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role of bio-inoculation in the biological treatment of groundwater salinity and its effect on some chemical and biological soil properties.

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Abstract

A study was conducted during the summer season of 2025 at the College of Agriculture, University of Kufa, with the aim of biologically treating groundwater. This experiment included the sequential addition of two types of bacteria and one type of fungus (Control, *Providencia rettgeri*, *Bacillus amyloliquefaciens* and *Rhizoctonia solari*) to saline groundwater, as well as adding them to squash seedlings irrigated with two types of water (saline and fresh). The bacteria were isolated from multiple locations in the College of Agriculture soil, and sites clearly affected by salinity were selected. The experiment was then carried out, which included adding treatments B1, B2, F, and C to a field planted with squash seedlings irrigated with two types of irrigation water (saline and fresh). Their role in reducing the effects of salinity, pH, and the total number of soil bacteria was monitored.

The addition of *Rhizoctonia solari* achieved superiority, recording the lowest soil electrical conductivity using fresh and saline water, reaching 79.7 and 184.7 ds m^{-1}, respectively, compared to the control treatment, which recorded the highest electrical conductivity, reaching 154.7 and 620.3 ds m^{-1}, respectively. The same treatment also achieved superiority by raising the acidity from 6.333 to 5.833 .

The bacteria *Providencia rettgeri*, using fresh and saline water, demonstrated significant superiority in increasing the total bacterial count in the soil, recording the highest count of 89,000,000 and 61,000,000 CFU g^{-1} dry soil, respectively, compared to the control treatment, which recorded the lowest count of 26,000,000 and 3,300,000 CFU g^{-1} dry soil, respectively.

Key words: Soil bacteria, *Rhizoctonia solari*, bio-inoculum, soil salinity, fresh water

Introduction

The use of well-known agricultural practices, including the addition of mineral fertilizers to increase crop production, has been shown in studies to have multiple negative effects on humans and the environment. Hence, it's of significance employing fertilization techniques that reduce the use of pesticides and mineral fertilizers, lowering environmental pollution and mitigate soil salinity (Han et al., 2021). One such technique is the use of bio fertilizers, as are environmentally friendly alternative and a promising fertilizer that helps reduce the problem of soil salinity (Singh et al., 2020). Beneficial, *Providencia rettgeri*, non-pathogenic bacteria have become among the prominent microorganisms receiving widespread attention in the field of sustainable agriculture. Some strains of this bacterium, isolated from soil and rhizosphere, were found avirulence, to be candidates for bio agricultural applications (Chaudhary et al., 2022). These non-pathogenic strains can breakdown soil complex compounds, facilitating iron and zinc availability, resulting in more abundance organic and inorganic phosphates, and consequently increase plant root and shoot growth, and directly impacting productivity (Rahman et al., 2022).

Another bacterium, *Bacillus amyloliquefaciens*, is also distinguished by its important role in reducing salinity. It is effective in combating diseases such as root rot caused by fungi such as *Rhizoctonia solani* and *Fusarium* spp., using multiple mechanisms such as the production of antibiotics and enhanced root competition (Al-Sharif, 2020).

B. amyloliquefaciens also showed positive effects in improving soil properties, reducing salinity, and increasing fertilizer use efficiency (Al-Najjar, 2017). Meanwhile, *Rhizoctonia solani* is a widespread soil-borne fungus with an importance as a major cause root rot, stem canker, and seedling wilt in a number of economic crops (Akber et al., 2023). This fungus is classified into hyphal association groups known as Anastomosis Groups (AGs), which currently include more than 14 groups that differ in genetic makeup, pathogenicity, and family specialization (Umer et al., 2014). In addition, recent research has indicated the potential for using these strains integrated with *Bacillus velezensis* for inhibiting the growth of pathogenic *R. solani* AG-4 (Ji et al., 2022).

Given that salinity is a todays significant problem, particularly in Iraq, and due to the increasing geographical area of desertified and saline lands, the current study aimed to determine the effect of adding *Providencia rettgeri*, *Bacillus amyloliquefaciens*, and *Rhizoctonia solari* on soil salinity, pH, and the total number of soil bacteria.

Experimental Site

An experiment was conducted in one of the greenhouses in the fields of Babylon Governorate, in the Al-Azzawiya/Al-Musayyab Project, located north of the governorate at latitude and longitude (44.3308664, 32.0316912). The experiment was conducted for the summer season of 2025 in a sandy loam soil. The dimensions of the plastic field were 6 x 25 m. Soil samples were taken randomly from different locations in the field and subjected to chemical and physical analyses before planting (Table 1).

Material and methods

Table 1. Some physical and chemical properties of the field soil before planting.

properties		Value	unit
pH 1:1		7. 8	-
EC 1:1		3.07	dS m^{-1}
Cation	Ca^{+2}	8.47	Meq mol^{-1}
	Mg^{+2}	5.8 1	
Anion	Cl^{-1}	5.60	
	$SO4^{-2}$	2.78	
	$HCO3^{-1}$	7.65	
CEC		19.07	$Cmol_c$ Kg^{-1} soil
$CaCO_3$		231.00	g Kg^{-1} soil
O.M		8.51	
N		19.7	mg Kg^{-1} soil
P		8.1	
K		158.9	
Texture			Sandy Loam

Preparing Experimental Factors

Providencia rettgeri

A group of salt-tolerant *Providencia rettgeri* bacteria was isolated from the College of Agriculture, University of Kufa, from the rhizosphere of *Suaeda* sp. plants, which is characterized by its high salinity tolerance, Media specific to the bacteria (Nutrient Agar) was prepared according to back age recommended concentrations. The media were placed in an autoclave for sterilization. Samples were taken, and seven dilutions were made using dilution tubes and distilled water. Samples were then taken from these dilutions and placed in their respective media. They were then placed in an incubator for 48 hours, after which they were identified.

Bacillus amyloliquefaciens.

Samples were taken from saline soil at the College of Agriculture at the University of Kufa. Seven dilutions were made, from which samples were taken and placed in their respective media, which had been prepared and sterilized in advance. The samples were then placed in an incubator for 48 hours, after which they were identified.

Rhizoctonia solani

The fungus *Rhizoctonia solani* obtained from the plant pathology laboratory, Department of Plant Protection, University of Kufa, was used and activated on clean millet seeds. The seeds were soaked for 6 hours in a glass beaker, left on cheese cloth for an hour to remove excess water. Then, 50 g of the seeds were placed in 250 ml glass beakers, autoclaved for one full hour. Same sterilization process was performed the next day after which the seeds cooled and refrigerated until use (Akber, 2023) .

Preparing the *inocula*

Liquid media for bacterial culture

Six glass bottles were prepared and washed thoroughly, filled with 190 ml of distilled water and 2.6 ml of Nutrient Broth medium according to manufacturer. The bottles were then autoclaved for one hour. Three bottles were then inoculated with 10 ml of Nutrient Broth medium previously inoculated with *Providencia rettgeri* bacteria. They were then incubated for 48 hours. The other three bottles were inoculated with 10 ml of Nutrient Broth medium previously inoculated with *Bacillus Amyloliquefaciens* bacteria, which were incubated at 37°C for 48 hours.

Field Experiment Design and Cultivation

The experiment was in the greenhouse designed according to a split-plot design, two types of irrigation water, four treatments, and six replicates (three for fresh water and three for salt water), a total of 48 experimental units. The soil was plowed and smoothed, then the greenhouse was divided into two sectors, for subsurface drip irrigation with saline well water (W1) and fresh water (W0). Each sector was irrigated with three lines of 10 meters each with 1meter line distance. Each line was divided into four experimental units, and the number of experimental units in the field was (24) experimental units. The irrigation laods were supplied with fresh water from a river near the field, while the saline water was supplied from a well near the field as well. Then zucchini, variety *Cucurbit pepo*

L., were planted during March 2025. The seeds were planted in cork plates filled with peat moss and maintained for 11 days, then in 3/21/2024 were transplanted into the field lines on both sides of the sub-lines at a rate of 20 seedlings per line.

Studied Traits

- Soil Moisture Content (%) : The soil moisture content was calculated using the gravimetric method on a dry weight basis. Moist soil samples were dried in an oven at 105°C for 24 hours. It was then calculated using the method proposed by Gardner (1965) using the following equation:

$$Pw = ((Msw - Ms)/Ms) \times 100$$

Where:

Pw%: gravimetric moisture content.

Msw: mass of moist soil in grams.

Ms: mass of dry soil in grams.

- Soil Electrical Conductivity (EC) : was determined in a (1:1) soil : water extract using an EC meter according to the method described in Black (1965b).

- Soil pH : was determined in a (1:1) soil : water extract using a pH meter according to Black (1965b).

- Total number of soil bacteria : The total bacterial counts in the soil were estimated by the dilution and plate count method according to Black's (1965b) method. 10 g of soil was weighed and placed in a 250 ml volumetric flask containing 90 ml of distilled water. The mixture was shaken well for 10 minutes, and 1 ml of the soil was transferred to a test tube containing 9 ml of distilled water. The dilution process continued to obtain the decimal dilution series from 10^1 to 10^7. 1 ml of the 10^7 dilution was then transferred to Petri dishes containing nutrient agar for bacterial growth. The plates were incubated at 28°C and then removed for colony counting. The plates were incubated at 28°C, and the colonies were then counted using a colony counting device. Statistical Analysis of Experimental Data

The Genstate statistical (VSN, 2009) analysis software was used for analysis of variance, and a split-plot design was used. The means of the treatments were compared for significant differences according to least significant difference LSD test at 0.05 probability level (Al-Rawi and Khalafallah, 1980).

Results and Discussion

Effect of biological treatments on soil moisture (%)

The results of Table (2) show a clear impact on soil relative moisture, both from the quality of irrigation water and the biological treatments used. Soil moisture increased significantly when irrigated with saline water compared to fresh water. This is attributed to reduced water loss resulting from increased salinity and changes in the surface structure of the soil. The presence of dissolved salts in saline water increases osmotic pressure, which hinders water evaporation from the soil and maintains a higher moisture content. Salts also create less permeable surface layers in the soil, which also reduces water loss and increases soil moisture. Treatment F (which contained fungi) recorded the highest moisture level (29.33%) under saline conditions, while treatment C produced the lowest moisture level under the influence of fresh water, demonstrating the effectiveness of this treatment in improving the physical properties of the soil and enhancing its water retention. This is because these fungi can improve soil structure (Arora et al., 2008) or secrete substances that contribute to the formation of more cohesive and moist soil.

Table (2) Effect of biological treatments on soil moisture content (%)

Treatment	Irrigation water quality		Average
	W0	W1	
C	17.57	28.10	22.83
B1	19.83	22.27	21.05
B2	22.50	21.23	21.87
F	19.73	29.33	24.53
Average	19.91	25.23	
LSD 0.05	Irrigation water quality	Treatment	Interaction
	0.78	1.10	1.55

C:control, B1:Providencia rettgeri, B2:Bacillus amyloliquefaciens, F:Rhizoctonia solani, W0:fresh water, W1:saline well water

Effect of Bioremediation on Soil Electrical Conductivity (EC)

The results in Table (3) showed significant differences in soil electrical conductivity (EC) values depending on the type of irrigation water and the biological treatments added to the water. Saline irrigation water (W1) significantly increased soil salinity compared to fresh water (W0). This was attributed to the accumulation of dissolved salts resulting from poor leaching and high ionic concentrations in the root zone, especially given the limited drainage of salts from the soil (Xu et al., 2021 and Rengasamy, 2010). However, the introduction of biological treatments into irrigation water, particularly treatment F, led to a significant reduction in EC, with the lowest value recorded for treatment (132.2 µS/cm) under saline irrigation conditions, compared to 620.3 µS/cm in treatment C. This indicates that the microorganisms used in this treatment—mostly nutrient-solubilizing bacteria and symbiotic fungi—played a pivotal role in improving soil properties and reducing salt accumulation.

These results are attributed to several mechanisms, most notably:

• Stimulating microbial activity responsible for converting salts into less soluble forms or improving their uptake by plants (Rodríguez & Fraga, 1999).

• Also, potassium and phosphorus-solubilizing organisms increase the uptake of essential nutrients, reducing their presence as free ions, which contributes to raising EC (Basak & Biswas, 2010; Yan et al., 2015).

It was also observed that both treatments B1 and B2 also showed good effectiveness in reducing EC. Electrical conductivity decreased from 620.3 µS/cm in treatment C to:

• 412.7 µS/cm in treatment B1.

• 269.3 µS/cm in treatment B2, which was the most effective.

These results indicate that the use of biological treatments in water—even before adding it to the soil—can improve its chemical properties during irrigation and reduce the harmful effects of salinity on soil and crops. This is also confirmed by Xu et al. (2021), who stated that the improvement in ionic balance resulting from microbial activity may contribute to reducing apparent salinity in the root system.

Table (3) Effect of bioremediation on soil electrical conductivity (EC)

Treatment	Irrigation water quality		Average
	W0	W1	
C	154.7	620.3	387.5
B1	133.3	412.7	273.0
B2	126.0	269.3	197.7
F	79.7	184.7	132.2
Average	123.4	371.8	

LSD 0.05	Irrigation water quality	Treatment	Interaction
	15.6	22.1	31.2
C:control, B1:Providencia rettgeri, B2:Bacillus amyloliquefaciens, F:Rhizoctonia solani, W0:fresh water, W1:saline well water			

The Effect of Bioremediation on Soil pH

The results of Table (4) showed that soil pH was significantly affected by the quality of irrigation water and treatments. A significant decrease in pH was observed when using saline water, with the overall average reaching 6.217 compared to 7.333 under irrigation with fresh water. At the treatment level, treatment B2 recorded the highest average reaction rate of 7.083, while treatment F recorded the lowest value of 6.517. These differences were significant according to the LSD value of 0.216.

This may be attributed to the fact that treatment B2 may contain bacteria that produce ammonia or basic compounds, which led to an increase in the pH value. In contrast, treatment F reduced the reaction rate, which may indicate the production of organic acids that contributed to the decrease in soil pH (Nguyen, 2003). Although a decrease in EC is often associated with an increase in pH, the results of the experiment showed that the relationship was not linear, but rather affected by the nature of the organisms used in each treatment:

- Treatment F reduced both EC and pH, indicating the production of organic acids.
- Treatment B2 reduced EC and increased pH, indicating an ionic balance that contributes to neutralizing the effect of salts. This difference indicates that the type of organisms (bacterial or fungal) and their metabolic mechanisms are the decisive factor in changing the soil's chemical properties, not just the amount of salts (Wong et al., 2010).

Table (4) Effect of bioremediation on soil pH

Treatment	Irrigation water quality		Average
	W0	W1	
C	7.167	6.333	6.750
B1	7.267	6.233	6.750
B2	7.700	6.467	7.083

F	7.200	5.833	6.517
Average	7.333	6.217	
LSD 0.05	Irrigation water quality	Treatment	Interaction
	0.153	0.216	0.306n.s
C:control, B1:Providencia rettgeri, B2:Bacillus amyloliquefaciens, F:Rhizoctonia solani, W0:fresh water, W1:saline well water			

The effect of bioremediation and irrigation water quality on the total bacterial count in soil.

The results of Table (5) showed that the use of treatment (B1) led to a significant and significant increase in the total bacterial count in soil compared to treatment (C), whether under the influence of freshwater or saltwater irrigation, i.e., under the influence of salt stress. This means that it supports the growth of beneficial microbes, such as plant growth-promoting bacteria. Irrigation with freshwater enhanced this effect more than irrigation with saltwater, but saltwater did not inhibit the microbial response.

Treatment B2 also increased microbial activity compared to treatment (C), but its effect was less significant than treatment (B1).

The significant effect of the interaction indicates that freshwater enhanced the effectiveness of the treatments, particularly B1-B2, while saltwater reduced the response but did not inhibit the microbial response.

Table (5) Effect of bioremediation and irrigation water quality on the total bacterial count in soil (CFU g^{-1} dry soil)

Treatment	Irrigation water quality		Average
	W0	W1	
C	26000000	3300000	14650000
B1	89000000	61000000	75000000
B2	41000000	30200000	35600000
F	2300000	1500000	1900000
Average	39575000	24000000	
LSD 0.05	Irrigation water quality	Treatment	Interaction
	2837875.9	1239778.5	2251074.1

C:control, B1:Providencia rettgeri, B2:Bacillus amyloliquefaciens, F:Rhizoctonia solani, W0:fresh water, W1:saline well water

Conclusion

We conclude that the water treated with the salt-tolerant bacteria *Providencia rettgeri* recorded the highest total number of soil bacteria using fresh and saline water, and the treatment of adding the fungus *Rhizoctonia solani* achieved the lowest electrical conductivity using fresh and saline water and also maintained lower soil pH. Therefore, this study may recommend the use of *Providencia rettgeri* bacteria in bioremediation of saline water. Meanwhile, more focus might be toward *Rhizoctonia solani* as an effective bio agent that can improve water and soil quality, maintain water pH and tolerate salinity.

Recommendations

Therefore, it is recommended to use the salt-tolerant bacteria *Providencia rettgeri* in soil bio management due to its high ability to increase the total number of beneficial bacteria in the soil, whether the water is fresh or saline. It is also recommended to add the fungus *Rhizoctonia solani* to the soil when irrigating saline water sources, due to its effective role in reducing electrical conductivity, which reduces soil salinity. The combination of *Providencia rettgeri* and *Rhizoctonia solani* is an effective strategy for enhancing soil fertility, especially in areas affected by water stress or salinity.

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