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Effect of inoculation with *Bacillus* spp., *A. chroococcum*, P. *fluorescens* and phosphorous levels on the amount of major nutrients in maize (*Zea mays* L.) irrigated with saline water

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ABSTRACT
A field experiment was carried out using a randomized complete block design (RCBD) to study the effect of bacterial inoculation (non-inoculation, inoculation with *Bacillus* spp., Inoculation with *A. chroococcum*, inoculation with *P. fluorescens*, and co-inoculation) on the amount of major nutrients in maize. Two levels of salinity of irrigation water were 3 and 6 dSm^{-1} and four levels of phosphate fertilizer were 0, 40, 80 and 120 kg P. ha^{-1} in the form of superphosphate fertilizer (21% P) to investigate the weight of 100 grain of maize plants and its leaves content of N, P and K nutrients. The results indicated that the co-inoculation with *Bacillus* spp., *A. chroococcum* and *P. fluorescens* was superior to increase the weight of 100 grains and the concentrations of nitrogen, phosphorus and potassium in the vegetative of maize plant by 44.99% and 12.98%, 156.5% and 39.5%, respectively, compared to the un-inoculated control irrigated with 6 dS m^{-1} saline water and not fertilized with phosphorus, and reduced the phosphate fertilizer recommend by 33%. The inoculation with *Bacillus* spp. treatment was not differ significantly from *P. fluorescens* inoculation in most of these traits.

Key words: Bacterial, Inoculation, *Zea mays*, Salt water**.**
Article type: Research Article.

INTRODUCTION
Phosphorus is one of the most important nutrients for plants and it is a limit element of agricultural production because of its important role in cell formation, division, cellular membranes, and the transfer of genetic properties, etc. Phosphorous availability depends on soil pH, it was decreases in the base medium as a result of its interaction with calcium carbonate and thus agricultural land had large deposits of phosphorus accumulated from successive annual additions of phosphate fertilizer (Omer *et al*. 2016). Soil salinity affects plant growth directly by increasing the osmotic pressure that leads to the plant's unable to absorb water and nutrients and affects the nutritional balance of the plant, which act the deficiency of some nutrients as well as the toxic effects of sodium and chlorine, whose concentrations increase with the increase in saline soils and the physiological effect of salinity on hormones and enzymes, The indirect effects of salinity on plant growth were on the physical properties of the soil, which leads to making it an unsuitable medium for plant growth (FAO, 2013). Biofertilizers technology was used as one of the scientific methods to improve available phosphorous under saline conditions by using Plant Growth Promoting Rhizobacteria (PGPR) among these microorganisms the phosphate-dissolving bacteria (*Bacillus* and *Pseudomonas*), which were proved their ability to dissolve the insoluble phosphorous compounds and increase its availability for absorption by the plant, as well as their ability to produce different types of growth hormones, including auxins, gibberellins and cytokinins. Thus reducing the harmful effects of the nutritional imbalance resulting due to the increased salt concentration in the soil and that some of genera had ability to produce antibiotics that contribute effectively, in protecting plants from pathogens (Mohamed & Gomaa 2012; Al

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Mousawi *et al.* 2022; Abed Almjalawi *et al.* 2022). the genus *Azotobacter* is one of the genera of free-living bacteria with a high ability to fix atmospheric nitrogen and had ability to secrete hormones, enzymes and vitamins, which were used as a biological fertilizer to improve plant growth, as well as it was secretion of resistance materials Osmosis such as ACC deaminase amino acids, proteins, and polysaccharides to improve the growth of plants growing under conditions of salt stress (Omer *et al*. 2016). In order to reduce the harmful effects of salinity on the availability of phosphorous in the soil and improve the growth and production of plants, the research aimed to study the effect of a single and co-inoculation with *Bacillus* spp., *P. fluorescens* and *A. chroococcum* bacteria in improving the some yield component of maize and its content of nitrogen, phosphorous and potassium.

MATERIALS AND METHODS

In the field experiment, a randomized complete block design (RCBD) was used with three replications, the field was plowed and 20 ton ha^{-1} organic fertilizer was added for all treatments. The field were divided into two large plots, then each plots was divided into small slabs of 2 × 3 m. It contained the all studied experimental treatments, and leaving a distance of 1 m between an experimental unit and another. The slabs had two farrow in each experimental unit with a length of 3 m, and the distance between the centre and the other was 75 cm. The seeds of maize, Baghdad variety (non-denatured) obtained from the General Authority for Inspection and Certification of Seeds - Abu Ghraib - Ministry of Agriculture, were washed with distilled water and then surface sterilized according to Nandini Kumari & Pushpanjali Khare (2019). The inoculates for *A. chroococcum, P. fluorescens* and *Bacillus* spp. were prepared, as the growth for a 24-hour-old slant bacterial culture was harvested with adding 3 mL of sterile distilled water to the slant, and transferring the inoculate to a sterile tube for the purpose of adjusting the turbidity to 0.85 with a spectrophotometer at a wavelength of 600 nm (this optical density is equal to the standard turbidity containing 1.5×10^8 CFU mL^{-1}) according to B-Evenhuis *et al*. (1976). Then 1 mL of the inoculum were taken and placed in one litre of the culture medium of the third bacteria, and a killed bacteria were used as un-inoculated treatment. Thereafter, incubating was performed in a vibrating incubator at a temperature of 30 °C for a period of 2-3 days, according to Page *et al*. (1982). The maize seeds were inoculated with the five prepared bacterial inoculates, which were non-inoculation, inoculation with *Bacillus* spp., *A. chroococcum*, *P. fluorescens* and their mixed inoculation which symbolized T0, T_1, T_2, T_3 and T_4 respectively. Inoculation was performed by drenching the maize seeds in each inoculates for one hour separately and the addition of gum arabic (10%) to increase the adhesion of the inoculate to the seeds (Ahmed & Hassan 2021). The soil was irrigated by calibration irrigation with river water (1.6 dS m^{-1}), then maize seeds were sown at the rate of 3 seeds per hole with a depth of 4 cm, and the distance between was 25 cm. Phosphorus was added in the form of concentrated superphosphate fertilizer (21% P) in four levels: 0, 40, 80 and 120 kg ha^{-1}, which is symbolized as P_0, P_1, P_2 and P_3, respectively. Nitrogen fertilizer (urea N 46%) was added in the amount of 280 kg N ha^{-1} in two doses (the first was added before germination and the second before the flowering stage, from 45 days after planting). Potassium fertilizer was added (Potassium Sulphate 51% K) in an amount of 80 kg ha^{-1} with two doses, the first at planting and the second at the flowering stage. The land were irrigated to the field capacity limits with water of different salinity 3 and 6 dS m^{-1}, Symbolized by S_1 and S_2, respectively. Irrigation was repeated after draining 50% of the prepared water using a tensiometer (Hawaldar & Agasimani 2012). The service operations were carried out by lightening the plants to one plant per burrow, controlled the insects and weed by pesticides. After the crop matured physiologically, according to B-Evenhuis *et al*. (1976), the yield was collected and the weight of 100 grains (g) after adjusting the humidity to 15% (Nandini Kumari & Pushpanjali Khare 2019). The plant samples were taken (the leaf directly below the sprouts) and the plants were dried at 65 °C for 48 hours and milled (Page *et al*. 1982). Afterward, 0.2 g of ground leaf samples were taken and then digested with concentrated sulfuric acid according to B-Evenhuis *et al*. (1976) in which the total nitrogen concentration was estimated by the Kjeldahl method according to Chapman & Parker (1961). The phosphorus concentration was determined using the method of ammonium molybdates, by the measurement with the Spectrophotometer, while potassium by the method of the flam photometer according to Page *et al*. (1982).

RESULTS AND DISCUSSION

The best treatment of the combined bacterial inoculation and level of 3 dS m^{-1} of the salinity of irrigation water with the level of 120 kg P ha^{-1} for fertilization Phosphate was ($T_4S_1P_3$) 34.55 g $grain^{-1}$ with an increase of 44.99% compared to $T_0S_2P_0$, which recorded the lowest average 23.83 g, for the weight of 100 grain (Table 1). The second best treatment was ($T_4S_1P_2$), which recorded an average of 33.90 g, of weight of 100 grains and is not different significantly with $T_4S_1P_3$ treatment. This indicates that the combined bacterial inoculation reduced the fertilizer application by 33%, which is consistent with Hanoon *et al*. (2020). They were indicated that the biological inoculate secretes growth-promoting hormones that plays an important role in increasing growth and yield components by increasing cell division and elongation, then increasing the number of root hairs. Consequently, an increase in the surface area of the roots and rises in the absorption of nutrients, positively reflect in the elevation of vegetative growth, dry matter formation and upraised grain yield.

Table 1. The effect of the bacterial inoculate and the two salinity levels of irrigation water and the levels of phosphate fertilizer in the weight of 100 grain (g) of the maize grains.

Bacterial inoculate	**Salinity levels**	**Levels of phosphate fertilizer**				**T× S**
		P_0	**P_1**	**P_2**	**P_3**	
T_0	S_1	26.52	28.34	30.76	31.64	29.32
Uninoculated	S_2	23.83	25.66	28.21	28.22	26.48
T_1	S_1	28.26	30.03	32.48	33.17	30.99
Bacillus spp.	S_2	25.12	26.81	29.37	30.06	27.84
T_2	S_1	26.73	28.58	30.93	31.40	29.41
A.chroococcum	S_2	24.20	25.94	28.47	28.51	26.78
T_3	S_1	27.58	29.28	31.81	32.53	30.30
P.fluorescens	S_2	27.04	28.81	31.30	30.97	29.53
T_4	S_1	29.63	31.46	33.90	34.55	32.39
Mixed inoculate	S_2	27.49	29.19	31.67	32.25	30.15
LSD 0.05		1.6096				.٠8048

The results in Table 2 showed that the triple interaction between the study factors had a significant effect on the rate (%) of nitrogen in the plant, the highest rate belonged to $T_4S_1P_2$ (2.341 % N), with an increase of 12.93% compared to the comparison treatment ($T_0S_2P_0$), which exhibited the lowest percentage of nitrogen 2.073%. Also, it was noticed that there was not significant difference between $T_4S_1P_2$ and $T_2S_1P_2$ (2.31), which confident the efficiency *A. chroococcum* as inoculation. The $T_4S_1P_2$ and $T_2S_1P_2$ reduced the fertilizer application for phosphorus by 33% due to the ability of mixed and *A. chroococcum* inoculation to stabilize atmospheric nitrogen by *A. chroococcum* and dissolve phosphate by *Bacillus* spp. and *P. fluorescens* which help the plant to form the energy compounds responsible for the absorption of nutrients, including nitrogen. Nandini Kumari & Pushpanjali Khare (2019) reported that the nitrogen content of the plant elevates when inoculating with *Bacillus* spp. because secretion of growth hormones such as cytokinin, gibberellin and auxin improve plant growth and encourage the plant to form a dense root system, leading to the upraised absorption of nutrients, including nitrogen.

Table 2. The effect of the bacterial inoculate, the salinity levels of irrigation water and the levels of phosphate fertilizer in% of nitrogen in the vegetative part of maize plants.

Bacterial inoculate	**Salinity levels**	**Levels of phosphate fertilizer**				**T× S**
		P_0	**P_1**	**P_2**	**P_3**	
T_0	S_1	2.180	2.207	2.251	2.277	2.228
Uninoculated	S_2	2.073	2.106	2.152	2.184	2.129
T_1	S_1	2.203	2.209	2.273	2.271	2.239
Bacillus spp.	S_2	2.087	2.110	2.172	2.191	2.140
T_2	S_1	2.247	2.236	2.31	2.295	2.272
A.chroococcum	S_2	2.165	2.183	2.233	2.240	2.205
T_3	S_1	2.197	2.208	2.259	2.269	2.233
P.fluorescens	S_2	2.142	2.147	2.191	2.197	2.169
T_4	S_1	2.268	2.268	2.341	2.339	2.304
Mixed inoculate	S_2	2.183	2.198	2.236	2.247	2.216
LSD 0.05		٠,٠5024				0.02512

According to the results of Table 3, the triple interaction of the three examined factors exhibited a significant effect in the rate (%) of phosphorus in the vegetative part of the maize. The treatment $T_4S_1P_3$ reached the highest rate (0.431%) of phosphorus with an elevation of 156.55% compared to the un-inoculated treatment ($T_0S_2P_0$) which exhibited the lowest rate of phosphorous (0.168%). Applying phosphate fertilization at the level of 120 kg P ha^{-1} (0.343%) did not differ significantly from 80 kg P ha^{-1} (0.314%). Also, $T_4S_1P_3$ (0.431% P) did not differ significantly from $T_4S_1P_2$ (0.393% P) indicating that the mixed bacterial inoculation reduced the application of phosphate fertilizer by 33%. This may be due to the ability of *Bacillus* spp. and *P. fluorescens* to increase the availability of phosphorus in the soil by dissolving phosphorus-containing compounds, hence by production of some organic acids and the secretion of enzymes such as phosphatase and phytase that elevates phosphorous availability for the plant, which is in agreement with Keston (2013) and Haran (2021). Based on the results, the triple interaction between the examined factors exhibited a significant effect on rate (%) of potassium in the vegetative part of maize plant (Table 4). The highest rate was due to the effect of the two co- inoculation treatments, at saline level of 3 dS m^{-1} and the levels of 80 and 120 kg P ha^{-1} ($T_4S_1P_2$ and $T_4S_1P_3$) (2.86) respectively with an increase of 39.51% compared to the un-inoculated treatment and the saline level 6 dS m^{-1} and non-phosphorous ($T_0S_2P_0$) which exhibited the lowest rate (2.05% K).

Table 3. The effect of the bacterial inoculate and the salinity levels of irrigation water and the levels of phosphate fertilizer in% of phosphorus in the vegetative part of maize plants.

Bacterial inoculate	Salinity levels	Levels of phosphate fertilizer				T× S
		P_0	P_1	P_2	P_3	
T_0	S_1	0.220	0.247	0.298	0.319	0.271
Uninoculated	S_2	0.168	0.202	0.250	0.278	0.225
T_1	S_1	0.267	0.313	0.339	0.376	0.324
Bacillus spp.	S_2	0.238	0.269	0.307	0.324	0.285
T_2	S_1	0.243	0.269	0.290	0.326	0.282
A.chroococcum	S_2	0.192	0.222	0.258	0.288	0.240
T_3	S_1	0.263	0.305	0.343	0.377	0.322
P.fluorescens	S_2	0.232	0.281	0.317	0.340	0.293
T_4	S_1	0.276	0.354	0.393	0.431	0.364
Mixed inoculate	S_2	0.281	0.329	0.349	0.373	0.333
LSD 0.05		0.0544				0.0272

Also, no significant difference was found between $T_4S_1P_2$ and $T_3S_1P_2$, $T_2S_1P_2$ and $T_1S_1P_2$. This indicated the ability of the single inoculation with *A. chroococcum, P. fluorescens, Bacillus* spp. and their mixed to improve the maize with potassium, while reducing the phosphorus fertilizer application by 33%. This may be attributed to the fact that the combined and single bacterial inoculation improved the plant's ability to resist salt stress by dissolving phosphate and helping the plant in the formation of energy compounds responsible for the absorption of nutrients, including potassium, followed by the secretion of osmotic resistance materials such as salicylic acid, and the production of various growth hormones that improve plant nutrition and resistance to stress conditions (Haran & Thaher 2019).

Table 4. The effect of the bacterial inoculate and the salinity levels of irrigation water and the levels of phosphate fertilizer in% of potassium in the vegetative part of the maize plants.

Bacterial inoculate	Salinity levels	Levels of phosphate fertilizer				T × S
		P_0	P_1	P_2	P_3	
T_0	S_1	2.24	2.37	2.51	2.60	2.43
Uninoculated	S_2	2.05	2.13	2.30	2.40	2.22
T_1	S_1	2.32	2.49	2.71	2.76	2.57
Bacillus spp.	S_2	2.20	2.28	2.42	2.46	2.34
T_2	S_1	2.32	2.45	2.63	2.71	2.53
A. chroococcum	S_2	2.12	2.22	2.38	2.44	2.29
T_3	S_1	2.33	2.47	2.67	2.74	2.55
P. fluorescens	S_2	2.25	2.33	2.46	2.48	2.38
T_4	S_1	2.40	2.56	2.86	2.86	2.67
Mixed inoculate	S_2	2.21	2.31	2.49	2.55	2.39
LSD 0.05		0.4012				0.2006

CONCLUSION

The combined bacterial inoculation led to an increase in the weight of 100 grains and the concentration of NPK elements in the vegetative part of maize plant by 44.99%, 12.44%, 156.5% and 39.5%, respectively, compared to the un-inoculated treatment and reduced the phosphate fertilizer application for maize by 33%.

RECOMMENDATIONS

We recommend to inoculate various plants with phosphate-dissolving and nitrogen-fixing microorganisms in order to enhance the number of beneficial microorganisms in the soil to encourage their bioactivities for the benefit of the plant.

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