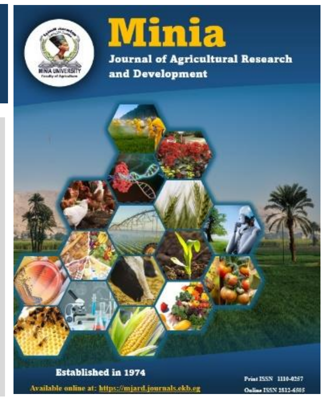
Minia J. of Agric. Res. & Develop., Minia Univ., Vol. 45 (4): 953 - 973, 2025

Minia Journal of Agricultural Research and Development

Journal homepage & Available online at:
https://mjard.journals.ekb.eg



Biofertilization of Barley with non-Symbiotic nitrogen-fixing bacteria and phosphate-solubilizing bacteria under different levels of salinity

Gaber Z. Breisha[1], Omar A. O. Saad[1], Afifi M. M. I[2] and Walaa M. Nagieb[1]

[1]Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Egypt.
[2] Soils, Water and Environmental Research Institute , Agricultural Research Center, Giza, Egypt.

Received: 23 Oct. 2025 Accepted: 30 Oct. 2025

ABSTRACT

Inoculating barley plant *(Hordeum vulgare L.)* with non-symbiotic nitrogen-fixing bacteria Azotobacter, Azospirillum and phosphate-solubilizing bacteria that were identified in previous study under different salinity levels was mainly attitude plant growth and enhanced salinity tolerance, especially when a combination of bacterial strains was applied.

Date showed that inoculation with these bacteria improved plant growth parameters, such as shoot, root biomass and nutrient absorption as well as total count of bacteria in barley rhizosphere, especially at moderate salinity levels. Also, inoculation was significantly enhanced plant hormone production, particularly in the mixed treatments. In addition , Organic acids such as citric acid, malic acid, and ascorbic acid were significantly influenced by salinity and bacterial inoculation. The activities of catalase (CAT), peroxidase (POX) enzymes, and proline content were significantly increased under salinity stress compared to the control treatment. The bacteria appeared to mitigate the negative impacts of salt stress by increasing the availability of nitrogen , phosphorus and essential nutrients for plant development.

Overall, the findings suggest that using non-symbiotic nitrogen-fixing and phosphate-solubilizing bacteria could be an effective, eco-friendly approach to improve barley plants cultivation in saline environments, potentially reducing dependence on chemical fertilizers and supporting sustainable farming practices.

Key words : Barley plant · Phosphate-solubilizing bacteria · Salinity stress · Biofertilizers · Nitrogen fixation · Salt tolerance.

INTRODUCTION:

Barley is rich in dietary fiber, proteins, vitamins, and minerals, contributing significantly to human health and nutrition (**Kumar *et al*., 2016).** Beyond its nutritional value, barley is widely used in the brewing industry for beer production, as well as in animal feed and various food products **(Khatri *et al*., 2017).** Its versatility and resilience have led to its extensive cultivation across the globe, particularly in regions with challenging climatic conditions **(Bouis and Welch, 2010).**

*** Corresponding author: Omar A. O. Saad**
E-mail address: omr.saad@gmu.edu.eg

Research efforts continue to focus on improving barley yield, disease resistance, and nutritional quality through breeding programs, aiming to enhance its role in sustainable agriculture and food security **(Kumar *et al*., 2018**). This aligns with global goals to reduce food loss and improve agricultural sustainability (**FAO, 2019**). Overall, barley remains a crucial crop with economic, nutritional, and industrial significance worldwide**.**

Soil salinity is a growing challenge in agriculture, especially in regions where poor-quality irrigation water and low rainfall are common. High salt concentrations in soil can impair plant growth by reducing water uptake, disturbing nutrient availability, and causing oxidative and ionic toxicity at the cellular level **(El-Sayed *et al*., 2023 and Masrahi *et al*., 2023**). As a result, even moderately salt-tolerant crops like barley (*Hordeum vulgare* L.) often exhibit reducing growth and yield under saline conditions (**Khandan Gorgan *et al*., 2024).**

In recent years, researchers have been exploring sustainable and eco-friendly alternatives to chemical fertilizers to improve plant tolerance to salinity. One promising solution is the use of biofertilizers beneficial microorganisms that enhance plant nutrition and stress resilience. Among these are non-symbiotic nitrogen-fixing bacteria such as *Azotobacter chroococcum* and *Azospirillum brasilense*, which fix atmospheric nitrogen and produce phytohormones like IAA and gibberellins (**Hayat *et al.*, 2010; Bhardwaj *et al*., 2014**). Similarly, phosphate-solubilizing bacteria (PSB) like *Bacillus megaterium* release organic acids that convert insoluble phosphorus into forms available to plants, improving root development and overall plant performance under stress (**Rodríguez, 1999; Bharti *et al*., 2022).**

This study investigates the effects of applying *Azotobacter chroococcum* , *Azospirillum brasilense* and *Bacillus megaterium* bacteria, as biofertilizers individually or combination on the growth and salt tolerance of barley plant grown under different salinity levels. The objective is to identify biological strategies that support barley production ,while preserving soil health in salt-affected areas.

2. MATERIALS AND METHODS:

Barely seeds:

Barely plant as a winter crop (Giza 123), seeds were obtained from Barley Research Department- Field Crops research Institute - Agricultural Research Center. Egypt.

2.1 Experimental design and site description

This experiment was conducted in pots under control in the Microbiology Laboratory and the green house of the faculty of Agriculture, University of Minia. Pot experiments were set up during winter season (2023). These study aime to investigate the effect of biofertilizers on barley (*Hordeum vulgar* L.) under different salinity levels and the experiment followed a completely randomized design (CRD) with five replicates for each treatment.

Soil samples analysis.

Soil was collected from the surface layer (0–30 cm), air-dried, sieved (2 mm) and analyzed for initial physicochemical properties as shown in Table (1)

Table (1) Physical and chemical analyses of the tested soil:

Physical analysis					Chemical analysis								Total Nitrogen %	Available Phosphorus %
pH	Sandy %	Salt %	Clay %	Texture	Anions				Cations					
					SO_4	CL	HCO_3	CO_3	K^+	Na^+	Mg^{+2}	Ca^{+2}		
8	8.7	29.5	61.9	Clay loam	0.7	2.2	2.8	0.5	0.4	1.6	2.3	1.6	14.6	0.72

Treatments:

Salinization of the used Soil

To induce salinity stress, artificial salinization of the soil was performed using two types of salts: sodium chloride (NaCl) and calcium chloride ($CaCl_2$). Different salinity levels were prepared corresponding to electrical conductivity (EC) values ranging from EC2 to EC8. Electrical Conductivity (EC) is a measure of the ability of a solution (such as soil and water) to conduct an electric current. It reflects the total concentration of soluble salts (ions) in the soil or irrigation water. Higher EC values indicate higher salinity levels. EC is commonly measured in dS/m (deci Siemens per meter) and is widely used as an indicator of soil salinity, which can influence plant water uptake, nutrient availability, and overall growth performance.

The following concentrations of salts were dissolved in 100 liters of water and applied to 250 kg of clay soil for each EC level: EC2: 156 g of NaCl + 156 g of $CaCl_2$ per 100 L of water, EC4: 312 g of NaCl + 312 g of $CaCl_2$ per 100 L of water, EC6: 468 g of NaCl + 468 g of $CaCl_2$per 100 L of water and EC8: 624 g of NaCl + 624 g of $CaCl_2$per 100 L of water.

This method allowed for the controlled simulation of salinity stress in the experimental soil. **Salinity levels**: Five levels of irrigation water salinity were used: each level was 6 replicates.

Treatment EC0: (control) without salinity or inoculation.

without salinity with *Azot.ch.* inoculum.
without salinity with *Azosp.br.* inoculum .
without salinity with *B.meg.* inoculum.
without salinity with mixed of *Azot.ch.*, *Azosp.br.* and *B.meg.* inoculums.

Treatment EC2 ds m^{-1}(control) with salinity and without inoculation

with salinity and inoculation *Azot.ch.*.
with salinity and inoculation *Azosp.br.* .
with salinity and inoculation *B.meg.*.
with salinity and inoculation mixed of *Azot.ch.*, *Azosp.br.* and *B.meg.* inoculums.

Treatment EC4 ds m^{-1}(control) with salinity and without inoculation

with salinity and inoculation *Azot.ch.*.
with salinity and inoculation *Azosp.br.*
with salinity and inoculation *B.meg*.
with salinity and inoculation mixed *Azot.ch.*., *Azosp.br.* and *B.meg*

Treatment EC6 ds m^{-1}(control) with salinity and without inoculation

with salinity and inoculation *Azot.ch*.
with salinity and inoculation *Azosp.br.*
with salinity and inoculation *B.meg*.
with salinity and inoculation mixed *Azot.ch.*., *Azosp.br.* and *B.meg*

Treatment EC8 ds m^{-1}(control) with salinity and without inoculation

with salinity and inoculation *Azot.ch.*
with salinity and inoculation *Azosp.br.* .
with salinity and inoculation *B.meg*.
with salinity and inoculation mixed *Azot.ch.*, *Azosp.br.* and *B.meg*

2.4 Pot experiment

Soil was collected from the surface layer (0–30 cm), air-dried, sieved (2 mm). Plastic pots (30 cm diameter) were filled with 5 kg of soil. Biofertilizers were applied by sowing at a rate of 10^5CFU/mL per strain. Initially, Barley seeds were surface-sterilized with 1% sodium hypochlorite, rinsed thoroughly multiple times with water, then soaked in inoculum solution and sown in pots (10 seeds per pot). After germination, seedlings were thinned to 5 per pot. Irrigation with water tap began 10 days after emergence and was adjusted according to pot weight to maintain field capacity.

2.5 Bacteria strains and preparation inoculum

Azotobacter chroococcum (Azoto.ch.), *Azospirillum brasilense* (Azosp.br.) and *Bacillus megaterium* (B. meg.) bacteria were isolated from soil rhizospher in Kafr Elshikh Governorate, Egypt . Strains were identified by 16S RNA by **Breisha *et al.* (2025)**. Pure isolates of Azoto. ch., Azosp.br. inoculation for 48 hours while, B. meg.) inoculation for 24 hours. Each bacterium was grown separately in nutrient broth medium at 28°C under sterile conditions. *Azotobacter* chroococcum and *Azospirillum brasilense* were cultured nitrogen-fixing and PSB isolates were propagated singly in conical flasks 250 ml containing 50 ml of the nutrient broth. The cultures were incubated in a shaking incubator at 150 rpm to ensure good aeration. After incubation, the bacterial cells were adjusted to a concentration of about 10^8 colony forming units per milliliter (CFU/mL) using sterile distilled water. For the mixed inoculum, equal volumes from the three bacterial cultures were combined before application. Barley seeds were soaked in the bacterial suspension for one hour before sowing. The same inoculum was also applied by soil drenching after the appearance of seedlings**.**

2.6 Planting and irrigation

Barley seeds were surface-sterilized with 1% sodium hypochlorite, rinsed thoroughly several times with water, then soaked in inoculum solution and sown in pots (10 seeds per pot). After germination, seedlings were thinned to 5 per pot. Irrigation with tap water began 10 days after emergence and was adjusted according to pot weight to maintain field capacity.

2.7 Growth Measurements

Barley plants grown in pots were carefully uprooted after 30, 45, 60, and 90 days of growing. Then, the plants were thoroughly hand-washed to remove soil particles from the roots, then, heights of plants, plant fresh weight and dry weight were recorded.

2.8 Bacterial measurements :

Rhizosphere samples were taken after 30-, 45-, 60-, and 90 days from sowing for determination total counts of bacteria Azotobacter , Azospirillum and PSB using the serial dilution and plate count method. **(Abdel-Malek** and **Ishac 1968** and **Subba**, **1999)** for total Azotobacter , Semi-solid Malate Medium **(Dobereiner, 1980)** was used for counting Azospirillum and **(Pikovskaya, 1948)** for total PSB. For Azotobacter and Azospirillum, incubation was carried out at 28–30°C for 14 days ,while PSB was done the same temperature for 48–72 hours. After incubation, colonies were counted and expressed as colony-forming units per milliliter CFU/mL

Determination of Phytohormones (IAA, IBA, GA3)

Endogenous phytohormones, including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and gibberellic acid (GA3), were quantified at 60 and 90 days after sowing. Plant tissues were homogenized in 80% ethanol for extraction. IAA and IBA were determined colorimetrically using the Salkowski reagent method **(Gordon and Weber, 1951)**. GA3 was estimated spectrophotometrically according to **Holbrook *et al.* (1961**).

2.10 Determination of Organic Acids (Citric, Malic, and Ascorbic)

Organic acids were extracted from plant tissues using ethanolic extraction. Citric and malic acids were determined using standard spectrophotometric or HPLC methods **(AOAC, 2005)**. Ascorbic acid content was quantified by titration with 2,6-dichlorophenolindophenol (DCPIP).

2.11 Determination of Antioxidive Enzymes and Proline

Catalase (CAT) activity was determined by monitoring the decrease in absorbance at 240 nm due to H_2O_2 decomposition **(Aebi, 1984)**. Peroxidase (POX) activity was assayed at 470 nm using guaiacol as substrate **(Chance and Maehly, 1955)**. While Proline content was determined according to the ninhydrin-based method of **Bates *et al.* (1973)**, measuring absorbance at 520 nm.

2.12 Chemical composition of plant:

Determination of plant mineral contents (N, P and K %): Fresh samples of 100 g of plant were oven dried at 70° C for 48 h till weight constant. The dry matter was finely ground to a fine powder then sub sample of 0.2 gm was wet digested with sulphuric acid - perchloric acid mixture (1:1) as described by **A.O.A.C (2000)**, to assay nutrient elements.

-Total nitrogen (N%) was determined by the modified microkjeldahl method as described by **Jones *et al*. (1991).**

-Total Phosphurus(P) molybdophosphoric blue colour which was determined photometrically using UV spectrophotometrically (model no. UV 2100 S/N: BH 16041603003) according to **Olsen *et al* . (1954)**

-Total potassium(K) was determined in colourless extract of plant obtained by digestion in sulphuric per-chloric (1:1) acids mixture. using Flame-Photometer (JENWAY PFP7 model) according to **Peters *et al*. (2003).**

2.13 Statistical analysis:

The experiment was arranged in a completely randomized design (CRD) with six replicates per treatment. The treatments included various salinity levels and bacterial inoculations. Data for plant height, fresh weight, and dry weight were subjected to analysis of variance (ANOVA) to evaluate the effects of salinity, bacterial treatments, and their interaction. Mean comparisons were performed using the Least Significant Difference (LSD) test at the 5% significance level ($P \leq 0.05$). Results are expressed as mean ± standard error (SE), and means followed by different letters indicate significant differences.

All statistical analyses were performed using CoStat version 6.400 (**CoHort Software, freely available since 2022**) for ANOVA, mean comparisons, and data manipulation.”

3-RESULTS AND DISCUSSION:

The five most efficient isolates of each bacterial type ***Azot. ch., Azosp. br*., and *B.meg*** were selected based on their growth performance and functional activity. These selected isolates were used to prepare the inoculants for seed treatment and soil application in the experiment.

Data in Table (2) demonstrated that the increase of levels salinity were synchronized with decline MPN/g of ***Azoto.ch., Azosp.br.* and *B. meg.*** The results shown the mix treatment was significantly the highest value of bacteria in all levels of salinity at period 60 day, the MPN/g ranged from 0.95,0.98 and 147 at level of EC0 then reduction to 0.90, 0.95 and 135 at level EC8 with ***Azoto.ch., Azosp.br.* and *B. meg,*** respectively.

Table (2) Bacterial count of *Azot. ch.*, *Azosp. br.*, and *B.meg* in the rhizosphere of Barley plant under different levels of salinity after 60 days from sowing.

Salinity Levels	Sample	*Azoto.ch.* (MPN ×10^6 /g)	Azosp.br. (MPN ×10^6/g)	*B.meg.* (CFU/plate)
EC0	1	0.33	0.43	56
	2	0.53	0.43	62
	3	0.35	0.85	93
	4	0.39	0.63	127
	5	0.95	0.98	147
EC2	6	0.23	0.33	59
	7	0.42	0.33	62
	8	0.24	0.75	86
	9	0.28	0.45	119
	10	0.95	0.95	140
EC4	11	0.23	0.30	52
	12	0.33	0.25	54
	13	0.23	0.43	66
	14	0.43	0.27	143
	15	0.82	0.93	139
EC6	16	0.20	0.25	51
	17	0.29	0.31	53
	18	0.23	0.40	63
	19	0.43	0.39	112
	20	0.75	0.75	137
EC8	21	0.18	0.23	47
	22	0.25	0.23	50
	23	0.20	0.39	59
	24	0.43	0.55	111
	25	0.90	0.93	135

So, after 60 day from sowing, the population densities of *Azot. ch.* and *Azosp.br.*, measured by the MPN technique, were relatively high, indicating an active establishment of these nitrogen-fixing bacteria and the same trend was *B. meg.* in the rhizosphere during the early growth stages of barley plants (**Bashan and de-Bashan, 2010).**

The total bacterial count after 90 days indicated a significant increase in rhizosphere microbial populations in barley plants inoculated with beneficial bacteria compared to uninoculated controls. The highest microbial density was recorded in the combined treatment (*Azoto. ch.* + *azosp.br.* + *Bacillus megaterium*), especially under moderate salinity levels (EC4 and EC6), as shown in Table (3). This suggests a strong synergistic effect among the inoculants that promoted microbial survival and colonization under saline conditions. These findings are consistent with previous studies ,which reported that mixed inoculation of salt-tolerant plant growth-promoting rhizobacteria (PGPR) enhances microbial population density and root colonization in saline soils (**Ruppel *et al*., 2013; Egamberdieva *et al*., 2017**). The resilience of these inoculants could be attributed to their ability to produce extracellular polysaccharide and oxidative enzymes, which help in maintaining microbial viability under stress **(Nautiyal *et al*., 2013; Vurukonda *et al*., 2016).** Moreover, The increases of bacterial count may also be associated with better root exudate by host plants under microbial stimulation, creating a favorable niche for microbial proliferation **(Bharti *et al*., 2016).** Plants inoculated with PGPR often exhibit enhanced rhizodeposition, which in turn supports the persistence of beneficial microbes in the rhizosphere **(Backer *et al*., 2018).**

Table (3): Bacterial count of *Azoto.ch.*, *Azosp.br.*, and *B.meg.* in the rhizosphere of Barley plant under different levels of salinity after 90 days from sowing.

Salinity Levels	Sample	*Azoto.ch.* (MPN $\times 10^6$ /g)	*Azosp.br.* (MPN $\times 10^6$ /g)	*B.meg.* (CFU/plate)
EC0	1	0.13	0.40	55
	2	0.23	0.13	58
	3	0.13	0.43	81
	4	0.23	0.23	85
	5	0.25	0.33	95
EC2	6	0.11	0.40	50
	7	0.43	0.23	55
	8	0.23	0.43	71
	9	0.13	0.13	82
	10	0.13	0.45	85
EC4	11	0.11	0.43	49
	12	0.43	0.23	51
	13	0.13	0.13	34
	14	0.13	0.43	67
	15	0.43	0.13	84
EC6	16	0.10	0.23	45
	17	0.23	0.23	49
	18	0.13	0.13	59
	19	0.15	0.13	85
	20	0.39	0.13	88
EC8	21	0.9	0.13	42
	22	0.23	0.23	45
	23	0.13	0.23	55
	24	0.13	0.13	80
	25	0.25	0.23	83

Table (4) Effect of inoculation with *Azoto.ch.,Azosp. br.*, *B.meg.* and mixture of them under salinity levels on growth parameters of barely plant after 60 days from sowing

Salinity Levels	Treatments	Plant Height ± SE (cm)	Fresh Weight ± SE (g)	Dry Weight ± SE (g)
Ec 0	Control	43.80 ^{e} ± 1.06	7.28 ^{d} ± 0.50	2.77 ^{e} ± 0.30
	Azoto. ch.	48.20 ^{d} ± 1.05	8.10 ^{cd} ± 0.45	3.05 ^{d} ± 0.28
	Azosp. br.	55.00 ^{c} ± 1.00	9.00 ^{bc} ± 0.42	3.50 ^{c} ± 0.27
	B. meg.	52.50 ^{cd} ± 0.95	9.40 ^{b} ± 0.40	3.70 ^{bc} ± 0.26
	Azoto. ch.+ Azosp. br.+ B. meg	60.50 ^{a} ± 0.90	12.80 ^{a} ± 0.48	4.60 ^{a} ± 0.25
EC2	Control	47.00^{e} ± 1.20	8.40 ^{d} ± 0.65	2.30 ^{f} ± 0.22
	Azoto. ch.	51.00^{d} ± 1.22	7.83 ^{cd} ± 0.40	2.22 ^{f} ± 0.18
	Azosp. br.	60.00^{b} ± 1.06	8.39 ^{dc} ± 0.33	2.49 ^{cf} ± 0.26
	B. meg.	52.00 ^{cd} ± 1.00	9.20 ^{b} ± 0.42	2.79 ^{c} ± 0.29
	Azoto. ch.+ Azosp. br.+ B. meg	62.00 ^{a} ± 1.02	13.79 ^{a} ± 0.54	4.81 ^{a} ± 0.31
EC4	Control	50.00 ^{d} ± 1.35	6.20 ^{d} ± 0.49	2.97 ^{e} ± 0.33
	Azoto. ch.	60.00 ^{ab} ± 1.10	9.83 ^{d} ± 0.36	2.68 ^{d} ± 0.27
	Azosp. br.	61.00^{b} ± 1.10	10.33 ^{b} ± 0.41	4.07 ^{b} ± 0.29
	B. meg.	57.00 ^{bc} ± 1.08	9.80 ^{b} ± 0.39	2.89 ^{e} ± 0.30
	Azoto. ch.+ Azosp. br.+ B. meg	63.00 ^{a} ± 0.90	13.64 ^{a} ± 0.48	4.80 ^{a} ± 0.26
EC6	Control	44.00 ^{e} ± 1.25	5.59 ^{e} ± 0.55	2.09 ^{f} ± 0.24
	Azoto. ch.	52.00 ^{cd} ± 0.94	8.72 ^{bc} ± 0.44	3.35 ^{c} ± 0.28
	Azosp. br.	59.00 ^{ab} ± 0.98	9.21 ^{bc} ± 0.37	4.01 ^{b} ± 0.35
	B. meg.	50.00 ^{d} ± 1.20	9.55 ^{bc} ± 0.41	3.19 ^{d} ± 0.27
	Azoto. ch.+ Azosp. br.+ B. meg	63.00 ^{a} ± 0.95	13.64 ^{a} ± 0.48	4.47 ^{a} ± 0.29
EC8	Control	39.00 ^{g} ± 1.15	4.74 ^{e} ± 0.50	0.98 ^{g} ± 0.16
	Azoto. ch.	51.80 ^{fg} ± 1.00	7.15 ^{cd} ± 0.36	2.50 ^{cf} ± 0.23
	Azosp. br.	40.80 ^{g} ± 0.92	7.35 ^{cd} ± 0.32	2.01 ^{f} ± 0.18
	B. meg.	41.00 ± 1.05 f	7.10 ± 0.38 c	2.10 ^{f} ± 0.20
	Azoto. ch.+ Azosp. br.+ B. meg	49.00 ± 1.10 e	9.11 ± 0.42 bc	3.77 ^{c} ± 0.30
LSD (0.05)		4.62	1.22	0.89

The integrated analysis shows that biofertilizer treatments, especially the mixed inoculation (Azotobacter + Azospirillum + PSB), significantly enhanced barley growth under all salinity levels. The mixture consistently received the highest group rank (a, b) and showed superior performance in plant height, fresh weight, and dry weight, indicating synergistic effects among the bacteria **(Egamberdieva *et al*., 2017; Aasfar *et al*., 2021; El-Saadony *et al*., 2022)**

At higher salinity levels (EC6 and EC8), plants without inoculation fell into the lowest statistical groups, confirming the detrimental effects of salt stress (**Shrivastava and Kumar, 2015)**. However, inoculation, particularly with the bacterial mixture, helped maintain growth, highlighting its potential in promoting salt tolerance **(Zhao *et al*., 2016; Bakhoum *et al*., 2022)**

These results emphasize the importance of microbial consortia in improving barley resilience under saline conditions and support their use as eco-friendly alternatives to chemical fertilizers (**Bharti *et al*., 2022; Kour *et al*., 2021**).

Data in Table (4) cleared that at period 60 day from sowing, the inoculated plants with the mixed bacterial showed significantly the highest plant height and biomass compared to control, particularly under moderate salinity levels (EC 2 and 4). However, growth was reduced under severe salinity (EC8), regardless of inoculation. These results suggest that biofertilizers mitigate salinity stress, likely by improving nutrient uptake and enhancing root development

This could be attributed to the favorable root exudates and soil conditions that support microbial growth during the initial plant development.

Similarly, PSB, counted on phosphate-solubilizing agar, showed substantial colony-forming units, which suggests efficient colonization and phosphate-solubilizing activity at this stage. These results align with earlier findings that beneficial rhizobacteria reach their peak densities during the vegetative stage, enhancing nutrient availability and plant growth.

This elevated microbial density plays a crucial role in improving nutrient cycling, hormone production and stress tolerance in the host plant, further emphasizing the importance of using mixed microbial inoculants as sustainable biofertilizers in saline agroecosystems **(Shrivastava and Kumar, 2015; Grover *et al*., 2020)**

The results of the present study demonstrated that inoculating barley plants with a mixture of Azotobacter, Azospirillum, and PSB significantly enhanced plant performance under different salinity levels. The combined inoculation led to a remarkable improvement in plant height, fresh weight, and dry weight compared to the uninoculated control, even under elevated salinity (EC6 and EC8). This enhancement in growth parameters suggests a synergistic effect among the three microbial strains used.

The observed improvement could be attributed to multiple beneficial mechanisms exerted by the bacterial consortium. Azotobacter and Azospirillum are well-known for their nitrogen-fixing capabilities and production of phytohormones such as indole-3-acetic acid (IAA), which promote root elongation and nutrient uptake (**Bharti *et al*., 2016; Egamberdieva *et a*l., 2017**). Meanwhile, *Bacillus megaterium* is recognized for its efficient phosphate-solubilizing ability, enhancing the bioavailability of phosphorus—a critical nutrient often limited under saline conditions (**Vurukonda *et al*., 2016)**

Moreover, the inoculated plants likely experience reduced ionic toxicity and oxidative stress, which are major constraints under saline soils. The bacterial consortium may have improved the plant's salt tolerance by producing exopolysaccharides, enhancing osmotic balance and triggering systemic tolerance responses **(Goswami *et al*., 2014; Rojas-Tapias *et al.*, 2012)**

The significant increase in dry matter accumulation in the inoculated plants under salinity stress also indicates an enhancing photosynthetic capacity and metabolic activity, likely due to better nutrient acquisition and hormonal regulation. This is in agreement with the findings of **(Vurukonda *et al*. (2016**), who reported that co-inoculation with PGPR strains under saline stress improves plant vigor and biomass production.

Table (5) Effect of inoculation with Azotobacter,Azospirillum, PSB and mixture of them under salinity levels on plant height, fresh and dry weight of barely plant after 90 days from sa.owing

Salinity Levels	Treatments	Plant Height ± SE (cm)	Fresh Weight ± SE (g)	Dry Weight ± SE (g)
Ec 0	Control	48.2 ± 1.10f	9.30 ± 0.55e	3.51 ± 0.30f
	Azoto. ch.	54.0 ± 1.15 e	10.20 ± 0.58 de	3.90 ± 0.31 ef
	Azosp. br.	60.5 ± 1.10 cd	11.50 ± 0.55 cd	4.30 ± 0.30 de
	B. meg.	57.8 ± 1.12 de	12.20 ± 0.57 c	4.60 ± 0.29 d
	Azoto. ch.+ Azosp. br.+ B. meg	65.0 ± 1.05 b	15.40 ± 0.60 b	5.60 ± 0.32 b
EC2	Control	51.5 ± 1.25ef	9.85 ± 0.62e	3.60 ± 0.33f
	Azoto. ch.	61.3 ± 1.30cd	11.80 ± 0.60cd	4.18 ± 0.32e
	Azosp. br.	66.1 ± 1.15bc	12.90 ± 0.58c	4.53 ± 0.29de
	B. meg.	56.7 ± 1.20de	13.40 ± 0.63c	4.80 ± 0.30de
	Azoto. ch.+ Azosp. br.+ B. meg	68.3 ± 1.10ab	17.30 ± 0.60a	6.00 ± 0.34a
EC4	Control	55.2 ± 1.40de	8.95 ± 0.58e	3.91 ± 0.32ef
	Azoto. ch.	65.5 ± 1.15bc	13.60 ± 0.61c	4.39 ± 0.29de
	Azosp. br.	67.0 ± 1.20ab	14.40 ± 0.65bc	5.35 ± 0.30bc
	B. meg.	62.2 ± 1.25cd	13.80 ± 0.62c	4.30 ± 0.33de
	Azoto. ch.+ Azosp. br.+ B. meg	70.4 ± 1.10a	17.10 ± 0.55a	5.98 ± 0.32a
EC6	Control	48.8 ± 1.30f	7.20 ± 0.57f	3.18 ± 0.31g
	Azoto. ch.	58.7 ± 1.25de	12.20 ± 0.60cd	4.65 ± 0.34de
	Azosp. br.	66.0 ± 1.10bc	13.00 ± 0.63c	5.30 ± 0.30bc
	B. meg.	56.5 ± 1.20de	12.50 ± 0.58c	4.80 ± 0.31de
	Azoto. ch.+ Azosp. br.+ B. meg	68.5 ± 1.00ab	15.00 ± 0.59b	5.81 ± 0.28ab
EC8	Control	36.1 ± 1.05h	6.00 ± 0.50g	1.40 ± 0.22h
	Azoto. ch.	40.9 ± 1.00g	9.20 ± 0.54e	3.00 ± 0.28f
	Azosp. br.	43.0 ± 1.10fg	9.40 ± 0.55e	2.91 ± 0.26f
	B. meg.	44.5 ± 1.20fg	9.10 ± 0.51e	3.10 ± 0.25f
	Azoto. ch.+ Azosp. br.+ B. meg	48.2 ± 1.15f	12.10 ± 0.57cd	4.85 ± 0.30cd
LSD (0.05)		4.62	1.22	0.89

3.Biochemical Parameters under Salinity Stress and Bacterial Inoculation

1. Plant Hormones (IBA, IAA and GA_3)

The results cleared that plant products including butyric acid (IBA) , indole-3-acetic acid (IAA), and gibberellic acid (GA_3), exhibited marked variations in response to salinity and bacterial inoculation. Salinity (EC8) generally reduced endogenous hormone levels, indicating the inhibitory effect of salt stress on growth-promoting hormones. However, inoculation with Azotobacter, Azospirillum, and *Bacillus megaterium* significantly enhanced hormone production, particularly in the mixed inoculation treatment. PGPR are well known to synthesize phytohormones such as IAA and gibberellins, which promote root development, cell elongation, and nutrient uptake. The observed increase in IBA further suggests stimulation of root initiation processes, contributing to improved plant adaptation under saline conditions. Similar results were reported by **Dobbelaere *et al*. (2003), Spaepen *et al.* (2014),** and **Egamberdieva *et al*.** (**2019),** who emphasized the role of PGPR in modulating phytohormonal balance under stress environments

Table (6): Effect of different inoculation on Phytohormones after 60 and 90 days

Salinity levels	Treatments	IBA Mean ± SE		IAA Mean ± SE		GA3 Mean ± SE	
		60days	90days	60days	90days	60days	90days
EC0	Control	15.0^{c}±0. 5	13.8^{c}±0.4	14.00^{c}±0.4	12.6^{c} ±0.4	9.5^{c} ±0.3	8.4^{c} ±0.3
	Azoto. ch.	16.5^{b} ±0.5	15.2^{b}±0.5	15.40^{b}±0.5	13.9^{b}±0.4	10.6^{b} ±0.3	9.20^{b} ±0.3
	Azosp.br.	16.2^{ab}±0.5	14.9^{ab}±0.5	15.12^{ab}±0.5	13.6^{ab}±0.4	10.26^{ab}±0.3	9.03^{ab}±0.3
	B. meg	15.9^{bc}±0.5	14.6^{bc}±0.4	14.84^{b} ±0.5	13.4^{bc}±0.4	10.1^{bc} ±0.3	8.7^{bc} ±0.3
	Mix	17.3^{a} ±0.5	15.9^{a} ±0.5	16.10^{a} ±0.5	14.5^{a} ±0.4	10.9^{a} ±0.3	9.6^{a} ±0.3
EC2	Control	13.8^{c} ±0.4	12.7^{c} ±0.4	12.88^{c} ±0.4	11.6^{c} ±0.4	8.7^{c} ±0.3	7.7^{c} ±0.2
	Azoto. ch.	15.2^{b} ±0.5	13.9^{b} ±0.4	14.17^{b} ±0.4	12.8^{b} ±0.4	9.6^{b} ±0.3	8.5^{b} ±0.3
	Azosp.br.	14.9^{ab}±0.4	13.7^{ab}±0.41	13.91^{ab}±0.4	12.5^{ab}±0.4	9.4^{ab} ±0.3	8.3^{ab} ±0.3
	B.meg	14.6^{bc}±0.4	13.5^{bc} ±0.4	13.6^{bc} ±0.4	12.3^{bc}±0.4	9.3^{bc} ±0.28	8.2^{bc} ±0.2
	Mix	15.9^{a} ±0.5	14.6^{a} ±0.4	14.81^{a} ±0.4	13.3^{a} ±0.4	10.1^{a} ±0.30	8.8^{a} ±0.3
EC4	Control	12.3^{c} ±0.4	11.3^{c} ±0.3	11.5c ±0.3	10.3^{c} ±0.3	7.8^{c} ±0.23	6.9^{c} ±0.2
	Azoto. ch.	13.5^{b} ±0.4	12.5^{b} ±0.4	12.63^{b} ±0.4	11.4^{b} ±0.3	8.6^{b} ±0.26	7.5^{b} ±0.2
	Azosp.br.	13.3^{ab}±0.4	12.2^{ab} ±0.4	12.4^{ab} ±0.4	11.2^{ab}±0.3	8.4^{ab} ±0.25	7.4^{ab} ±0.2
	B.meg	13.0^{bc}±0.4	11.9^{bc} ±0.4	12.2^{bc} ±0.4	10.9^{bc}±0.3	8.26^{bc} ±0.3	7.27^{bc}±0.2
	Mix	14.1^{a} ±0.4	13.0^{a} ±0.4	13.2^{a} ±0.4	11.88^{a}±0.4	8.96^{a} ±0.3	7.88^{a} ±0.2
EC6	Control	10.8^{c} ±0.3	9.9^{c} ±0.3	10.1^{c} ±0.3	9.07^{c} ±0.3	6.84^{c} ±0.2	6.02^{c} ±0.2
	Azoto. ch.	11.9^{b} ±0.4	10.9^{b} ±0.3	11.1^{b} ±0.3	9.98^{b} ±0.3	7.52^{b} ±0.2	6.62^{b} ±0.2
	Azosp.br.	11.7^{ab}±0.4	10.7^{ab} ±0.3	10.9^{ab} ±0.3	9.80^{ab}±0.3	7.39^{ab}±0.2	6.5^{ab} ±0.2
	B.meg	11.5^{bc}±0.3	10.5^{bc} ±0.3	10.7^{bc} ±0.3	9.62^{bc}±0.3	7.25^{bc} ±0.2	6.38^{bc}±0.2
	Mix	12.4^{a} ±0.4	11.4^{a} ±0.3	11.6^{a} ±0.4	10.43^{a}±0.3	7.87^{a} ±0.24	6.92^{a} ±0.2
EC8	Control	9.00^{c} ±0.3	8.3^{c} ±0.3	8.4^{c} ±0.3	7.56^{c} ±0.3	5.70^{c} ±0.2	5.02^{c} ±0.2
	Azoto. ch.	9.9^{b} ±0.3	9.1^{b} ±0.3	9.24^{b} ±0.3	8.32^{b} ±0.3	6.27^{b} ±0.2	5.52^{b} ±0.2
	Azosp.br.	9.7^{ab} ±0.3	8.9^{ab} ±0.3	9.07^{ab} ±0.3	8.16^{ab}±0.3	6.16^{ab} ±0.2	5.4^{ab} ±0.2
	B.meg	9.5^{bc} ±0.3	8.8^{bc} ±0.3	8.9^{bc} ±0.3	8.01^{bc}±0.3	6.04^{bc} ±0.2	5.32^{bc}±0.2
	Mix	10.4^{a} ±0.3	9.5^{a} ±0.3	9.66^{a} ±0.3	8.69^{a} ±0.3	6.55^{a} ±0.2	5.77^{a} ±0.2
LSD (5%)		IBA : 1.10		IAA:1.05		GA3: 0.70	

Mix (*Azoto.ch. +Azosp.br. + B. meg*.)

Table (7) Effect of inoculation with Azotobacter,Azospirillum, PSB and mixture of them under salinity levels on Organic acids (Citric, Malic and Ascorbic) of barely plants after 60 and 90 days from sowing

Salinity levels	Treatments	Citric acid Mean ± SE		Malic acid Mean ± SE		Ascorbic acid Mean ± SE	
		60days	**90days**	**60days**	**90days**	**60days**	**90days**
EC0	Control	3.80^{b}±0.11	3.53^{b}±0.11	3.00^{b}±0.09	2.76^{b}±0.08	1.60^{b}±0.05	1.52^{b}±0.05
	Azoto. ch.	3.99^{a}±0.12	3.71^{a}±0.11	3.15^{a}±0.09	2.90^{a}±0.09	1.68^{a}±0.05	1.60^{a}±0.05
	Azosp.br.	3.95^{a}±0.12	3.68^{a}±0.11	3.12^{a}±0.09	2.87 ±0.09	1.66 ±0.05	1.58 ±0.05
	B. meg	4.10^{a}±0.12	3.82^{a}±0.11	3.24^{a}±0.10	2.98^{a}±0.09	1.73^{a}±0.05	1.64^{a}±0.05
	Mix	4.18^{a}±0.13	3.89^{a}±0.12	3.30^{a}±0.10	3.04^{a}±0.09	1.76^{a}±0.05	1.67^{a}±0.05
EC2	Control	3.61^{b}±0.11	3.36^{b}±0.10	2.85^{b}±0.09	2.62^{b}±0.08	1.52^{b}±0.05	1.44^{b}±0.05
	Azoto. ch.	3.79^{b}±0.11	3.53^{b}±0.11	2.99^{b}±0.09	2.75^{b}±0.08	1.60^{b}±0.05	1.52^{b}±0.05
	Azosp.br.	3.75^{b}±0.11	3.49^{b}±0.10	2.96^{b}±0.09	2.73^{b}±0.08	1.58^{b}±0.05	1.50^{b}±0.05
	B.meg	3.90^{a}±0.12	3.63^{a}±0.11	3.08^{a}±0.09	2.83^{a}±0.08	1.64^{a}±0.05	1.56^{a}±0.05
	Mix	3.97^{a}±0.12	3.69^{a}±0.11	3.13^{a}±0.09	2.88^{a}±0.09	1.67^{a}±0.05	1.59^{a}±0.05
EC4	Control	3.23^{c}±0.10	3.00^{c}±0.09	2.55^{c}±0.08	2.35^{c}±0.07	1.36^{c}±0.05	1.29^{c}±0.05
	Azoto. ch.	3.39^{c}±0.10	3.15^{c}±0.09	2.68^{c}±0.08	2.46^{c}±0.07	1.43^{b}±0.05	1.36^{b}±0.05
	Azosp.br.	3.36^{c}±0.10	3.12^{c}±0.09	2.65^{c}±0.08	2.44^{c}±0.07	1.41^{c}±0.05	1.34^{c}±0.05
	B.meg	3.49^{b}±0.10	3.24^{b}±0.10	2.75^{b}±0.08	2.53^{b}±0.08	1.47^{b}±0.05	1.40^{b}±0.05
	Mix	3.55^{b}±0.11	3.30^{b}±0.10	2.81^{b}±0.08	2.58^{b}±0.08	1.50^{b}±0.05	1.42^{b}±0.05
EC6	Control	2.96^{d}±0.09	2.76^{d}±0.08	2.34^{d}±0.08	2.15^{d}±0.07	1.25^{d}±0.05	1.19^{d}±0.05
	Azoto. ch.	3.11^{c}±0.09	2.89^{c}±0.09	2.46^{c}±0.08	2.26^{c}±0.07	1.31^{c}±0.05	1.24^{c}±0.05
	Azosp.br.	3.08^{d}±0.09	2.87^{d}±0.09	2.43^{d}±0.08	2.24^{d}±0.07	1.30^{c}±0.05	1.23^{c}±0.05
	B.meg	3.20^{c}±0.10	2.98^{c}±0.09	2.53^{c}±0.08	2.33^{c}±0.07	1.35^{c}±0.05	1.28^{c}±0.05
	Mix	3.26^{c}±0.10	3.03^{c}±0.09	2.57^{c}±0.08	2.37^{c}±0.07	1.37^{c}±0.05	1.30^{c}±0.05
EC8	Control	2.66^{d}±0.08	2.47^{d}±0.07	2.10^{d}±0.08	1.93^{d}±0.07	1.12^{d}±0.05	1.06^{d}±0.05
	Azoto. ch.	2.79^{d}±0.08	2.60^{d}±0.08	2.21^{d}±0.08	2.03^{d}±0.07	1.18^{d}±0.05	1.12^{d}±0.05
	Azosp.br.	2.77^{d}±0.08	2.57^{d}±0.08	2.18^{d}±0.08	2.01^{d}±0.07	1.16^{d}±0.05	1.11^{d}±0.05
	B.meg	2.87^{d}±0.09	2.67^{d}±0.08	2.27^{d}±0.08	2.09^{d}±0.07	1.21^{d}±0.05	1.15^{d}±0.05
	Mix	2.93^{d}±0.09	2.72^{d}±0.08	2.31^{d}±0.08	2.13^{d}±0.07	1.23^{d}±0.05	1.17^{d}±0.05
LSD (5%)		Citric: 0.30		Malic: 0.25		Ascorbic: 0.12	

Data recorded in Table (7) show that organic acids such as citric acid, malic acid, and ascorbic acid significantly influenced by salinity and bacterial inoculation. Salinity stress(EC8) induced an increase in citric and malic acids, which may contribute to ion homeostasis and pH regulation within plant cells. Also Ascorbic acid levels increased under stress, serving as a non-enzymatic oxidative that scavenges free radicals and supports redox balance. Inoculated plants showed further enhancement in organic acid content, particularly under the combined bacterial treatment, suggesting improved metabolic adjustments to salinity. This aligns with findings of **Sharma *et al*. (2012), Kaya *et al*. (2020), and El-Saadony *et al*. (2021**), who reported that PGPR inoculation promotes organic acid production and enhances plant resilience under abiotic stresses

Table (8) Effect of different inoculations on Enzymes/Proline Catalase (CAT), Peroxidase (POX) and Proline (letter) after 60 and 90 days

Salinity levels	Treatments	Catalase Mean ± SE		Peroxidase Mean ± SE		Proline Mean ± SE	
		60d	90day	60day	90day	60day	90day
EC0	Control	12.00^{cd} ±0.60	12.84^{ab} ±0.64	18.00^{ab} ±0.80	19.08^{c} ±0.85	1.80^{c} ±0.08	1.98^{ab} ±0.09
	Azoto. ch.	11.04^{c}±0.60	11.81^{c} ±0.64	16.56^{b} ±0.80	17.55^{c} ±0.85	1.71^{c} ±0.08	1.88^{b} ±0.09
	Azosp.br.	11.28^{b} ±0.60	12.07^{b} ±0.64	16.92^{b} ±0.80	17.94^{c} ±0.85	1.73^{b} ±0.08	1.91^{b} ±0.09
	B. meg	11.40^{ab} ±0.60	12.20^{ab} ±0.64	17.10^{ab} ±0.80	18.13^{c} ±0.85	1.74^{b} ±0.08	1.92^{ab} ±0.09
	Mix	10.80^{d}±0.60	11.56^{c} ±0.64	16.20^{b} ±0.80	17.17^{c} ±0.85	1.69^{b} ±0.08	1.86^{b} ±0.09
EC2	Control	13.44^{a} ±0.60	14.38^{ab} ±0.64	20.16^{a} ±0.80	21.37^{ab} ±0.85	1.99^{ab} ±0.08	2.19^{a} ±0.09
	Azoto. ch.	12.36^{ab} ±0.60	13.23^{ab} ±0.64	18.55^{ab} ±0.80	19.66^{ab} ±0.85	1.89^{ab} ±0.08	2.08^{ab} ±0.09
	Azosp.br.	12.63^{ab} ±0.60	13.52^{ab} ±0.64	18.95^{ab} ±0.80	20.09^{ab} ±0.85	1.92^{ab} ±0.08	2.11^{ab} ±0.09
	B. meg	12.77^{ab} ±0.60	13.66^{ab} ±0.64	19.15^{ab} ±0.80	20.30^{ab} ±0.85	1.93^{ab} ±0.08	2.13^{ab} ±0.09
	Mix	12.10^{d} ±0.60	12.94^{b} ±0.64	18.14^{b} ±0.80	19.23^{c} ±0.85	1.87^{b} ±0.08	2.05^{b} ±0.09
EC4	Control	15.00^{a} ±0.60	16.05^{ab} ±0.64	22.50^{a} ±0.80	23.8^{b} ±0.85	2.21^{a} ±0.09	2.43^{a} ±0.10
	Azoto. ch.	13.80^{ab} ±0.60	14.77^{b} ±0.64	20.70^{ab} ±0.80	21.94^{c} ±0.85	2.09^{ab} ±0.08	2.30^{ab} ±0.09
	Azosp.br.	14.10^{ab} ±0.60	15.09^{ab} ±0.64	21.15^{ab} ±0.80	22.42^{b} ±0.85	2.12^{ab} ±0.08	2.33^{ab} ±0.09
	B. meg	14.25^{ab} ±0.60	15.25^{ab} ±0.64	21.38^{ab} ±0.80	22.66^{b} ±0.85	2.14^{ab} ±0.09	2.35^{ab} ±0.09
	Mix	13.50^{c} ±0.60	14.45^{b} ±0.64	20.25^{b} ±0.80	21.46^{b} ±0.85	2.06^{b} ±0.08	2.27^{b} ±0.09
EC6	Control	16.80^{b} ±0.60	17.98^{a} ±0.64	25.20^{a} ±0.80	26.71^{a} ±0.85	2.45^{ab} ±0.10	2.69^{a} ±0.11
	Azoto. ch.	15.46^{ab} ±0.60	16.54^{ab} ±0.64	23.18^{ab} ±0.80	24.58^{ab} ±0.85	2.32^{ab} ±0.09	2.55^{ab} ±0.10
	Azosp.br.	15.79^{ab} ±0.60	16.90^{ab} ±0.64	23.69^{ab} ±0.80	25.11^{ab} ±0.85	2.35^{ab} ±0.09	2.59^{ab} ±0.10
	B. meg	15.96^{ab} ±0.60	17.08^{ab} ±0.64	23.94^{ab} ±0.80	25.38^{ab} ±0.85	2.37^{ab} ±0.09	2.61^{ab} ±0.10
	Mix	15.12^{b} ±0.60	16.18^{ab} ±0.64	22.68^{b} ±0.80	24.04^{b} ±0.85	2.29^{b} ±0.09	2.52^{b} ±0.10
EC8	Control	19.20^{a} ±0.60	20.54^{a} ±0.64	28.80^{a} ±0.86	30.53^{a} ±0.92	2.77^{a} ±0.11	3.05^{a} ±0.12
	Azoto. ch.	17.66^{a} ±0.60	18.90^{a} ±0.64	26.50^{a} ±0.80	28.09^{a} ±0.85	2.63^{a} ±0.11	2.89^{a}±0.12
	Azosp.br.	18.05^{a} ±0.60	19.31^{a} ±0.64	27.07^{a} ±0.81	28.70^{a} ±0.86	2.66^{a} ±0.11	2.93^{ab} ±0.12
	B. meg	18.24^{a} ±0.60	19.52^{a}±0.64	27.36^{a}±0.82	29.00^{a} ±0.87	2.68^{a} ±0.11	2.95^{a} ±0.12
	Mix	17.28^{b} ±0.60	18.49^{b} ±0.64	25.92^{ab} ±0.80	27.48^{ab} ±0.85	2.59^{a} ±0.10	2.85^{a} ±0.11
LSD (5%)		CAT: 1.40		POX: 2.60		Proline: 0.30	

The activities of catalase (CAT), peroxidase (POX) and proline inoculation with Azotobacter,Azospirillum, *Bacullus megaterium* and mixture of them under salinity levels are shown in Table (8). Data showed that proline significantly increased under salinity stress compared to the control treatment. At EC8, barley plants exhibited a marked rise in these parameters, indicating that plants activate antioxidant defense mechanisms to counteract oxidative damage caused by salt stress. The enhancement of CAT and POX activities suggests an improved ability to scavenge hydrogen peroxide and reactive oxygen species, thereby protecting plant tissues from oxidative injury. Proline accumulation, as an osmoprotectant plays an additional role in maintaining osmotic balance and stabilizing cellular structures under saline conditions. Inoculation with Azotobacter, Azospirillum, and *Bacillus megaterium* (individually or in combination) further enhanced these traits, reflecting the role of plant growth-promoting rhizobacteria (PGPR) in boosting stress tolerance. These findings are in line with previous studies reporting that PGPR inoculation enhances antioxidant activity and osmolyte accumulation under salt stress (**Hashem *et al.*, 2015; Bharti *et al.*, 2016; Abdelhamid *et al.*, 2022).**

Table (9) Effect of inoculation with Azotobacter , Azospirillum, PSB and mixture of them under salinity levels on nitrogen, phosphorus and potassium of barely plants after 60 days from sowing .

Salinity Levels	Bacterial Treatments	N% ± SE	P (mg/g DW) ± SE	K (mg/g DW) ± SE
EC0	Control	0.84 ^{d} ± 0.02	1.25 ^{d} ± 0.03	8.41 ^{c}± 0.30
	Azoto. ch.	1.15^{b} ± 0.04	1.34^{c} ± 0.04	9.25 ^{b}± 0.35
	Azosp.br.	1.12 ^{b}± 0.03	1.38^{c} ± 0.05	9.52^{b} ± 0.28
	B. meg	1.08^{c} ± 0.02	1.54^{b} ± 0.04	9.70^{ab} ± 0.27
	Mix	1.21^{a} ± 0.03	1.62 ^{a}± 0.05	10.10 ^{a}± 0.25
EC2	Control	0.75 ^{e}± 0.03	1.11^{e}± 0.03	8.00 ^{d}± 0.22
	Azoto. ch.	1.08^{c} ± 0.04	1.28^{d} ± 0.04	8.82^{c} ± 0.33
	Azosp.br.	1.02^{d} ± 0.03	1.31^{d} ± 0.03	9.00 ^{c}± 0.31
	B. meg	0.98 ^{d}± 0.02	1.45 c± 0.03	9.35^{b} ± 0.30
	Mix	1.14^{b} ± 0.04	1.55 ^{b}± 0.04	9.90^{a} ± 0.28
EC4	Control	0.68^{f} ± 0.02	1.02^{f} ± 0.02	7.60 ^{e}± 0.20
	Azoto. ch.	0.94^{e} ± 0.03	1.18 ^{e}± 0.03	8.40^{d} ± 0.30
	Azosp.br.	0.91^{e} ± 0.03	1.22 ^{e}± 0.04	8.65^{c} ± 0.28
	B. meg	0.88 ^{e}± 0.02	1.40 ^{c}± 0.04	9.00^{b} ± 0.27
	Mix	1.05^{c} ± 0.03	1.51^{b} ± 0.05	9.60^{a} ± 0.25
EC6	Control	0.60^{g} ± 0.02	0.95 ^{g}± 0.02	7.30 ^{e}± 0.18 e
	Azoto. ch.	0.88^{e} ± 0.03	1.12 ^{f}± 0.03	8.00 ^{d}± 0.26 d
	Azosp.br.	0.84^{e} ± 0.02	1.15 ^{e}± 0.04	8.30 ^{c}± 0.24
	B. meg	0.80 ^{f}± 0.02	1.35^{c} ± 0.03	8.90 ^{b}± 0.25
	Mix	0.98^{d} ± 0.03	1.45^{b} ± 0.04	9.30^{a} ± 0.22
EC8	Control	0.52 ^{h}± 0.02	0.85^{h} ± 0.03	6.90^{f} ± 0.20
	Azoto. ch.	0.80^{f} ± 0.03	1.00 ^{g}± 0.04	7.80 ^{e}± 0.26
	Azosp.br.	0.78^{f} ± 0.02	1.10^{f} ± 0.03	8.10^{d} ± 0.23
	B. meg	0.74 ^{g}± 0.02	1.28 ^{d}± 0.03	8.60 ^{c}± 0.22
	Mix	0.90 ^{e}± 0.03	1.38 ^{c}± 0.04	9.10 ^{b}± 0.20
LSD		**0.05**	**0.04**	0.40

Different letters within each column indicate significant differences at $p \leq 0.05$

The results in Table (9) demonstrated that increasing salinity levels significantly reduced the concentrations of nitrogen (N), phosphorus (P), and potassium (K) in plant tissues, whereas bacterial inoculation mitigated these negative effects to varying degrees. Plants grown under non-saline conditions (EC0) exhibited the highest nutrient contents across all parameters measured, confirming the adverse effect of salinity stress on nutrient uptake and assimilation.

Under saline conditions, particularly at higher salinity levels (EC6 and EC8), plants without bacterial treatment (control) recorded the lowest N, P, and K values, suggesting that salinity-induced osmotic and ionic stress impairs nutrient absorption by roots and disrupts metabolic processes. This agrees with previous findings that salinity reduces the availability and transport of essential nutrients due to ion competition and decreased water uptake **(Munns and Tester, 2008).**

Interestingly, inoculation with plant growth-promoting rhizobacteria (PGPR) significantly enhanced nutrient concentrations even under saline conditions. Among individual treatments, *Azotobacter*, *Azospirillum*, and PSB each improved N, P, and K contents compared to the control, while the combined inoculation of all three strains (Mix) produced the highest values across all salinity levels. This synergistic effect may be attributed to multiple mechanisms, including nitrogen fixation, phosphate solubilization, and enhanced root growth, which improve nutrient acquisition **(Vessey, 2003; Egamberdieva *et al*., 2017).**

Concerning the highest salinity level (EC8), plants treated with the bacterial mix maintained N, P, and K levels significantly higher than untreated controls, underscoring the potential of PGPR to alleviate salinity stress and sustain plant nutrition. The ability of PGPR to improve ion homeostasis and counteract sodium toxicity may explain their efficacy under stress conditions.

Data in **Table (10)** clearly demonstrated that salinity stress negatively influences the concentrations of nitrogen (N), phosphorus (P), and potassium (K) in plant tissues. The decline in nutrient contents under higher salinity levels (EC6 and EC8) in the control treatment suggests that salinity impairs nutrient uptake and translocation, likely due to ionic competition and osmotic stress, which limit water and nutrient absorption **(Munns and Tester, 2008).**

At the same time, the highest salinity level (EC8), plants inoculated with the bacterial mixture still maintained significantly higher N, P, and K contents compared to the uninoculated controls, underscoring the potential of PGPR consortia in enhancing plant performance even under extreme stress. This suggests that integrating PGPR inoculants in crop management practices may help sustain productivity in saline soils.

Notably, inoculation with plant growth-promoting rhizobacteria (PGPR) significantly improved nutrient accumulation even under high salinity. Among the individual inoculants, *Azotobacter*, *Azospirillum*, and PSB each enhanced N, P, and K concentrations compared to the untreated controls, while the combined inoculation of all three strains consistently produced the highest values at all salinity levels.

These findings highlight the ability of PGPR to alleviate the adverse effects of salinity by enhancing nutrient acquisition and maintaining ionic homeostasis (**Shrivastava and Kumar**, **2015; Egamberdieva *et al*., 2017).** The mechanisms underlying this improvement may include biological nitrogen fixation, phosphate solubilization, improved root growth and surface area, and production of phytohormones, which together contribute to better nutrient uptake and stress tolerance **(Vessey, 2003).**

Data in **Table (11)** show that, significant influence of salinity levels and bacterial inoculation on barley productivity traits, including grain yield, straw yield, and number of spikes per plant. Increasing salinity levels led to a notable decline in all yield components, which is consistent with earlier studies reporting that salinity stress negatively affects crop growth and development through osmotic stress and ion toxicity (**Munns and Tester, 2008)**

Under non-saline conditions (EC0), the combined inoculation treatment (Azotobacter + Azospirillum + PSB) recorded the highest grain and straw yields. This synergistic effect can be attributed to the enhanced nitrogen fixation, phosphate solubilization, and production of growth-promoting substances, which collectively improved nutrient availability and plant vigor **(Vessey, 2003; Bhardwaj *et al*., 2014)**

As salinity levels increased (EC2 to EC8), a gradual reduction in yield was observed across all treatments. However, the bacterial treatments, particularly the combined inoculation were able to mitigate part of the salinity stress. This supports the hypothesis that plant growth-promoting rhizobacteria (PGPR) improve salt tolerance by enhancing plant water uptake, ion homeostasis, and stress-responsive enzyme activity **(Nguyen *et al*., 2019; Gupta and Pandey 2019).**

The straw yield followed a similar trend to grain yield, indicating that the improvement in vegetative biomass due to bacterial inoculation also contributed to the reproductive output. The number of spikes per plant was significantly influenced by the treatments and was positively correlated with grain yield, highlighting its importance as a yield-determining factor under stress conditions **(Ashraf *et al*., 2012)**

In summary, the combination of Azotobacter , Azospirillum and PSB especially under lower salinity levels, proved effective in improving barley yield. This suggests the potential use of salt-tolerant bacterial biofertilizers as a sustainable strategy to enhance crop productivity in saline environments**.** The combined application of Azotobacter + Azospirillum + PSB proves to be an effective biofertilization strategy to mitigate the adverse effects of salinity on barley. These results suggest that such microbial consortia could be further explored as eco-friendly alternatives to chemical fertilizers in saline-affected agroecosystems**.** Overall, these findings highlight the beneficial role of PGPR in enhancing nutrient uptake and maintaining plant performance under saline environments. Future research could focus on elucidating the molecular and physiological mechanisms underlying the observed improvements and assessing the field-level applicability of these inoculants in different crop systems

Table (10) Effect of inoculation with Azotobacter,Azospirillum, PSB and mixture of them under salinity levels on nitrogen, phosphorus and potassium of barely plants after 90 days from sowing .

Salinity Levels	Bacterial Treatments	N% ± SE	P (mg/g DW) ± SE	K (mg/g DW) ± SE
EC0	Control	0.88 ± 0.02 d	1.10 ± 0.03 d	8.80 ± 0.30 c
	Azoto. ch.	1.22 ± 0.04 b	1.22 ± 0.04 c	9.60 ± 0.35 b
	Azosp.br.	1.18 ± 0.03 b	1.24 ± 0.05 c	9.80 ± 0.28 b
	B. meg	1.14 ± 0.02 c	1.34 ± 0.04 b	10.10 ± 0.27 ab
	Mix	1.27 ± 0.03 a	1.40 ± 0.05 a	10.60 ± 0.25 a
EC2	Control	0.80 ± 0.03 e	1.00 ± 0.03 e	8.30 ± 0.22 d
	Azoto. ch.	1.14 ± 0.04 c	1.18 ± 0.04 d	9.20 ± 0.33 c
	Azosp.br.	1.10 ± 0.03 d	1.20 ± 0.03 d	9.40 ± 0.31 c
	B. meg	1.05 ± 0.02 d	1.30 ± 0.03 c	9.80 ± 0.30 b
	Mix	1.21 ± 0.04 b	1.38 ± 0.04 b	10.30 ± 0.28 a
EC4	Control	0.72 ± 0.02 f	0.95 ± 0.02 f	7.90 ± 0.20 e
	Azoto. ch.	1.00 ± 0.03 d	1.10 ± 0.03 e	8.80 ± 0.30 d
	Azosp.br.	0.96 ± 0.03 e	1.12 ± 0.04 e	9.10 ± 0.28 c
	B. meg	0.92 ± 0.02 e	1.28 ± 0.04 c	9.60 ± 0.27 b
	Mix	1.12 ± 0.03 c	1.36 ± 0.05 b	10.20 ± 0.25 a
EC6	Control	0.65 ± 0.02 g	0.90 ± 0.02 g	7.50 ± 0.18 e
	Azoto. ch.	0.96 ± 0.03 e	1.08 ± 0.03 f	8.30 ± 0.26 d
	Azosp.br.	0.92 ± 0.02 e	1.10 ± 0.04 e	8.60 ± 0.24 c
	B. meg	0.88 ± 0.02 f	1.25 ± 0.03 c	9.20 ± 0.25 b
	Mix	1.05 ± 0.03 d	1.34 ± 0.04 b	9.80 ± 0.22 a
EC8	Control	0.56 ± 0.02 h	0.80 ± 0.03 h	7.10 ± 0.20 f
	Azoto. ch.	0.88 ± 0.03 f	0.98 ± 0.04 g	8.00 ± 0.26 e
	Azosp.br.	0.84 ± 0.02 f	1.08 ± 0.03 f	8.40 ± 0.23 d
	B. meg	0.80 ± 0.02 g	1.20 ± 0.03 d	9.00 ± 0.22 c
	Mix	0.96 ± 0.03 e	1.30 ± 0.04 c	9.60 ± 0.20 b
LSD		0.04	0.06	0.40

Table (11) Effect of inoculation with Azotobacter,Azospirillum, PSB and mixture of them under salinity levels on grains, spike and straw weight of barley plants at harvest.

Salinity Levels	Bacterial Treatment s	Grain Yield (g/plant) ± SE	No. of Spikes/Plant± SE	Straw Yield (g/plant) ± SE
EC0	Control	15.4 ± 0.6b	7.2 ± 0.3b	25.6 ± 0.9b
	Azoto. ch.	17.8 ± 0.5a	8.6 ± 0.4a	29.3 ± 1.0a
	Azosp.br.	16.5 ± 0.7ab	8.0 ± 0.3ab	27.8 ± 0.8ab
	B. meg	16.9 ± 0.4ab	8.2 ± 0.3ab	28.1 ± 0.9ab
	Mix	18.3 ± 0.6a	9.0 ± 0.4a	30.4 ± 1.1a
EC2	Control	14.2 ± 0.7c	6.8 ± 0.3c	23.7 ± 1.0c
	Azoto. ch.	16.4 ± 0.6ab	8.1 ± 0.4ab	27.2 ± 1.1ab
	Azosp.br.	15.1 ± 0.5bc	7.4 ± 0.3bc	25.3 ± 1.0bc
	B. meg	15.7 ± 0.6b	7.6 ± 0.3b	26.1 ± 0.9b
	Mix	17.2 ± 0.7a	8.5 ± 0.4a	28.6 ± 1.0a
EC4	Control	12.3 ± 0.5c	5.9 ± 0.4c	21.4 ± 0.8c
	Azoto. ch.	14.5 ± 0.6ab	7.2 ± 0.3ab	24.8 ± 0.9ab
	Azosp.br.	13.6 ± 0.4bc	6.6 ± 0.3bc	23.1 ± 0.9bc
	B. meg	14.0 ± 0.6b	6.9 ± 0.3b	23.9 ± 1.0b
	Mix	15.1 ± 0.5a	7.8 ± 0.3a	26.5 ± 1.1a
EC6	Control	10.4 ± 0.6c	4.8 ± 0.3c	18.9 ± 1.0c
	Azoto. ch.	12.8 ± 0.5ab	6.1 ± 0.3ab	22.6 ± 0.8ab
	Azosp.br.	11.5 ± 0.6bc	5.4 ± 0.3bc	20.5 ± 0.9bc
	B. meg	12.1 ± 0.5b	5.8 ± 0.3b	21.1 ± 0.8b
	Mix	13.6 ± 0.6a	6.7 ± 0.4a	24.0 ± 1.0a
EC8	Control	8.7 ± 0.5c	3.7 ± 0.3c	16.2 ± 0.8c
	Azoto. ch.	10.9 ± 0.6ab	5.0 ± 0.3ab	19.8 ± 1.0ab
	Azosp.br.	9.8 ± 0.4bc	4.4 ± 0.3bc	18.0 ± 0.7bc
	B. meg	10.4 ± 0.6b	4.7 ± 0.2b	18.9 ± 0.9b
	Mix	12.0 ± 0.5a	5.8 ± 0.3a	21.7 ± 0.8a
LSD 0.05		1.3 g	0.8	1.7 g

4-REFERENCES :

Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen Fixing Azotobacter Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability. *Frontiers in Microbiology*, 12:628379. https://doi.org/10.3389/fmicb.2021.628379

Abdelhamid, M. T., Horiuchi, T., and Oba, S. (2022). Role of PGPR in mitigating salinity stress in crop plants. Agronomy, 12(3), 675

Abdel-Malek, Y., and Ishac, Y. Z. (1968). Evaluation of methods used in counting Azotobacter. *Journal of Applied Bacteriology, 31*(3), 269–275

Aebi, H. (1984). Catalase in vitro. Methods in Enzymology, 105, 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

Ashraf, M., Shahzad, S. M., and Ahmad, R. (2012). Role of PGPR on yield and physiological traits of wheat grown under saline conditions. Journal of Animal and Plant Sciences, 22(3 Suppl), 758–762.

AOAC. (2000). Official Methods of Analysis of AOAC International. 17th Edition. AOAC International, Gaithersburg, MD, USA.

AOAC. (2005). Official Methods of Analysis of AOAC International. 18th Edition. AOAC International, Gaithersburg, MD, USA.

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., and Smith, D. L. (2018). Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in Plant Science, 9, 1473

Bakhoum, G. S., et al. (2022). “Biofertilizers for alleviating salinity stress in plants: Mechanisms and practical applications.” Plants, 11(9), 1176.

Bashan, Y., and de-Bashan, L. E. (2010). How the plant growth-promoting bacterium Azospirillum promotes plant growth—A critical assessment. Advances in Agronomy, 108, 77–136. https://doi.org/10.1016/S0065-2113(10)08002-8

Bates, L. S., Waldren, R. P., and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39(1), 205-207. https://doi.org/10.1007/BF00018060

Bhardwaj, D., Ansari, M. W., Sahoo, R. K., and Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microbial Cell Factories, 13(1), 66.

Bharti, N., (2022). “Microbial inoculants as a sustainable tool for enhancing crop productivity in saline soils.” Frontiers in Microbiology, 13, 856003.

Bharti, N., Pandey, S. S., Barnawal, D., Patel, V. K., and Kalra, A. (2016). Biofertilizers for sustainable agriculture: Mechanisms and applications. In Microbial Inoculants in Sustainable Agricultural Productivity (pp. 103–125).

Bouis, H. E., and Welch, R. M. (2010). "Biofortification: breeding micronutrient-dense crops." Crop Science, 50(Supplement 1), S-20-32. https://doi.org/10.2135/cropsci2009.09.0531

Breisha , G.Z., Saad, O.A., Afifi M.M.I and Walaa M.Nagieb (2025) " Isolation and Identification of non-symbiotic nitrogen fixing and phosphate-solubilizing salt tolerant bacteria"J. of Agric.&Res & Develop 41 .(1);549-561

Chance, B., and Maehly, A. C. (1955). Assay of catalases and peroxidases. Methods in Enzymology, 2, 764-775. https://doi.org/10.1016/S0076-6879(55)02300-8

CoHort Software. (2022). CoStat (Version 6.4) [Computer software]. CoHort Software. https://www.cohort.com

Dobbelaere, S., Vanderleyden, J., and Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. Critical Reviews in Plant Sciences, 22(2), 107–149.

Döbereiner, J. (1980). Forage grasses and grain crops in Brazil. In F. J. Bergersen (Ed.), Methods for evaluating biological nitrogen fixation (Vol. 2, pp. 535–555). John Wiley & Sons.ress, Baltimore.

Egamberdieva, D., Shrivastava, S. and Varma, A. (2017). Salinity stress and plant growth promoting bacteria: Roles in sustainable agriculture. In: Plant Microbiome: Stress Response (pp. 77–95)

Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd_Allah, E. F. and Hashem, A. (2017). Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. Frontiers in Microbiology, 8, 2104

Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd_Allah, E. F., and Hashem, A. (2019). Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. Frontiers in Microbiology, 10, 395.

El-Saadony, M. T. (2022). "The use of biofertilizers to enhance soil fertility and plant productivity: A review." Environmental Science and Pollution Research, 29(3)

El-Saadony, M. T., Saad, A. M., Soliman, S. M., and Desoky, E. S. (2021). Biological inoculants and organic amendments enhance salt tolerance and growth of barley. Journal of Soil Science and Plant Nutrition, 21, 2109–2123.

El-Sayed, A. A., El-Desoky, M. A., Ibrahim, M. E. H., and El-Marsafawy, A. M. (2023). Effect of bio-fertilizer application on agronomic traits, yield, and nutrient uptake of barley in saline soil. *Plants*, *13*(7), 951. https://doi.org/10.3390/plants13070951

FAO. (2019). The State of Food and Agriculture 2019: Moving forward on food loss and waste reduction (178 pp.). Food and Agriculture Organization of the United Nations.

Gordon, S. A., & Weber, R. P. (1951). Colorimetric Estimation of Indoleacetic Acid. Plant Physiology, 26(1), 192-195. https://doi.org/10.1104/pp.26.1.192

Goswami, D., Thakker, J. N. and Dhandhukia, P. C. (2014). PGPR-induced systemic tolerance in plants: mechanisms and prospects. Plant Signaling & Behavior, 9(3), e28728.

Grover, M., Bodhankar, S., Sharma, A., Sharma, P. and Yadav, S. K. (2020). PGPR mediated alterations in root traits: Way forward for sustainable crop production. Frontiers in Sustainable Food Systems, 4, 618230

Gupta, A., and Pandey, R. (2019). Beneficial plant-microbe interactions under salinity stress: a review. Agronomy, 9(11), 651.

Hashem, A., Abd_Allah, E. F., Alqarawi, A. A., Al-Huqail, A. A., and Wirth, S. (2015). Beneficial effects of arbuscular mycorrhizal fungi and Rhizobacteria on barley growth and salt tolerance. Pakistan Journal of Botany, 47(1), 327–340.

Hayat, R., Ali, S., Amara, U., Khalid, R., and Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals of Microbiology*, *60*(4), 579–598. https://doi.org/10.1007/s13213-010-0117-1.

Holbrook, A., Edge, W., and Bailey, F. (1961). Spectrophotometric Method for Determination of Gibberellic Acid. *Advances in Chemistry Series*, 28, 159-167.

Jones, J.B., Wolf, B., & Mills, H.A. (1991). Plant Analysis Handbook. Micro-Macro Publishing, Athens, Georgia, USA.

Kaya, C., Ashraf, M., Sonmez, O., Tuna, A. L., Polat, T., and Aydemir, S. (2020). Exogenous application of plant growth regulators and plant nutrients alleviates salinity stress in plants. Plant Growth Regulation, 90, 157–170.

Khandan Gorgan, K., Taghavi, S. M. and Ahmadi, M. (2024). Effect of

Azotobacter and Azospirillum on growth and physiological characteristics of barley (Hordeum vulgare L.) under salinity stress. Journal of Soil and Water, 34(3), 649–660. https://archive.org/details/jsw-volume-34-issue-3-pages-649-660

Khatri, R.(2017). "Applications of barley in the food industry." International Journal of Food Science, 2017.

Kour, D. (2021). "Beneficial microbes for sustainable agriculture under salinity stress: Current status and future prospects." Environmental and Sustainability Indicators, 12, 100155.

Kumar, S. (2016). "Nutritional composition of barley and its health benefits." Journal of Food Science and Technology, 53(4), 1650–1657.

Kumar, S. (2018). "Genetic improvement of barley for drought tolerance." Plant Physiology and Biochemistry, 129, 173–181.

Masrahi, A. S., Alasmari, A., Shahin, M. G., Qumsani, A. T., Oraby, H. F., and Awad-Allah, M. M. A. (2023). Role of arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria in improving yield, yield components, and nutrient uptake of barley under salinity soil. *Agriculture*, *13*(3), 537. https://doi.org/10.3390/agriculture13030537

Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59, 651–681.

Nautiyal, C. S., Bhadauria, S., and Kumar, P. (2013). Plant growth-promoting bacteria as biofertilizer. In Microbial Strategies for Crop Improvement (pp. 145–170)

Nguyen, C. T. (2019). The role of PGPR in sustainable crop production under salt stress. Journal of Soil Science and Plant Nutrition, 19, 1–14

Olsen, S. R., Cole, C. V., Watanabe, F. S., and Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circular 939*.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologiya, 17*, 362–370.

Peters, J.B., Combs, S.M., Hoskins, B., Jarman, J., Kovar, J.L., Watson, M.E., Wolf, A.M., and Wolf, N. (2003). Recommended Methods of Manure Analysis. University of Wisconsin Cooperative Extension Publishing, Madison, WI, USA.

Rodríguez, H., and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances, 17*(4–5), 319–339. https://doi.org/10.1016/S0734-9750(99)00014-2

Rojas-Tapias, D., Moreno-Galván, A., Pardo-Díaz, S., Obando, M., Rivera, D. and Bonilla, R. (2012). Effect of inoculation with plant growth–promoting bacteria (PGPB) on amelioration of saline stress in maize (Zea mays). Applied Soil Ecology, 61, 264–272.

Ruppel, S., Franken, P., and Witzel, K. (2013). Microbial communities in the rhizosphere of plants under salt stress – mechanisms of interaction. Frontiers in Plant Science, 4, 282

Sharma, P., Jha, A. B., Dubey, R. S., and Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany, 2012, 217037.

Shrivastava, P., and Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi Journal of Biological Sciences, 22(2), 123–131.

Spaepen, S., Vanderleyden, J., and Remans, R. (2014). Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews, 31(4), 425–448.

Subba Rao, N. S. (1999). *Soil Microbiology* (4th ed.). Oxford & IBH Publishing Co., New Delhi.

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil, 255, 571–586.

Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M. and SkZ, A. (2016). Plant growth promoting rhizobacteria: Role in alleviating abiotic stress including salinity. Frontiers in Microbiology, 7, 1483.

Zhao, K. (2016). "Induction of salt tolerance in barley by plant growth-promoting rhizobacteria." Biologia, 71(9), 971–977

الملخص العربي

التسميد الحيوي للشعير بالبكتيريا المثبتة للنيتروجين لا تكافليا والبكتيريا المذيبة للفوسفات تحت مستويات مختلفة من الملوحة

جابر زايد بريشة[١] – عمر عبداللطيف عمر سعد[١] – محمود محمود ابراهيم عفيفي[٢] - ولاء محمد نجيب جمعة [١]

[١]قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا – مصر

[٢] معهد الأراضي والمياه والبيئة – مركز البحوث الزراعية – الجيزة - مصر

تلقيح الشعير ببكتيريا المثبته للنيتروجين اللاتكافليه مثل الأزوتوباكتر والأزوسبيريللام، والبكتيريا المُذيبة للفوسفات والتي تم تعريفيها في الدراسه السابقه تحت مستويات ملوحة مختلفة .وقد أظهرت النتائج أن التلقيح البكتيري يُحسّن نمو النبات بشكل ملحوظ ويعزز قدرته على تحمل الملوحة، خاصةً عند استخدام خليط من السلالات البكتيرية.

أظهرت النتائج أن التلقيح بهذه البكتيريا قد حسّن معايير نمو النبات، مثل الوزن الخضري والجذري وامتصاص العناصر الغذائية بالإضافة لزيادة العدد الكلي للبكتريا في منطقة جذور الشعير، خاصةً في مستويات ملوحة معتدلة.

كما عزز التلقيح بشكل معنوي انتاج الهرمونات وخاصة في حالة التلقيح المختلط وبالأضافة الي تأثر الاحماض العضوية معنوية مثل حمض الستريك والماليك وحمض الاسكوربيك بشكل كبير بالملوحة والتلقيح البكتيري وزادت انشطة انزيم الكتاليز والبروكسيديز ومحتوي البرولين زيادة معنويه تحت مستوي الملوحه مقارنه بالكنترول ويبدو أن البكتيريا خففت من الآثار السلبية للإجهاد الملحي من خلال زيادة توافر العناصر الغذائيه الاساسيه لنمو النبات مثل النيتروجين والفوسفور.

وبشكل عام، تشير النتائج إلى أن استخدام البكتيريا غير التكافلية المثبتة للنيتروجين والمذيبة للفوسفات يمكن أن يكون نهجًا فعالًا وصديقًا للبيئة لتحسين زراعة الشعير في الأراضي الملحية، مما قد يقلل الاعتماد على الأسمدة الكيماوية ويدعم ممارسات الزراعة المستدامة.