, so it leads to inhibits the formation of Mycorrhizal fungi and decreases its colonization of roots (Sheng *et al.,* 2008). The salinity also affected the decrease in the of *Azotobacter* count from $1.29*10^5$ to $0.43*10^5$ cfu gm^{-1} where irrigation water increases from 1.5 to 4.4 dS m^{-1} (Salman 2016).

The maize crop is a moderate sensitive to salinity, the high levels of salinity have severely affected on its growth and production (Sothar *et al.,* 2021). Farmers often use large quantities of mineral fertilizers in an attempt to increase yield where excessive application of fertilizers especially nitrogen and phosphate decrease in the efficiency of nitrogen, phosphate and occur pollution of adjacent waters (Yoo *et al.,*2014). that encouraged scientists to apply some techniques to ensure continued growth and better production of crops under stress conditions of water shortage, including the use of beneficial microorganisms that promote plant growth and improve the plant’s ability to tolerant salt stress which are effective environmentally, safe friendly, where microbial strains added as bio-fertilizers in the rhizosphere of plant growth in order to increase the availability of nutrients as N, P (Nadeem *et al.,* 2014). *Arbuscular Mycorrhiza* naturally reproduce in saline soils with symbiotic 80% of the plants (Abdel-Fattah *et al.,*2012).

The free-living *Azotobacter* bacteria that fixing-N from atmospheric is efficient in stimulating plant growth and improving its tolerance to stressful conditions through several mechanisms, directly and indirectly where these increasing the availability of nutrients in the soil such as nitrogen, as well increasing the dissolution of unavailable phosphorous, production of plant hormones of native isolates that have proven their efficiency compared to the imported bio-fertilizer (Al-Hilali 2015), and when using biotechnologies has become promising in a significant reduction in the levels of chemical fertilizers, in addition to the plant's tolerance of some plant pathogens (Kumar *et al.,*2021). The combined (*Azotobacter + Mycorrhizal*) had a significant positive effect in all stages of plant growth, as they enhanced shoot and root growth compared to non- inoculants plants (Behl *et al.,* 2007), and improved positive assimilation among organisms either or plants (microbe-plant) (Ishac 2000). The study aimed to show the effect of native isolates (*Mycorrhizal* and *Azotobacter*) and their relationship with the ready-made bio-fertilizers with or without mineral fertilizers on reducing the salinity and some chemical and biological properties in soil .

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 Mycorrhiza fungus

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 were fumigated with arabic gum suspension. The bacterial inoculum was carried on sterilized peat moss 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 in Page *et al.,* (1982) .

The land was divided into three blocks, each block divided into three equal main plots, which divided into nine experimental unit with dimensions of 3.5 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

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

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	15	66

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 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	**EC**	**pH**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**K^+**	**Cl^-**	**$\mathrm{HCO3}^{=}$**	**$\mathrm{SO4}^{=}$**	**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:

Electrical Conductivity (EC) and (pH) in soil:

The results of Table 3 indicated that there is a significant effect in the average of soil salinity by adding bio- fertilization treatments where it decreased from 5.86 dS m^{-1} at B0 to 4.55 dS m^{-1} with the effect of B6 with decrease of 22.35% compared to B0 and did not give any significant difference with each B7, B4 and M except for B5 the mean was 4.70, 4.83, 4.85, and 4.99 dS m^{-1} respectively. The decrease in soil salinity is due to the addition of the bio-fertilizer which contributed as a supplement to the mineral recommendation and improved the physical properties of the soil. and the movement and washing of salts away from the root zone which can result from the secretions of these compounds that helped,to reduce the effect of salinity in the Grover *et al.,*(2011) ; Rojas- Tapias *et al.,*(2012) where the reason behind bio-fertilizers produce exopolysaccharides which expellud sodium (Na^+) from the rhizosphere and increasing potassium(K^+) absorption and increase in moisture content

The results of the statistical analysis and the data in Table 3 showed that the increase in the salinity level of the irrigation water had a significant effect on the average of salinity the soil at the end of the growing season, As the electrical conductivity (ECe) increased in the soil from 3.41 to 4.74 , 7.17 dS m^{-1} to S2 and S3 in comparison with S1, This may be due to the increase in the ionic concentration of salts in the water (Table 2) and the continuation of the irrigation application which caused the accumulation of ions in the root zone with an increase in water evaporation due to the high temperature and lack of rain in the study area and this study agree with Haran and Thaher (2019) where concluded that the increase in the salinity of irrigation water added to the maize plant led to an impact on all biological processes and caused a disturbance in the vital activity in the plant rhizosphere, While the statistical analysis (Table 3) did not give any significant effect of the interaction between studied trait on the soil salinity rate.

Table 3: Effect of fertilizers treatments and irrigation water salinity on electrical conductivity (ECe) and (pH) in soil

traits	Water salinity		Fertilizers treatments									The mean
			B0	M	B1	B2	B3	B4	B5	B6	B7	
ECe	**S1**	**2**	3.89	3.40	3.78	3.55	3.47	3.20	3.32	2.99	3.12	**3.41**
	S2	**4**	5.73	4.37	5.20	5.06	4.83	4.42	4.65	4.10	4.27	**4.74**
	S3	**8**	7.95	6.78	7.84	7.58	7.25	6.87	7.01	6.56	6.70	**7.17**
The mean			**5.86**	**4.85**	**5.61**	**5.40**	**5.18**	**4.83**	**4.99**	**4.55**	**4.70**	
pH	**S1**	**2**	8.21	7.81	8.13	7.91	7.87	7.78	7.82	7.75	7.89	**7.91**
	S2	**4**	8.02	7.73	7.98	7.86	7.80	7.63	7.76	7.60	7.58	**7.77**
	S3	**8**	7.70	7.46	7.76	7.59	7.54	7.43	7.60	7.41	7.37	**7.53**
The mean			**7.98**	**7.67**	**7.93**	**7.79**	**7.74**	**7.61**	**7.73**	**7.59**	**7.61**	
LSD (P < 0.05)			traits			Fertilizers		Water salinity		interaction		
			ECe			0.344		0.20		N.S.		
			pH			N.S.		0.19		N.S.		

The results also showed that bio- fertilization treatments did not affect the average of (pH) soil where most of the pH values ranged between 7.59-7.98 , Although there is decrease in soil pH but doesn't reach to the significant degree , Al-Mousawi *et al*., (2017) obtained an insignificant decrease in soil pH for all the bio-inoculant treatments used with the mineral recommendation compared to the non-inoculant treatment at the harvest stage of maize and he justified this to the ability of the regulatory soil to resist temporary changes to some of its chemical properties .Table 3 showed that the treatments irrigated with a salinity of 2 dS m^{-1} gave the highest average soil pH of 7.91 which did not differ significantly from the of salinity 4 dS m^{-1} (7.77) and both of them differed significantly from 8 dS m^{-1} gave the lowest average soil pH was 7.53, the reason behind that the high buffering of the soil in the presence of salts like calcium carbonate ($CaCO_3$) which prevents the change towards lower soil pH . In connection with the impact of the interaction among the treatments in soil pH that the reults in Table 3 showed that there is not a significant difference

The Azotobacter counts (CFU gm^{-1} soil) and the Mycorrhizal colonization (%) in the soil after the end of the growing season

The results of Figures 1 and 4 showed the effect of bio-fertilization in increasing the average of *Azotobacter* counts and the Mycorrhizal colonization fungi , The treatment B6 excel over all treatments by giving 70.39×10^4 Cfu gm^{-1}soil and 60.81% respectively with an increase of 118.13% and 96.16% over the treatment of 100%FR . the treatments of B4 and B5 were superior to the treatment of the recommendation (100%FR) 54.06, 35.72, 32.27×10^4 Cfu gm^{-1}soil, 53.82, 50.44, and 31.00% For the above measurements respectively. while treatment B0 gave lowest averages 11.05 x 10^4 Cfu gm^{-1}soil and 19.22% respectively. The increase may be due to the presence of a positive synergy that achieved the interaction among (bacteria and mycorrhizal), as the secretions of one encourage the growth of the other compared with mineral recommendation and this agreed with the results of Cameron *et al*., (2013). Which obtained an increase in the activity of plant growth stimulating bacteria (PGPR) by means of the symbiosis that occurred in the presence of Mycorrhizal in the rhizosphere stimulated the vital activity between these organisms and called it mycorrhizosphere.

Statistical analysis in Figures 2 and 5 showed that increasing the salinity level of the irrigation water led to decrease in the counts of *A. chroococcum* and the infection with *Glomus* spp. in the soil at the end of the growing season which decreased from 50.24 x 10^4 Cfu gm^{-1}soil and 61.94% for level S1 to 40.91 x 10^4 CFU gm^{-1}soil and 40.18% and to 14.66 x 10^4 CFU gm^{-1}soil and 23.33% for level S2, and S3 respectively, These results are supported by what was obtained by Salman (2014).

The interaction between the studied treatments (Figures 3 and 6) had a significant effect on the counts of *A.chroococcum* and infection mycorrhiza, as the S2B6 treatment achieved the best values of 99.49 x 10^4CFU gm^{-1}soil and 88% Whereas the treatment of S3B0 achieved the lowest values of 07.94 x10^4 CFU gm^{-1} soil and 11.00 % for the studied traits respectively .

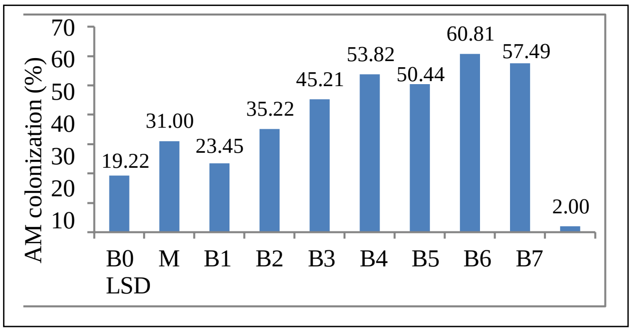

Figure 4: The effect of fertilizers treatments on Mycorrhiza (AM) colonization (%)

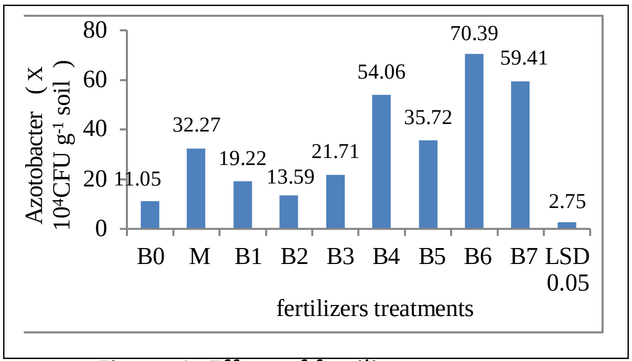

Figure 1: Effect of fertilizers treatments on azotobacter counts ($\times10^4$CFUgm^{-1} soil)

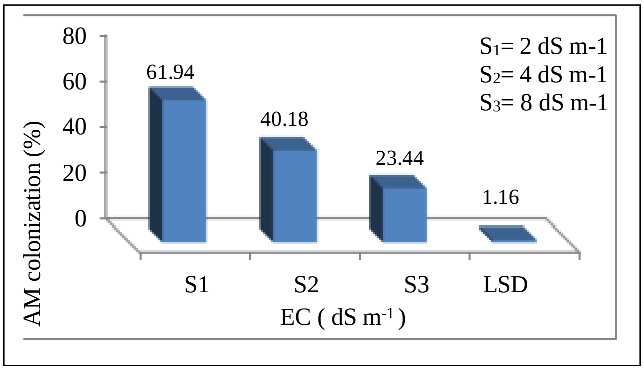

Figure 5: Effect of water salinity level (dS m^{-1}) on Mycorrhizal (AM) colonization (%)

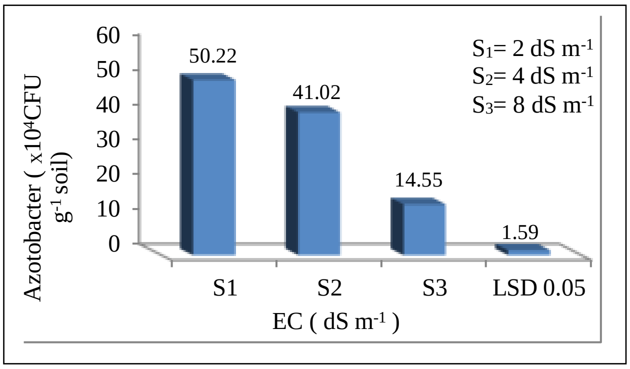

Figure 2: Effect of water salinity level (dS m^{-1}) on azotobacter counts (×10^4CFUgm^{-1} soil)

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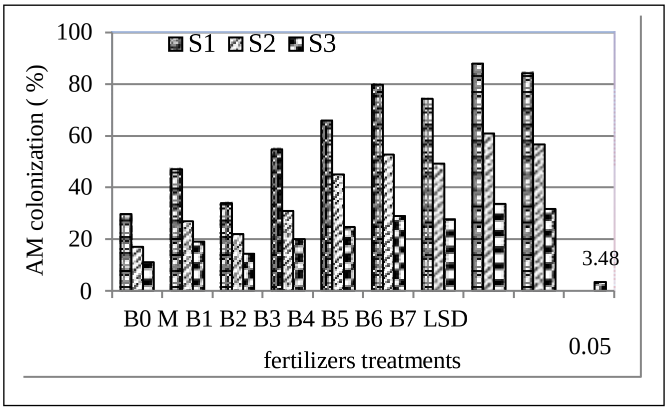

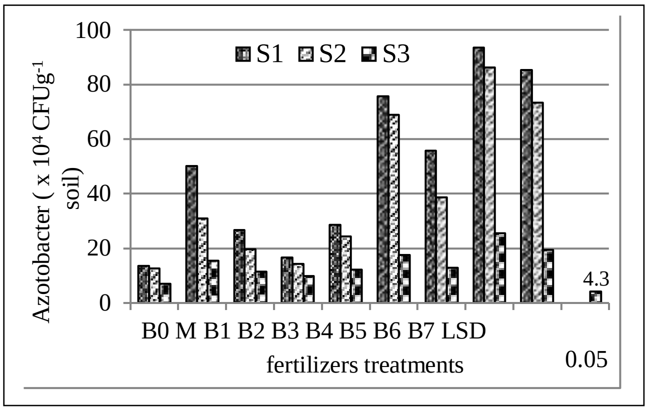

Figure 6: Effect of fertilizers treatments and water salinity level (dS m^{-1}) on Mycorrhizal (AM) colonization (%)

Figure 3: Effect of fertilizers treatments and water salinity level (dS m^{-1}) on azotobacter counts (x10^4CFU gm^{-1} soil)

Amount of Nitrogen, phosphorous and potassium residual in the soil (mg kg^{-1} soil) after the end of the growing season

The results of Table 4 showed that the addition of bio- fertilizer with or without mineral fertilizer led to an increase in the residual of nitrogen, phosphorous and potassium in the soil compared with control , The treatments of B6 achieved high averages where it reached 82.97, 22.18 , 284.73 mg kg^{-1} of dry soil respectively, and there is no significant difference of B7 , the M treatment did not give any significant effect on the characteristic of nitrogen and potassium residual compared to B4 and B5 with averages of 74.80, 74.61,
72.71 mg kg^{-1} in dry soil and 239.37, 251.24, 234.18 mg kg^{-1} in dry soil, and they outperformed them in the of ready phosphorous concentration 17.68, 20.70 and 19.82 mg kg^{-1} dry soil for treatments respectively. while the comparison B0 gave the lowest values measured for all treatments which were 48.82, 6.57 and 12.48 mg kg^{-1} dry soil for each of the concentrations of N, P and K, respectively this increase is attributed to the role of the biological inoculation used and their synergy in reducing soil salinity and regulating the degree of interaction (Table 3) .
Statistical analysis in Table 4 showed that increasing the salinity level of the irrigation water led to an increase in the residual of nitrogen, phosphorous and potassium where the values increased from 61.24, 12.59, 181.33 mg kg^{-1} of dry soil for S1 treatment to 70.17,
16.37 , 211.19 and 77.39, 18.75 , 252.65 mg kg^{-1} of dry soil for S^2 and S3 respectively,
this increase may be due to weak growth of roots and their spread and reducing to absorption , as well as the ionic effect of Na^+ and Cl^- which inhibited plant growth and the accumulation of nutrients in the soil solution .
The results of the statistical analysis in Table 4 showed that there was a significant effect of the interaction between the fertilizer treatments and the salinity levels of irrigation water on the residual of nitrogen and potassium. where S3B6 treatment gave the highest averages of 96.57 and 326.12 mg kg^{-1} soil, while the S1B0 gave the lowest averages of
43.47 and 98.66 mg kg^{-1} soil respectively , and the interaction did not give any significant
effect among the studied treatments on the residual of phosphorous in soil.

Conclusion

Combined bio-fertilization (*Azotobacter* + *Mycorrhizae*) and 75% FR has a main role in reducing the negative relics of salinity of irrigation water and save 25% of fertilization dose which leads to reduce the economical coast of mineral fertilizer.

Table 4: Effect of fertilizers treatments and irrigation water salinity on the amount of nitrogen, phosphorous and Potassium residual in the soil at the end of the growing season

Nutrient	Water salinity		Fertilizers treatments									The mean
			B0	M	B1	B2	B3	B4	B5	B6	B7	
N	**S1**	**2**	33.47	63.23	56.33	51.13	61.80	63.90	62.00	70.67	68.63	**59.02**
	S2	**4**	39.20	80.00	64.50	59.13	72.83	77.90	75.83	81.67	80.50	**70.17**
	S3	**8**	43.80	81.17	71.67	65.67	75.33	82.03	80.30	96.57	90.00	**76.28**
The mean			**38.82**	**74.80**	**64.17**	**58.64**	**69.99**	**74.61**	**72.71**	**82.97**	**79.71**	
P	**S1**	**2**	4.58	15.61	6.42	8.10	11.00	16.70	15.84	18.15	16.93	**12.59**
	S2	**4**	6.75	17.58	8.61	12.41	14.39	21.86	20.59	23.00	22.10	**16.37**
	S3	**8**	8.38	19.84	13.78	14.68	16.00	23.54	23.02	25.39	24.12	**18.75**
The mean			**6.57**	**17.68**	**9.61**	**11.73**	**13.80**	**20.70**	**19.82**	**22.18**	**21.05**	
	S1	**2**	98.66	200.90	134.75	138.36	201.64	210.31	190.85	241.07	229.35	**181.3**
	S2	**4**	114.43	229.00	159.32	165.77	201.64	246.57	235.74	287.00	271.22	**211.1**

K	**S3**	**8**	124.43	288.22	197.54	219.94	250.38	296.83	275.94	326.12	316.54	**252.6**
The mean			**112.48**	**239.37**	**163.87**	**174.69**	**217.88**	**251.24**	**234.18**	**284.73**	**272.37**	
LSD (P < 0.05)			Nutrient			Fertilizers		Water salinity		interaction		
			N			4.07		2.35		7.05		
			P			1.55		0.9		N.S.		
			K			16.78		9.69		29.06		

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