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Effect Bio-fertilizer of *Bacillus*, *Azotobacter* and *Pseudomonas floresence* in the Growth and Production of Corn Plant (*Zea mays* L.)

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Abstract A field experiment was carried out with the use of complete randomized block design (CRBD) to study the effect of five bacterial inoculations (non- inoculation, inoculation with phosphate soluble bacteria, inoculation with *Azotobacter* bacteria, inoculation with *Pseudomonas fluorescens* and Bacterial mixture). Two levels of salinity of irrigation water 3 and 6 dS.m^{-1} and four levels of phosphate fertilizer (0, 40, 80 and 120 kgP.ha^{-1}). The results showed that the mixed inoculation of phosphate soluble bacteria, *Azotobacter* and *P. fluorescens* bacteria were superior to the rest of the single bacterial inoculation in plant height, dry weight of the shoot part, total grain yield, weight of 100 grains, grain weight in cop, leaf surface area, nitrogen, phosphorus and potassium concentrations in the vegetative growth and grain with 33.08%, 31.90%, 61.07%, 44.99%, 62.06%, 36.94%, 12.8%, 31.5%, 156.5%, 272.3%, 39.5% and 75.1%, respectively, in comparison with the non- inoculation and irrigated water treatment of 6 dS.m^{-1} (T0S2P0), while the inoculation of phosphorus soluble bacteria did not differ significantly from the *P.fluorescence* inoculation in most of these traits. T3S2P3 was not significantly differ from T4S2P3 combined in the increase of plant height and total grain yield compared to the non- inoculation and non-phosphorous treated at the salt level 3and6 dS.m^{-1}. The single-dose T2S2P3 did not differ significantly from the combined T4S2P3 3 and 6 dS.m^{-1}.

Keywords: Bio-fertilizer, *Bacillus*, *Azotobacter*, *Pseudomonas florescence*, *Zea mays*.

Introduction

Bio-fertilizers are defined as the use of living microorganisms as fertilizers. Micro-organisms used in Bio-fertilizers include Phosphate solubilizing bacteria, Mycorrhiza fungus, non and Symbiotic Nitrogen Fixation bacteria, Plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas fluorescens*, *Trichoderma* and others. These bio-fertilizers provide the plant with various nutrient elements such as nitrogen and phosphorus, as well as production of various types of growth hormones such as Auxins, Gibberellins, and cytokinines, thus reducing the harmful effects of nutrient imbalances in soil resulting from salt stress. Some also have the ability to produce antibiotics that effectively contribute to the protection of Plant from pathogens (Siddikee *et al*., 2010).

Phosphorus is considered the second most important mineral nutrient after nitrogen, the plant needs it in large quantities. It is one of the determinants of agricultural production for its important role in cell formation, cell division, cellular membrane formation and transmission of genetic traits (Xiao e*t al*., 2009). However, its availability for the plant depends on the change in soil pH, where Phosphorus availability reduces in the alkaline media as a result of its deposition with calcium carbonate, thus most of the alkaline agricultural soils have large deposits of accumulated phosphorus resulting from the successive annual additives for the phosphate fertilizer (FNCA, 2006). Bio-fertilization was used individually (e.g. inoculation with Phosphate solubilizing bacteria) as a method to improve phosphorus availability in soil (El-Komy, 2005; Abd El-Ghany *et al*., 2010).

However, recent studies have suggested that different types of bacteria used in bio-fertilization to inoculate seeds or soil should be mixed at the same time, these organisms encourage plant growth according to the method of inoculation (Al-Shamma, 2013; Uddin *et al*., 2014). Yazdani *et al*. (2009) found that the mixture inoculation for the corn plants with *P. putida, p. lentus, A. chrococcum*, and *A. brasilanse* led to an increase in grain yield compared with non-inoculation treatment. Gholami *et al*. (2008) found that the mixture inoculation for corn plants with *Azotobacter* and *Azospirillum* led to increase the plant height. The mixture inoculation with plant growth-promoting bacteria led to improve the growth of plants growing under saline stress due to the synthesis of osmosis resistance materials such as polysaccharides, amino acids, proline, salicylic acid, ACC deaminase and growth regulators (Khan *et al*., 2009).

This study aims to study the effect of single and mixture inoculation with Phosphate solubilizing bacteria, *Azotobacter* and *P. floresence* on growth and production of corn under salt stress conditions and reduce the amount of phosphate fertilizer added less than the recommended fertilization.

Materials & Methods

Phosphate solubilizing bacteria (B6) isolated from the rhizosphere of alfalfa plant which showed high efficiency in the dissolving of the insoluble phosphate (Haran & Thaher, 2019). In addition, isolates of *P. floresence* and isolates of *Azotobacter* bacteria were obtained from the Department of Plant Protection and the Department of Soil Sciences and Water Resources, College of Agriculture, University of Basrah. The field experiment was conducted according to the Complete Randomized Block Design (CRBD), with three replicates. The soil of the field, that it traits are shown in table (1) was plowed, then organic material (cows manure) was added to the soil with the level of 2 tons.ha^{-1} for all treatments. The soil is then settled, smoothed and divided into two plots. Each plot divided into small plots, with dimensions of 2×3 m containing the studied experimental treatments and left 1 m between each experimental unit and other. Two furrows were planned in each experimental unit with a length of 3 m and the distance between the furrow and other is 75 cm.

The inoculation of the phosphate solubilizing bacteria, *Azotobacter* and *P. floresence* were separately prepared. The seeds of corn were inoculated through soaking them in the bacterial inoculating for half an hour with the addition of Arabic gum (10%) to increase the adhesion of the inocula to the seeds. Another group of corn seeds is soaked in a non-inoculation solution as a control treatment. After the calibration irrigation with the river water, the seeds of the inoculated and non-inoculated corn were planted in the holes at a depth of approximately 4 cm and the distance between the holes is 25 cm. Phosphorus was added in the form of trisuperphosphate fertilizer (21% P) with four levels of 0, 40, 80, 120 kg P. ha^{-1} in one dose at cultivation time by feeding method which summarized by placing it in a holes under the line of cultivation at a depth of 10 cm and then covered with soil. Nitrogen fertilizer was added as urea (46% N), with the level of 280 kg N.ha^{-1} in two doses, the first dose was in the stage of vegetative growth and the second before the flowering stage, after 45 days of cultivation. Potassium fertilizer (potassium sulfate) (51% K) was added with the level of 80 kg K.ha^{-1} in two dose, the first one at cultivation and the second in the flowering stage.

Table (1): Some chemical, physical and biological properties for studied soil before cultivation.

Soil properties	Units	Values
Electrical conductivity	dS m^{-1}	8.92±0.06
pH		7.4±0.06
Cation exchange capacity	$Cmoil^{+}$ kg^{-1} Soil	14.1±0.07
Organic matter	gm kg^{-1} Soil	4.46±0.07
$CaCO_3$		236.45±5.11
Available Nitrogen	mg kg^{-1} Soil	21.31±0.69
Available Phosphorus		18.46±0.26
Available Potassium		155.6±9.45
Soil separators		
Sand	gm kg^{-1}	254.20±12.27
Silt		518.80±22.66
Clay		227.00±14.57
Texture	Loam	
Total number of soil biota CFU (g $soil^{-1}$ Dry weight)		
Total bacteria	4.1×10^{9}	
Total fungi	0.83×10^{5}	
Azotobacter bacteria	3.2×10^{4}	
P.floresence bacteria	1.6×10^{4}	
Phosphate solubilizing bacteria	0.58×10^{5}	

The crop was irrigated with two levels of irrigation water S1, S2. After maturation stage on 4/7/2018, ten plants were randomly selected for each experimental unit to study the field traits of plant height and dry weight of the vegetative part. As for the amount of total yield for the treatment, 5 additional plants were harvested and their yield was estimated.

Results & Discussion

The results showed that the mixture inoculation, salinity of irrigation water (3 dS.m^{-1}) and the phosphorus level (120 kg P.ha^{-1}) (T4S1P3) led to an increase in plant height with percentage of 33.08% compared to the non-inoculated, non-fertilization with phosphorus and the 6 dS.m^{-1} saline level of irrigation water (T0S2P0). This treatment was not significantly differ from (T4S1P2, T4S1P1, T4S2P2, T4S2P3, T3S1P3, T1S1P3, T1S1P2) as shown in table (2). The table also indicated that there is no significant difference between T4S1P1, T4S1P2 and

T4S1P3, indicating a reduction in the nominal recommendation by half or less. This applies to all single and joint vaccination, and improved plant growth under saline stress conditions due to the susceptibility of the combined inoculation bacteria to increasing the nutrient availability for the plant and excretion it to different growth regulators such as Gibberellins and cytokinines which promote elongation and plant growth (Schoebitz *et al*., 2012). There was also no significant difference between T4S1P3 and T4S2P3, indicating an increase in plant tolerance to salinity during joint bacterial pollination.

Also, the treatment of single inoculation with Phosphate solubilizing bacteria (T1S1P3) led to an increase in plant height with percentage of 20.03% compared to the non-inoculated and non-fertilization with phosphorus treatment at 3 $ds.m^{-1}$ the level of salinity of irrigation water, which did not differ from the same treatment at 80 kg $P.ha^{-1}$ of phosphate fertilizer (T1S1P2) as shown in table (2).

Table (2): Effect of bacterial inoculation, phosphate fertilizer levels and different irrigation water salinity on plant height (cm) For the corn plant.

Bio-fertilizer	Salinity level	phosphate fertilizer levels			
		P0	P1	P2	P3
Non-inoculation T0	S1	182.2	190.0	199.1	207.5
	S2	170.8	178.6	187.8	195.6
PSI (T1)	S1	196.2	204.1	213.4	218.7
	S2	182.6	189.2	197.4	206.8
Azotobacter T2	S1	185.3	192.1	201.6	208.6
	S2	173.0	180.9	189.5	197.0
P.floresence T3	S1	194.6	203.7	210.9	217.6
	S2	185.4	193.8	202.1	209.9
The mixture Inoculation T4	S1	205.8	213.7	222.0	227.3
	S2	192.9	202.5	211.8	219.2
RLSD 0.05		16.2			

Table (3): Effect of bacterial inoculation, phosphate fertilizer levels and different irrigation water salinity on shoot dry weight ($g.plant^{-1}$) for the corn plants at harvest.

Bio-fertilizer	Salinity level	phosphate fertilizer levels			
		P0	P1	P2	P3
Non-inoculation T0	S1	258.1	270.1	6.082	291.8
	S2	239.5	249.8	265.3	271.0
PSI (T1)	S1	268.3	281.3	293.8	299.4
	S2	254.2	265.2	281.1	286.7
Azotobacter T2	S1	275.7	287.9	302.2	307.8
	S2	263.1	276.3	288.6	294.8
P.floresence T3	S1	265.8	277.7	291.3	296.8
	S2	256.6	270.6	281.1	286.5
The mixture inoculation	S1	282.9	294.9	310.7	315.9

T4	S2	270.0	283.4	295.3	301.7
RLSD 0.05			12.2		

Table (3) explained that T4S1P3 gave the highest shoot dry weight (315.9 g. Plant^{-1}) with a parent increase of 31.90% compared to the non-inoculated and non-fertilization with phosphorus treatment at 6 ds.m^{-1} the salinity level of irrigation water (T0S2P0) as shown in table (3). This is due to the role of bacterial inoculation in increasing the nutrients availability for the plants and inducing them to absorb water and increase plant resistance to high concentrations of salt, which is reflected in the dry weight of the plant (Rahmayani *et al*., 2017).

The same table showed no significant differences between the treatment (T4S1P3) and the treatment (T4S1P2) in the dry weight of vegetative part. It is also noted that the single inoculation with the *Azotobacter* (T2S1P3) led to an increase the shoot dry weight with percentage 19.5% compared to the control treatment (T0S1P0), which did not differ significantly from the treatment T2S1P2. This is due to the role of *Azotobacter* in the fixing of atmospheric nitrogen, which is used to build cells and chlorophyll, thus increase plant growth (Havlin *et al*., 2005).

Table (4): Effect of bacterial inoculation, phosphate fertilizer levels and different irrigation water salinity on the total yield of grains (mag.ha^{-1}) for the corn plants.

Bio-fertilizer	Salinity level	phosphate fertilizer levels			
		P0	P1	P2	P3
Non-inoculation T0	S1	5.746	6.158	6.562	6.910
	S2	5.019	5.409	5.848	6.180
PSI (T1)	S1	6.420	6.795	7.248	7.549
	S2	5.779	6.157	6.610	6.942
Azotobacter T2	S1	5.820	6.206	6.660	6.998
	S2	5.106	5.535	5.979	6.316
P.floresence T3	S1	6.375	6.754	7.209	7.498
	S2	5.963	6.342	6.799	7.076
The mixture inoculation T4	S1	6.967	7.352	7.809	8.084
	S2	6.283	6.682	7.041	7.350
RLSD 0.05		61.05			

Table (4) indicated that the mixture inoculation (T4S1P3) gave the highest average for grain yield of 8.084 mag.ha^{-1} with a relative increase of 61.07% compared to the control treatment (non-inoculation) T0S2P0, which recorded the lowest average for grain yield of 5.019 mag.ha^{-1}. T4S1P3 treatment did not differ significantly from T4S1P2 treatment (7.809 mag.ha^{-1}) in its effect on the grain yield of corn, also, they were not differ from the two treatments (T4S2P3, T4S2P2), which amounted their average to (7.350, 7.041 mag.ha^{-1}), respectively.

This indicates that the mixture of bacterial inoculation reduces the phosphatic fertilizer recommendation with a percentage of 33% and improves plant resistance against salt stress conditions. In addition, the single inoculation treatment with phosphate solubilizing bacteria (T1S1P3) and *P. fluorescens* (T3S1P3) led to an increase of 31.38% and 30.49%, in the yield of cron grains, respectively compared to the non-inoculation, non-fertilization with phosphorus at 3 ds.m^{-1} level of saline water (Abd El-Gany *et al*., 2013). These treatments did not differ significantly from the treatments T1S1P2 and T3S1P2, indicating the ability of these isolates to provide phosphorus for the plant and reduce the fertilizer recommendation of phosphorus to 33%. This is due to the ability of the common vaccine bacteria to analyze phosphorus-containing metals and to release available phosphorus. These species produce mineral acids that dissolve phosphorus, such as carbonic, nitric and sulfuric acid, as well as many organic acids (Wu *et al*., 2012).

In addition, the mixture of bacterial inoculation is characterized by its high capacity to provide the plant with nutrient, provided that the environmental conditions are suitable for the effectiveness of the bacterial vaccine. In addition, the bacterial inoculation enhances the number of bacteria and prevents them from adverse environmental effects such as drought and high temperature during the growth season (Abd El-Gany *et al*., 2013).

Table (5) displayed that the treatment of the mixture bacterial inoculation and saline level of 3 dS.m^{-1} and phosphorus the level of 120 kg P.ha^{-1} (T4S1P3) led to an increase in the average leaf area of 6309 cm^2 and an increase of 36.94% compared to the control treatment (T0S2P0), which gave the lowest average of leaf area of 4607 cm^2. The T4S1P3 treatment did not differ significantly from the same treatment at the phosphoras level of 80 kg P.ha^{-1} (T4S1P2). This indicates that the mixture of bacterial inoculation reduced the phosphatic fertilizer recommendation to 33%. This treatment (T4S1P3) did not differ significantly from the single inoculation treatment with *Azotobacter* and saline level of 3 dS.m^{-1} and phosphoras level of 120 kg P.ha^{-1} (T2S1P3). This indicates to the efficiency of the *Azotobacter* bacteria in the fixation of the nitrogen entering the cells and increasing the volume of the total vegetative and the leaf area of plant. This agrees with Zafar *et al*. (2011) when indicated that the use of biofertilizers in soil led to increasing the efficiency of plant to absorb nutrient elements, increases cell division, expands leaf cells, and then increases the leaf area of the plant.

Table (5): Effect of bacterial inoculation, phosphate fertilizer levels and different irrigation water salinity on the leaf area (cm^2) for the corn plants.

Bio-fertilizer	Salinity level	phosphate fertilizer levels			
		P0	P1	P2	P3
Non-inoculation T0	S1	5004	4252	5512	5628
	S2	4607	4856	5140	5269
PSI (T1)	S1	5375	5594	5863	5968
	S2	5024	5236	5499	5617
Azotobacter T2	S1	5503	5709	6074	6226
	S2	5255	5466	5752	5839
P.floresence T3	S1	5310	5527	5774	5953
	S2	5189	5390	5678	5779
The mixture inoculation T4	S1	5736	5982	6221	6309
	S2	5370	5570	5842	5946
RLSD 0.05		234			

Conclusions

1. The mixture bacterial inoculation increased plant height, dry weight and total yield of corn by 33.08%, 31.90% and 61.07%, respectively, compared to un inoculated treatments.

2. The mixture bacterial inoculation and single inoculation with bacteria dissolving phosphate and *P. fluorescens* bacteria led to reduce of phosphate fertilizer recommendation by 33% compared to Un inoculated.

3. The mixture bacterial inoculation and single inoculation has improved the growth and production of corn plant at against salt stress.

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