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Response of Barley (*Hordeum vulgare*) to Bio-Fertilizers Under Salt Stress Conditions and Different Irrigation systems in South Sinai

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Abstract: The two field experiments were conducted to study the effect of bio-fertilizer application, irrigation system, and varieties on barley production in the saline, arid conditions of South Sinai during the two growing seasons 2023-2024 and 2024-2025. The field experiments were carried out on a private farm in Al-Wadi village, Al-Tour city, South Sinai, Egypt (28.21089°N, 33.35450°E, at an elevation of 14 m above sea level). The data showed that the use of biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) significantly improved all parameters under both irrigation techniques, however the impact was greater under drip irrigation. improvements in yield of 1.5–1.7 t/ha. Additionally, drip irrigation produced taller plants, higher tillering rates, larger grains, better water usage efficiency, and higher yields than spray irrigation in every evaluated parameter. However, there were clear varietal differences, with Giza 134 performing better in the majority of treatment combinations and water use efficiency more than doubling between the least and most efficient treatment combinations. Therefore, the combination of drip irrigation, improved varieties, and biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) is a viable path toward more resilient and productive cereal agriculture for the farming communities of South Sinai and similar arid regions facing soil salinity and water scarcity.

Key words: Faba bean, productivity, biofertilizers, salt stress, *Azospirillum brasilense* & *Paenibacillus mucilaginosus.*

1. Introduction

According to (**FAO**) the Food and Agriculture Organization's predicts, the world's population reach to 10 billion by 2050, and the need for food will increase. Therefore, the production of staple crops must nearly quadruple, particularly in developing countries, to meet these growing demands. Global food security and social stability, especially in the Mediterranean, Middle East, and North Africa regions, depend heavily on cereal crops (**FAO, 2017**). However, several factors, including low soil fertility, salinity of the water and soil, and erratic rainfall, can cause changes in agricultural production

in these areas. Climate change is predicted to make these issues worse, making it extremely difficult for agricultural researchers to create high-yielding cultivars that can withstand harsh weather conditions and low soil fertility. A substantial amount of land worldwide is impacted by salinity, and this issue is increasing. Utilization of salt-affected soils for crop cultivation is severely limited because most crops cannot withstand high salt levels, and salt stress is a significant growth-limiting factor. There are currently 400,000 hectares of salinized land worldwide, with 20% agricultural and 50% crop lands experiencing salt stress, which poses a serious threat to agricultural productivity (**Emami *et al*., 2019**).

Barley is the fourth most extensively grown cereal crop worldwide (**FAO, 2021**), where barley, in contrast to other grains, can yield well in marginal soil and with water scarcity. It is a great species for arid and semi-arid regions with low rainfall and high precipitation irregularity since it is a versatile crop that can be grown in both irrigated and rainfed systems (**Abdelghany *et al.*, 2024**). Barley is primarily cultivated for its feed value and for malting, which is used in the production of alcoholic beverages worldwide. Additionally, both developed and developing countries use barley straw for animal bedding and feeding (**Miralles *et al*., 2021**).

Scientists are also employing several approaches to produce salt-tolerant plants to mitigate and overcome the consequences of salinity, these methods take a lot of time and money. It is still unclear exactly how salt tolerance works. An alternate tactic is to use rhizosphere microorganisms, especially beneficial bacteria, which can boost plant development through various processes and increase plant performance under stress. Certain plant growth promoting rhizobacteria (PGPR) can stimulate plant growth and development directly or indirectly by giving plants soluble phosphate, phytohormones, fixed nitrogen, and iron that has been sequestered by bacterial siderophores (**Parida & Das 2005; Dimkpa *et al*., 2009; Hayat *et al*., 2010**).

For many years, soil bacteria have been utilized to produce crops and are crucial to biogeochemical cycles. Plant health and soil fertility are determined by interactions between bacteria and plants in the rhizosphere. By colonizing the plant root, free-living soil bacteria known as plant growth promoting rhizobacteria (PGPR) can stimulate plant development. These are connected to the rhizosphere, a crucial biological soil setting for interactions between microbes and plants. PGPR may help promote the growth of plants in a sustainable manner. In general, PGPR works in three ways: they help the plants synthesize specific molecules, they make it easier for the plants to absorb specific nutrients from the soil, and they reduce or prevent plant diseases. Both direct and indirect methods can be used to promote the growth and development of plants. Preventing phytopathogenic organisms' harmful impacts is one way to indirectly promote plant growth. On the other hand, PGPR contributes to direct plant growth promotion by producing plant hormones such as auxins, cytokinins, gibberellins, ethylene, and abscisic acid. Auxin-related substances, indole-3-ethanol and indole-3-acetic acid (IAA), have been discovered to be produced by several bacterial taxa (**Hayat *et al*., 2010; Jin *et al*., 2025**). Additionally, PGPR improves soil structure and organic matter content, stabilizes soil aggregates, increases tolerance to stress, and aids in the solubilization of mineral phosphates and other nutrients. PGPR improves nutrient release and lessens the requirement for fertilizer N and P by retaining more organic N and other nutrients in the plant-soil system (**Jin *et al*., 2025**). Under the hyper-arid and saline conditions of South Sinai, the co-inoculation of plant growth-promoting rhizobacteria (PGPR) emerges as a sustainable strategy to mitigate the detrimental effects of osmotic stress and ionic toxicity in barley (*Hordeum vulgare*) **(Giannelli *et al*., 2023)**. Among these, *Azospirillum brasilense* plays a pivotal role in fixing atmospheric nitrogen, synthesizing indole-3-acetic acid (IAA) to enhance root architecture, and producing ACC deaminase to suppress stress-induced ethylene. ***Azospirillum brasilense*** was selected due to its proven capacity for associative nitrogen fixation, organic acid-mediated phosphate solubilization, and heavy exopolysaccharide (EPS) secretion, which mitigates drought and salt stress in the barley rhizosphere under hyper-arid desert conditions. **(Omer *et al*., 2022)**. Concurrently, *Paenibacillus mucilaginosus* supplements this synergy by weathering soil minerals through organic acid secretion, thereby solubilizing fixed potassium and silicon within the alkaline, calcareous matrix **(Afridi *et al*., 2023)**. Together, this multi-strain consortium secretes a dense matrix of exopolysaccharides (EPS) that acts as a biological hydrogel under drip and sprinkler irrigation systems, physically entrapping toxic sodium ions (Na^+) and maintaining a favorable K^+ / Na^+ ratio to sustain barley growth and photosynthetic efficiency. Among potassium-solubilizing rhizobacteria, *Paenibacillus mucilaginosus* is widely

recognized for its high capability to weather potassium-bearing minerals (such as mica and feldspar). Through the secretion of organic acids and exopolysaccharides (EPS), ***P. mucilaginosus*** solubilizes bound potassium into plant-available forms (K+), promoting root uptake and biofertilization efficacy in hyper-arid desert soils. **(Afridi *et al*., 2023)**. This study was conducted to evaluate the role and effect of biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) on barley productivity under salt stress conditions and two irrigation systems.

2. Materials and Methods

Field experiments and site descriptions

The field experiments were carried out on a private farm in Al-Wadi village, Al-Tour city, South Sinai, Egypt (28.21089°N, 33.35450°E, at an elevation of 14 m above sea level) throughout two consecutive growing seasons, 2023/2024–2024/2025. The farm where the experiments were conducted is located within the arid climate zone, characterized by an average annual rainfall of no more than 7 mm and an average temperature of 23.2°C. A drip and sprinkler irrigation system powered by groundwater was used for the irrigation. The soil at the site is classified as sandy loam, with a pH of 8.16, an electrical conductivity (EC) of 9.55 dS m^{-1}, and an organic matter content of 0.39 percent. The soil at the location is categorized as sandy loam (Table 1).

Table (1). Physicochemical properties of the experimental soil

Chemical analysis		Texture	Cations & anions content (meq/l)						
pH	EC ds/m	Sandy loam	Ca	Mg	Na	K	HCO_3	SO4	Cl
8.16	9.55		6.77	8.97	19.44	1.88	2.33	16.41	18.7

Irrigation water source and its quality

Irrigation was carried out using well water, as is the practice in the study area, where agriculture relies on groundwater. The following table shows the characteristics of well water used for irrigation.

Table (2). Water quality indicators from the well in Al-Wadi village (ppm)

pH	EC µmohs/cm	TDS	Ca	Mg	Na	K	CO3	HCO3	SO4	Cl
7.8	832	540	68	8	110	3.5	12	240	135	80

Experimental design

The field experiments were laid out in a split-split plot design, where the irrigation system was in the main plots, barley varieties were in the sub-plots, and grains had pre-sowing treatment by biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) in the sub-sub-plots in 4 replications. Each plot measured 50 m^2 (5 × 10 m), with a net harvested area of (10 m^2). Treatments included two irrigation systems (drip and sprinkler), two varieties of barley (Giza 134 and Giza 135) with and without grains pre-sowing treatment by biofertilizer (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) application. Experiment plots were arranged randomization to reduce experimental error and increased the data' dependability while enabling legitimate statistical comparisons between treatments. Cultivation was conducted during the first week of November, and harvesting occurred in the first week of April, during the two growing seasons.

Fertilization characteristics

Organic fertilization

Compost, as organic fertilizer, was incorporated into the soil during soil preparation (15 days prior to sowing) at 17 tons/ha as recommended by the Egyptian Ministry of Agriculture and Land Reclamation (MALR) for barley (*Hordeum vulgare* L.) in newly reclaimed sandy soils. The compost was evenly

spread over the experimental units and thoroughly ploughed into the upper surface layer (15-30 cm) to enhance soil organic matter, improve moisture retention, and increase nutrient availability in the newly reclaimed sandy soil. The chemical properties of compost are shown in Table 3.

Table (3). The chemical properties of the organic compost

Compost	OM (%)	Moisture content (%)	pH	Organic carbon (%)	Total N (%)	P_2O_5 (%)	K_2O (%)	C/N ratio
	45.8	24	7.2	26.6	1.4	0.81	1.12	19

Mineral fertilization

Mineral fertilizers were applied based on the standard recommended doses set by the Egyptian Ministry of Agriculture and Land Reclamation (MALR) for barley (*Hordeum vulgare* L.) in newly reclaimed sandy soils; Nitrogen: Applied at a total rate of 143 kg N/ha using Ammonium Sulphate (20.5% N). The total nitrogen requirement was split into 3 equal doses; at sowing, at the tillering stage (approx. 21–25 days after sowing) and at the elongation/booting stage (approx. 45–50 days after sowing). Phosphorus: Applied at a rate of 73 kg (P_2O_5)/ha (equivalent 475 kg/ha of ordinary calcium superphosphate, 15.5% P_2O_5). The dose was incorporated into the soil during pre-sowing tillage alongside the organic compost. Potassium: Applied at a rate of 57 kg K_2O/ha (equivalent to 119 kg/ha of potassium sulphate, 48% K_2O). It was split into two equal applications: the first during soil preparation and the second at the tillering stage.

Bio-fertilization

A. Isolation of Bio-fertilizer Strains

Local 55 halotolerant strains were isolated from the rhizospheric soil of native desert plants collected from salt-affected areas in South Sinai, Egypt, using the serial dilution and spread plate technique (**El-Shazly, 2021**).

Azospirillum brasilense; Isolated from fresh roots of barley (*Hordeum vulgare*) grown under arid conditions. Root segments were surface-sterilized with 70% ethanol for 1 min and 1% sodium hypochlorite for 3 min, rinsed five times in sterile distilled water, and macerated in sterile saline (0.9% NaCl). Serial dilutions were inoculated into semi-solid Nitrogen-Free Malate (NFM) medium and incubated at 30ºC for 72 h. The formation of a characteristic white pellicle 1-2 mm below the surface was subcultured onto solid NFM agar containing 0.002 Congo Red. Typical scarlet-red, dry, compact colonies were isolated and identified as *A. brasilense* (**Döbereiner *et al*., 1976**). *Paenibacillus mucilaginosus* (syn. *Bacillus mucilaginosus*): Isolated from rhizospheric soil using Aleksandrov's selective agar medium containing insoluble potassium aluminosilicate (feldspar) as the sole K source. Soil suspensions were pasteurized at 80º C for 15 min to select for endospore-forming bacteria before serial dilution and spread-plating. Plates were incubated at 30ºC for 5 days. Large, convex, smooth, transparent, viscous, and mucoid colonies exhibiting potassium solubilization zones were picked and purified (**Aleksandrov *et al*., 1967**).

B. Selection of promising biofertilizers

Nitrogenase activity; Determined using the Acetylene Reduction Assay (ARA). Liquid cultures of nitrogen fixing bacteria medium at 30º C for 48 h. Vials were sealed with rubber stoppers, and 10% v/v of the air phase was replaced with acetylene (C_2H_2) gas. After incubation at 30º C for 24 h, 1 ml of the headspace gas was analyzed using a gas chromatograph equipped with a flame ionization detector (FID) and a Porapak N column. Nitrogenase activity was expressed as n mole C_2H_4 ml^{-1} culture h^{-1} (**Hardy *et al*., 1968**).

Phosphate-dissolving activity; Quantified by inoculating 1 ml of bacterial suspension into 100 ml of liquid Pikovskaya's medium containing insoluble tricalcium phosphate. Cultures were incubated on a rotary shaker at 30º C and 150 rpm for 7 days. The culture broth was centrifuged at 10,000 g for 15

min, and soluble phosphorus in the supernatant was measured colorimetrically using the chlorostannous-reduced molybdophosphoric acid blue method at 660 nm and expressed in mg/l (**Jackson, 1973**). **Potassium-solubilizing activity;** Evaluated by inoculating bacterial strains into liquid Aleksandrov's medium containing insoluble potassium aluminosilicate (feldspar) as the sole potassium source. After incubation on a shaker at 30º C and 150 rpm for 7 days, the suspension was centrifuged at 10,000 g for 15 min. The released soluble potassium (K^+) concentration in the supernatant was determined using a flame photometer and expressed in mg/l (**Jackson, 1973**). **Exopolysaccharide (EPS) production;** Bacterial cultures were grown in sucrose-supplemented broth at 30º C for 5 days. Cultures were centrifuged at 10,000 g for 20 min at 4º C to yield cell-free supernatants. Exopolysaccharides were precipitated by adding two volumes of ice-cold absolute ethanol (96%) and stored overnight at 4º C. The precipitated EPS was collected by centrifugation, washed with 70% ethanol, oven-dried at 60º C to a constant weight, and quantified gravimetrically as g L^{-1} (**Dubois *et al*., 1956**). **Antioxidant activity;** the microbial osmoprotectant (DPPH Free Radical Scavenging Assay) in which, radical scavenging activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. 1 ml of extract or cell-free supernatant was mixed with 2 ml of 0.1 mM DPPH solution in ethanol. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 min. The decrease in absorbance was measured spectrophotometrically at 517 nm against an ethanol blank. Scavenging percentage was calculated using the formula: DPPH Radical Scavenging Activity (%) = (control – sample)/ control x100 as described by (**Brand-Williams *et al*., 1995**).

C. Identification of biofertilizers

Promising bacterial isolates were cultured in sterile test tubes containing 10 ml of nutrient broth media (**Zimbro *et al*., 2009**). Cultures were incubated at 28ºC for 48 hours prior sending to the molecular Biology Research Unit, Assiut University for DNA extraction. Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company; South Korea was used. The extracted DNA samples were sent to Sol Gent Company, Daejeon South Korea for polymerase chain reaction (PCR) and gene sequencing of 16S rRNA gene. Sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of 16S rRNA gene sequences and the tree were constructed using sequence alignment tool in MEGA software version 11.01.

D. Inoculant Preparation and seed inoculation

A. brasilense Inoculant: Purified cultures were grown in liquid NFM broth supplemented with 0.1% NH_4Cl and 0.5% sodium malate as the primary carbon source. Broth cultures were incubated in an orbital shaking incubator at 30ºC and 180 rpm for 48 h until reaching the late exponential phase, yielding a minimum cell density of 10^8 CFU mL^{-1} (**Döbereiner *et al*., 1976**). While, *P. mucilaginosus* Inoculant: Purified strains were cultured in modified Aleksandrov's liquid medium containing 0.5% sucrose, 0.1% Na_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.0005% $FeCl_3$, and 0.2% finely ground potassium alumino-silicate or Potassium feldspar. The culture was incubated on a rotary shaker at 30ºC and 150 rpm for 72 h until maximum endospore formation and exopolysaccharide production were achieved, standardizing the cell concentration to 10^8 CFU ml^{-1} (**Aleksandrov *et al*., 1967**). Furthermore, carrier loading and seed inoculation; Equal volumes (1:1 v/v) of the standardized *A. brasilense* and *P. mucilaginosus* liquid cultures were mixed to form a multi-strain bio-fertilizer consortium. The liquid consortium was mixed with sterilized neutralized vermiculite carrier (2:1 w/v) neutralized with $CaCO_3$ (pH 7.0). Barley seeds were coated using a 10% gum arabic solution as a sticker to ensure a final density of approximately 10^7 CFU $seed^{-1}$ prior to sowing (**Vincent, 1970**).

Microbial soil characteristics

1. Microbiological soil content

Soil microbial populations were enumerated using the serial dilution and spread plate technique under aseptic conditions. Ten grams of fresh rhizospheric soil was suspended in 90 ml of sterile physiological saline solution (0.9 % NaCl) and shaken at 150 rpm for 30 min. Serial dilutions up to 10^-

^{7} were prepared, and 0.1 ml aliquots were spread uniformly onto specific culture media. Total Microbial Count (TMC): Estimated on Nutrient Agar (NA) medium after incubation at 30° C for 48 h and expressed as x10^{5} CFU g^{-1} dry soil (**Allen, 1957**). Nitrogen-Fixing Bacteria (NFB); Enumerated on nitrogen-free malate (NFM) agar medium after incubation at 30° C 4 days and expressed as x10^{3} CFU g^{-1} dry soil (**Döbereiner *et al*., 1976**). Phosphate-Dissolving Bacteria (PDB); Quantified using Pikovskaya's agar medium containing tricalcium phosphate. Plates were incubated at 30° C for 5 days, and halo zone-forming bacterial colonies were counted as x 10^{3} CFU g^{-1} dry soil (**Pikovskaya, 1948**). Potassium-Solubilizing Bacteria (KSB); Enumerated on Aleksandrov's agar medium containing insoluble potassium aluminosilicate (feldspar) as the sole potassium source. Plates were incubated at 30° C for 5 days and expressed as x10^{3} CFU g^{-1} dry soil (**Aleksandrov *et al*., 1967**).

2. Soil enzymatic Activity

Dehydrogenase Activity (DHA); Soil dehydrogenase activity was determined colorimetrically using the 2,3,5-triphenyltetrazolium chloride (TTC) method. Briefly, 5 g of moist soil was incubated with 1 ml of 3% TTC substrate and 2.5 ml of distilled water at 37º C for 24 h in the dark. The formed triphenylformazan (TPF) was extracted with methanol, measured spectrophotometrically at 485 nm, and expressed as µg TPF g^{-1} dry Soil 24 h^{-1} (Casida et al., 1964).

3. Soil available mineral content

Soil samples were air-dried, crushed, and passed through a 2 mm sieve prior to chemical evaluation: Available Nitrogen (N); Estimated using the alkaline permanganate distillation method. Soil was distilled with 0.32 % $KMnO_4$ and 2.5% NaOH solutions, and the liberated ammonia gas was absorbed in 2% boric acid solution containing mixed indicators and titrated against standardized 0.02 N H_2SO_4, expressed in ppm (**Subbiah & Asija, 1956**). Available Phosphorus (P); Extracted with 0.5 M $NaHCO_3$ solution adjusted to pH 8.5 (Olsen's method) suitable for calcareous saline soils. Phosphorus concentration in the filtrate was determined colorimetrically at 882 nm using the ascorbic acid-molybdate blue method and expressed in ppm (**Olsen *et al*., 1954**). Available Potassium (K); Extracted by shaking soil samples with 1.0 N neutral ammonium acetate (pH 7.0) at a 1:5 ratio for 30 min. The exchangeable K^{+} content in the extract was quantified using a flame photometer and expressed in ppm (**Jackson, 1973**).

Plant characterizations

- Plant height (PH cm): Samples individual plants were randomly chosen at the time of harvest to estimate, the height of the plants at maturity is measured from ground to the base of the spike.
- Tillering index (TI) (%): was calculated as follows:

$$TI = \left[\frac{Total\ Spikes/m2}{Total\ rillers/m2}\right] \times 100.$$

- 1000-grain weight (g): was expressed as an average weight of 1000 clean grains in gram taken from each sub-sub plot.
- Grain yield (ton/ha): The grain yield of the plot was measured after harvesting of the rest of plot area (Kg/plot) and then was transferred into t/ha.
- Water Use Efficiency (WUE; kg grain/m^{3}): was calculated as follows:

$$WUE = \left[\frac{Grain\ yield\ (Kg)}{Irrigation\ water\ (m^{3})}\right].$$

Data analysis

All experiments were performed in four replicates, data collection and analysis of variance (ANOVA) were carried out with SPSS (IBM Corp., 2019). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$. Means sharing the same letter within the same column or parameter are not significantly different, according to Duncan's test (**Duncan, 1955**).

3. Results and Discussion

Selection and Identification of the most promising biofertilizers

1- Selection of the most promising biofertilizers

Table (4). Characteristics of the most promising biofertilizers

Characteristics \ Biofertilizer	*Azospirillum brasilense*	*Paenibacillus mucilaginosus*
Nitrogenase activity (n mole C_2H_4 ml^{-1} culture h^{-1})	384.3	98.6
Phosphate Dissolving activity (mg L^{-1})	53.4	36.1
Potassium-Solubilizing activity (mg L^{-1})	18.8	69.7
Polysaccharide (EPS) production (g L^{-1})	3.8	12.1
Antioxidant Activity (%)	44.5	61.3

Out of an initial pool of 55 bacterial isolates evaluated, *Azospirillum brasilense* and *Paenibacillus mucilaginosus* were selected as the two most promising isolates based on their superior biofertilizers multi-functional plant growth-promoting rhizobacteria (PGPR) capabilities as presented in table (4). Rather than acting via isolated mechanisms, these two strains demonstrated complementary functional specialization across all tested parameters, establishing strong scientific rationale for their co-inoculation as a biofertilizer mixture designed to enhance nutrient availability and mitigate soil salinity stress.

A. brasilense proved to be the primary driver for biological nitrogen fixation and phosphorus mobilization, exhibiting the highest nitrogenase activity (384.3 n mole C_2H_4 ml^{-1} culture h^{-1}) and phosphate-dissolving capacity (53.4 mg L^{-1}) among all 55 isolates. This rapid enzymatic reduction of atmospheric nitrogen and organic acid secretion for phosphate solubilization directly supplements host metabolic requirements (**Boleta *et al.*, 2020; Zeffa *et al*., 2019**). Conversely, *Paenibacillus mucilaginosus* dominated in delivering superior potassium-solubilizing activity (69.7 mg L^{-1}) (**Paz *et al*., 2021**). Under salt-stress conditions, the co-application of these two isolates offers critical bioprotective mechanisms. *P. mucilaginosus* yielded exceptionally high levels of exopolysaccharides (EPS) (12.1 g L^{-1}). EPS biosynthesis forms a hydrated rhizo-sheath surrounding plant roots, which traps sodium ions, reduces osmotic shock, preserves water in the rhizosphere, and maintains structural aggregation in salt-affected soils (**Naseem *et al*., 2020; Netrusov *et al*., 2023**). Concurrently, *P. mucilaginosus* exhibited high antioxidant activity (61.3 %) with enhanced radical-scavenging capacity to neutralize reactive oxygen species (ROS) and prevent oxidative cell damage under severe salt stress (**Habib *et al*., 2021; Bulegon *et al*., 2016**). Integrating *A. brasilense* and *P. mucilaginosus* as a dual-strain microbial inoculant creates a synergistic bio-consortium. By pairing the potent N_2-fixing and P-solubilizing power of *A. brasilense* with K-solubilizing, EPS-forming, and antioxidant features of *P. mucilaginosus*, this combination delivers comprehensive plant growth promotion while improving crop resilience against saline environments (**Siddika *et al*., 2022; Qin *et al*., 2024**).

2- Identification of the most promising biofertilizers

The phylogenetic tree, as demonstrated in Figure (1), reveals that isolate DRC 17 forms closely aligned with *Azospirillum brasilense*. Concurrently, isolate DRC 32 clusters tightly in a robust lineage adjacent to *Paenibacillus mucilaginosus* based on 16S rRNA gene sequences analysis. The high bootstrap values at these critical nodes validate the reliability of the taxonomic placement for both novel isolates within their respective genera. This phylogenetic topology highlights the distinct evolutionary divergence and functional potential of these two native isolates for agricultural promising applications.

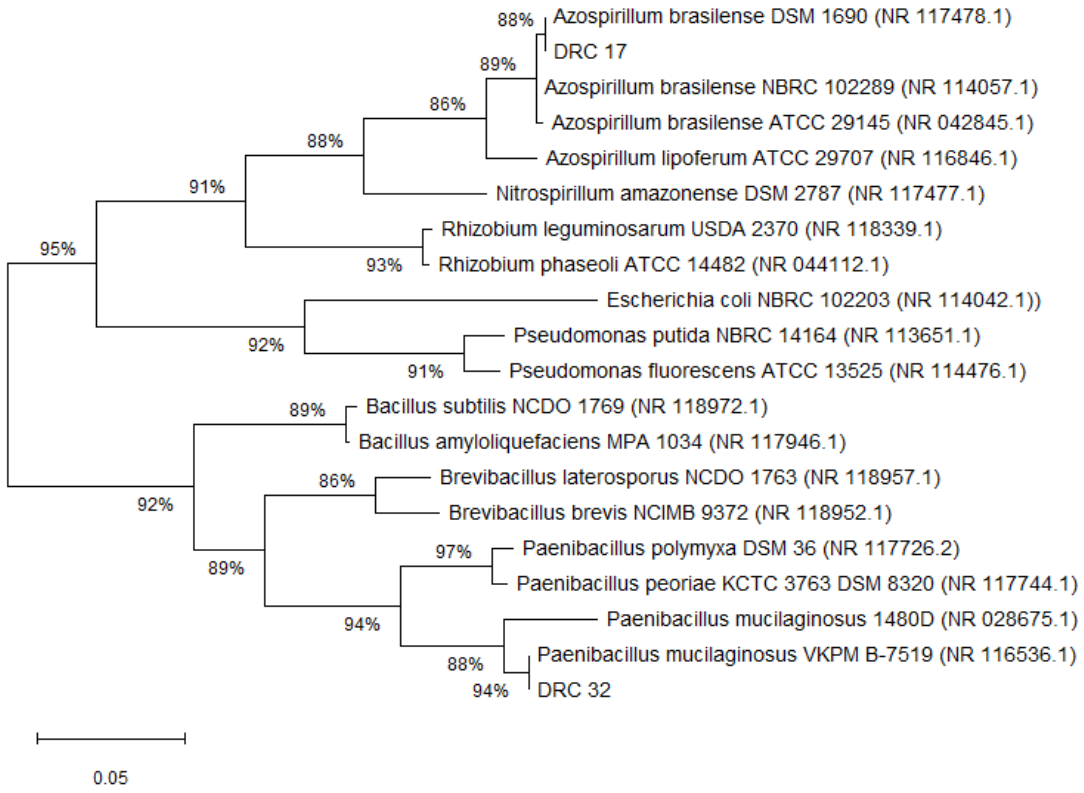


Fig. (1). Neighbor-Joining phylogenetic tree of identified microbial isolates (DRC 17 & DRC 32) based on 16S rRNA gene sequences analysis

Plant Characteristics

Plant Height (cm)

The data in Figure (2) illustrates the effect of biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) and irrigation system on plant height (cm) for two barley varieties during the 2023/2024 and 2024/2025 growing seasons. Plant height, a key indicator of vegetative growth, was significantly influenced by all three experimental factors. Across both seasons, plants grown under drip irrigation consistently outperformed those under sprinkler irrigation. The tallest plants were recorded for the Giza 134 variety treated with biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) under drip irrigation, reaching 107.2 cm in the first season and 106.4 cm in the second. In contrast, the shortest plants were observed for the untreated Giza 135 control under sprinkler irrigation, measuring just 79.2 cm and 81.6 cm in the first and second seasons, respectively. The superiority of drip irrigation was expected. In arid environments like South Sinai, where water is scarce and salinity poses a constant challenge, drip irrigation delivers water directly to the root zone with minimal evaporative loss. This not only conserves water but also maintains lower salt concentrations around the active roots compared to sprinkler systems, where foliar salt absorption can further stress plants. The data bears out across all variety and treatment combinations, drip irrigation gave plant height advantages ranging from roughly 2 to 15 cm. Bio-fertilizer application also clearly enhanced vegetative growth. For Giza 134 under drip irrigation, bio-fertilizer-treated plants reached 107.2 cm compared to 98.6 cm in the untreated control during the first season, a difference of nearly 9 cm. Similar patterns held for Giza 135 and under sprinkler systems, though the magnitude of response varied. This growth promotion likely stems from several PGPR-mediated mechanisms described in the literature. As **Hayat *et al.* (2010)** and **Jin *et al.* (2025)** have documented, beneficial rhizobacteria can produce phytohormones like indole-3-acetic acid (IAA) that directly stimulate root and shoot elongation. They may also enhance nutrient availabili ty through phosphate solubilization and nitrogen fixation, which would be particularly valuable in the low-fertility sandy loam soils of the study site. Varietal differences

were also apparent. Giza 134 generally exhibited greater plant height than Giza 135 across most treatment combinations, suggesting inherent genetic differences in growth potential or stress tolerance. This aligns with **Abdelghany *et al.* (2024)**, who noted substantial variability among barley genotypes in their response to environmental constraints.

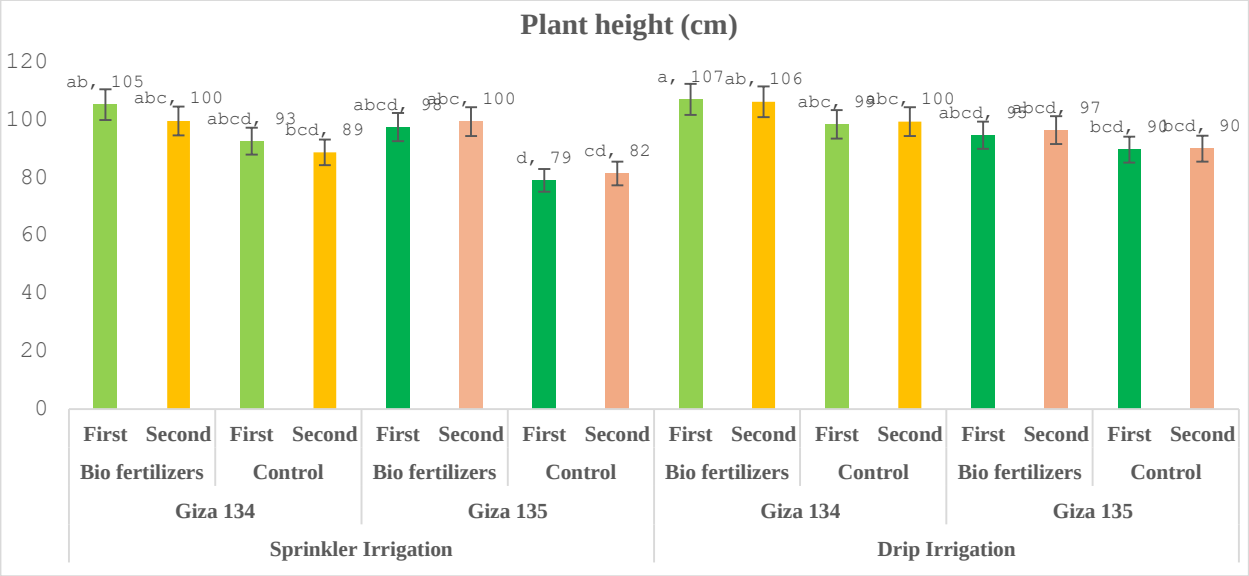


Fig. (2). Effect of biofertilizer *(A. brasilense and P. mucilaginosus)* and irrigation system on plant height (cm) of two barley cultivars during the two growing seasons 2023/2024 and 2024/2025

Tillering Index (TI %)

Tillering capacity, expressed as a percentage, is critical for yield determination in cereals as it directly influences ear-bearing tillers per unit area. The tillering index showed interesting patterns across treatments. According to data in Figure (3), data illustrates that drip irrigation with bio-fertilizer application, both varieties maintained tillering indices above 94% across seasons. Giza 134 with bio-fertilizer under drip irrigation recorded 94.1% and 95.6% in the first and second seasons, respectively. The corresponding values for Giza 135 were 92.0% and 98.2%. These relatively high and stable tillering rates suggest that the combination of efficient irrigation and biological stimulation created favorable conditions for tiller production and survival. The control treatments, particularly under sprinkler irrigation, showed more variable tillering. Giza 135 control under sprinkler irrigation in the first season dropped to 88.7%, the lowest recorded. This reduction likely reflects the compounded stress of saline irrigation water applied through sprinklers, which can lead to foliar salt accumulation and increased physiological stress during the critical tillering phase. What caught our attention was the seasonal variation in tillering response. In several treatment combinations, tillering indices actually increased in the second season compared to the first for instance, Giza 135 with bio-fertilizer under drip irrigation went from 92.0% to 98.2%. This might reflect cumulative soil improvements from PGPR colonization, as beneficial bacteria can persist and multiply in the rhizosphere over time, gradually improving soil structure and organic matter content. The positive effect of biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) on tillering was consistent but modest in absolute terms, typically 2-5 percentage points above controls. While not dramatic, such improvements can translate into meaningful yield increases when multiplied across thousands of tillers per hectare. **Dimkpa *et al.* (2009)** suggested that PGPR-induced stress tolerance may allow plants to maintain better tiller survival under adverse conditions, which could explain the pattern observed here.

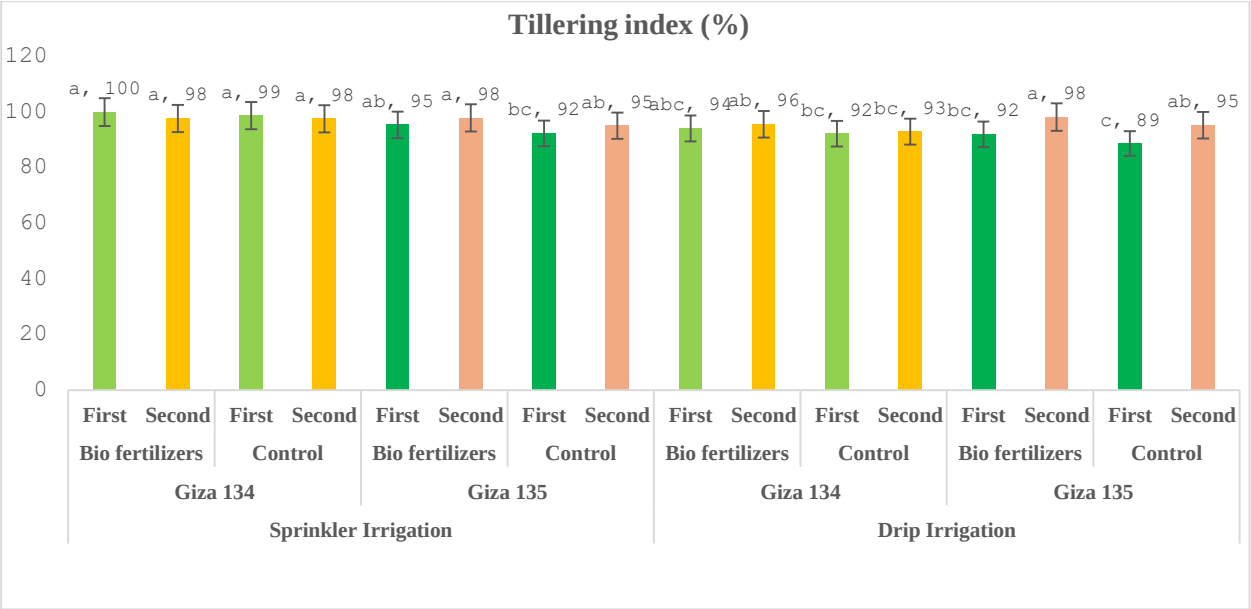


Fig. (3). Effect of biofertilizer *(A. brasilense and P. mucilaginosus)* and irrigation system on tillering index (%) of two barley cultivars during the two growing seasons 2023/2024 and 2024/2025

Thousand-Grain Weight (g)

Thousand-grain weight represents the final product of grain filling and integrates the plant's photosynthetic performance during the reproductive phase. The data in figure (4) illustrates that the parameter showed some of the clearest treatment effects in the study.

The combination of drip irrigation and bio-fertilizer application produced the heaviest grains. Giza 134 with bio-fertilizer under drip irrigation achieved 36.16 g and 35.84 g in the first and second seasons, respectively. Giza 135 under the same treatment combination recorded 34.45 g and 33.98 g. These values compare favorably with typical barley thousand-grain weights reported in the literature for non-stressed conditions.

In contrast, the lightest grains were produced under sprinkler irrigation without bio-fertilizer. Giza 135 control under sprinkler irrigation dropped to 30.24 g and 29.42 g across the two seasons. The reduction of roughly 6 g per thousand grains between the best and worst treatments represents a substantial yield penalty approximately 17% lower kernel weight.

What's particularly instructive is comparing treatments that isolate specific factors. Looking at Giza 134 under drip irrigation, bio-fertilizer application increased thousand-grain weight by about 5 g compared to the control (36.16 vs. 31.24 g in season one). Under sprinkler irrigation, the bio-fertilizer effect was smaller but still positive 34.69 vs. 29.81 g in season one for Giza 134. This suggests that while PGPR can mitigate stress effects, they work best when irrigation management is already optimized.

The physiological explanation likely involves both source and sink dynamics. biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*), through improved nutrient availability and hormone-mediated effects, may enhance photosynthetic capacity during grain filling. Simultaneously, better root health and function under PGPR colonization could maintain water and nutrient uptake even as soil salinity increases during the growing season. **Parida and Das (2005)** reviewed how salt stress disrupts multiple plant processes; mitigating any of these through PGPR activity could preserve grain filling.

Seasonal effects on thousand-grain weight were minimal, suggesting that the treatments established consistent conditions across both years and that the observed patterns are reliable rather than artifacts of particular weather patterns.

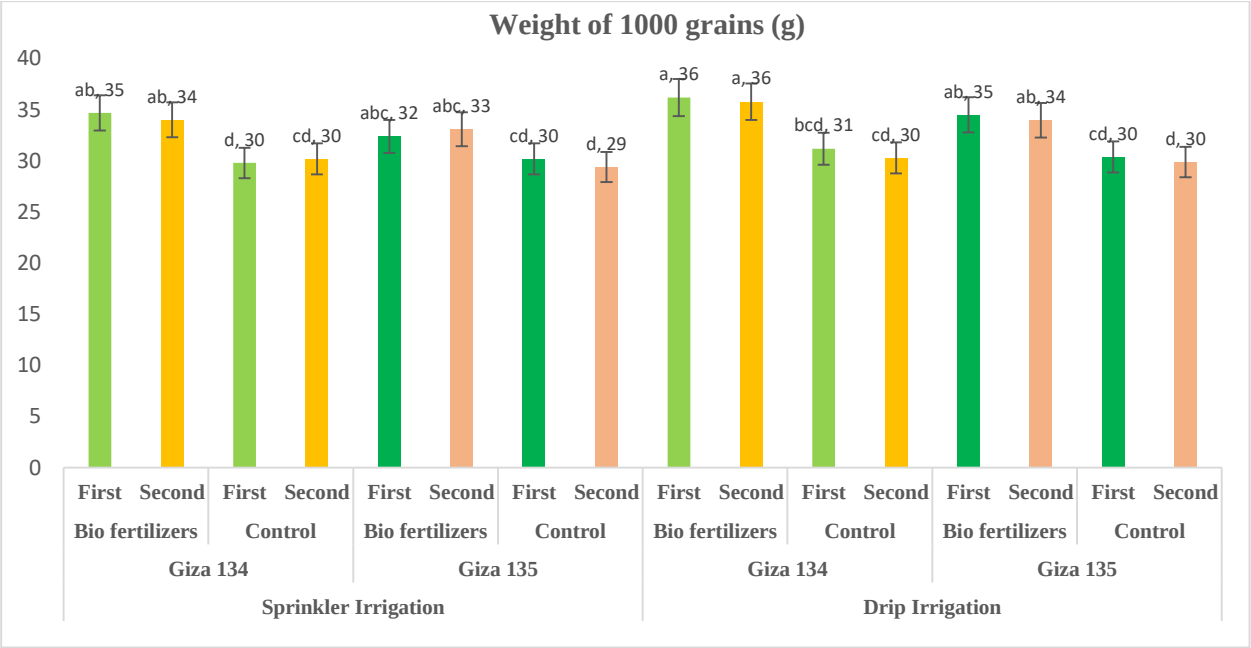


Fig. (4). Effect of biofertilizer *(A. brasilense and P. mucilaginosus)* and irrigation system on 1000 grains weight (g) of two barley cultivars during the two growing seasons 2023/2024 and 2024/2025

Water Use Efficiency (WUE kg/m^3)

The data in Figure (5) illustrates the effect of both the irrigation system used and the application of biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) on water use efficiency in barley fields. Water use efficiency, expressed as kilograms of grain produced per cubic meter of water consumed, is arguably the most critical parameter for agriculture in water-scarce regions like South Sinai. The values we obtained ranged from 0.93 kg/m³ up to 2.19 kg/m³, representing more than a doubling of efficiency between the least and most effective treatment combinations. The highest WUE values were achieved with drip irrigation combined with bio-fertilizer application. Giza 134 under this combination reached 2.13 and 2.19 kg/m³ across the two seasons, while Giza 135 recorded 1.78 and 1.86 kg/m³. These figures are impressive for barley grown under saline conditions and approach values reported for unstressed cereal production in more favorable environments. The lowest WUE values around 0.92-1.03 kg/m³ were consistently observed in sprinkler-irrigated control treatments. This dramatic difference stems from two factors. First, sprinkler systems lose more water to evaporation and wind drift, meaning less of the applied water actually enters the soil profile and becomes available for transpiration. Second, the salt stress imposed by sprinkler irrigation with saline water reduces yield per unit of water transpired, compounding the efficiency loss. Bio-fertilizer effects on WUE were substantial. Taking Giza 134 under drip irrigation as an example, bio-fertilizer application increased WUE from approximately 1.17-1.26 kg/m³ in controls to 2.13-2.19 kg/m³ roughly 80% improvement. Even under the less favorable sprinkler system, biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) boosted WUE from about 0.92-1.03 kg/m³ to 1.81-1.93 kg/m³. These are not marginal improvements; they fundamentally alter the productivity of each unit of water. The mechanism likely operates through both water capture and water use. PGPR-enhanced root systems can explore more soil volume, accessing water that might otherwise remain unavailable. Simultaneously, hormone-mediated effects on stomatal regulation and osmotic adjustment may allow plants to maintain photosynthesis with less water loss. Varietal differences in WUE were apparent, with Giza 134 consistently outperforming Giza 135 across irrigation systems and treatments. This suggests that genetic improvement programs have successfully incorporated traits that enhance either stress tolerance or water capture, and that these traits interact positively with PGPR applications.

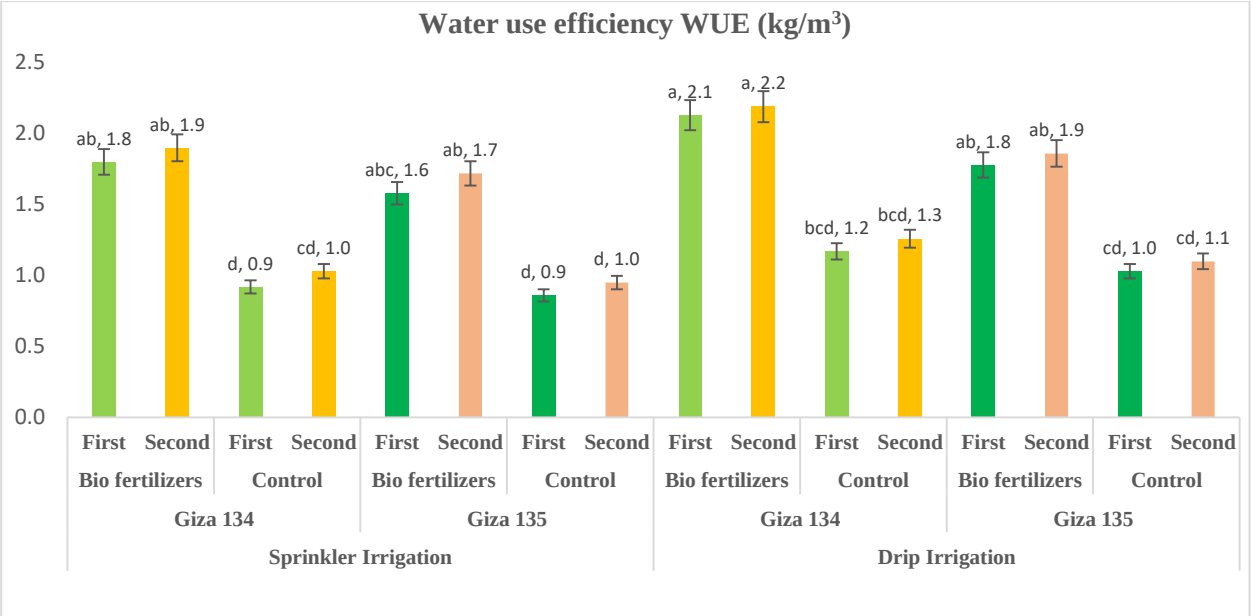


Fig. (5). Effect of biofertilizer *(A. brasilense and P. mucilaginosus)* and irrigation system on water use efficiency (kg/m^3) of two barley cultivars during the two growing seasons 2023/2024 and 2024/2025

Grain Productivity (t/ha)

The productivity data showed clear, consistent patterns across both growing seasons (Fig. 6). The highest yields were achieved with drip irrigation combined with bio-fertilizer application. Giza 134 under this treatment combination produced 5.76 t/ha in the first season and 5.92 t/ha in the second. Giza 135 followed the same pattern but at slightly lower levels 4.81 and 5.01 t/ha across the two seasons. These yields are respectable for barley grown under saline conditions in an arid environment and suggest that the combination of efficient irrigation and biological stimulation can substantially ameliorate the yield-limiting effects of salinity. The lowest yields around 3.02-3.33 t/ha were recorded for sprinkler-irrigated control treatments, particularly for Giza 135. The gap between best and worst treatments exceeded 2.5 t/ha, representing more than 80% yield improvement from optimized management. Several patterns in the yield data deserve comment. First, the response to biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) was larger under drip irrigation than under sprinkler irrigation. This might seem counterintuitive; one might expect greater relative benefits where stress is most severe but it likely reflects the fact that PGPR need reasonably favorable conditions to establish and function effectively. Under severe stress, even beneficial bacteria may struggle to colonize roots and express their growth-promoting traits. Second, the consistency between seasons was remarkable. For most treatment combinations, yields in the second season either matched or slightly exceeded those in the first. This is encouraging from a practical standpoint, as it suggests that the benefits of bio-fertilizer application are not ephemeral but can be relied upon across years. Third, varietal differences persisted across all treatment combinations but narrowed under optimal management. Giza 134 outperformed Giza 135 consistently, but the gap was smaller under drip irrigation with bio-fertilizer than under sprinkler irrigation without. This implies that good management can partially compensate for genetic limitations, though genetic improvement remains valuable. These yield levels compare favorably with those reported by **Emami *et al.* (2019)** for wheat and barley treated with PGPR under salinity stress. They reinforce the conclusion that integrated management—combining efficient irrigation, adapted varieties, and biological amendments offers a viable pathway to maintain cereal production in marginal environments.

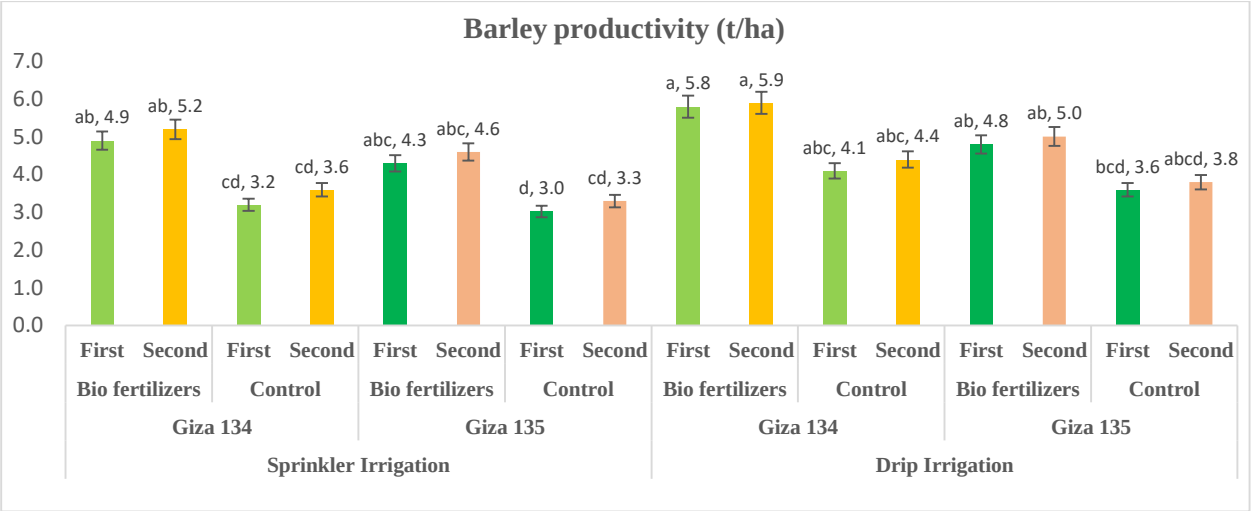


Fig. (6). Effect of biofertilizer *(A. brasilense and P. mucilaginosus)* and irrigation system on productivity (t/ha) of two barley cultivars during the two growing seasons 2023/2024 and 2024/2025

Soil fertility characteristics

Biological content

Total Bacterial Count

The results presented in Figure (7) demonstrate the total Bacterial count which was significantly higher in treatments of bio-fertilizer *(A. brasilense and P. mucilaginosus)* compared to uninoculated controls, with drip irrigation system under Barley variety Giza 135 reaching the maximum value (151 x10^5 CFU/g dry soil) in the second season. Drip irrigation system consistently outperformed sprinkler irrigation system across cultivars, and Giza 135 variety supported greater microbial densities than Giza 134 variety. The inoculation with biofertilizers *(A. brasilense and P. mucilaginosus)* introduces beneficial plant-growth-promoting rhizobacteria (PGPR) that thrive in the rhizosphere under saline conditions. Drip irrigation system creates a stable moisture localized around roots, preventing salt accumulation and enhancing root exudation, which improves microbial proliferation compared to sprinkler irrigation system overhead leaching (**Alotaibi *et al.*, 2024; Manda *et al.*, 2023**).

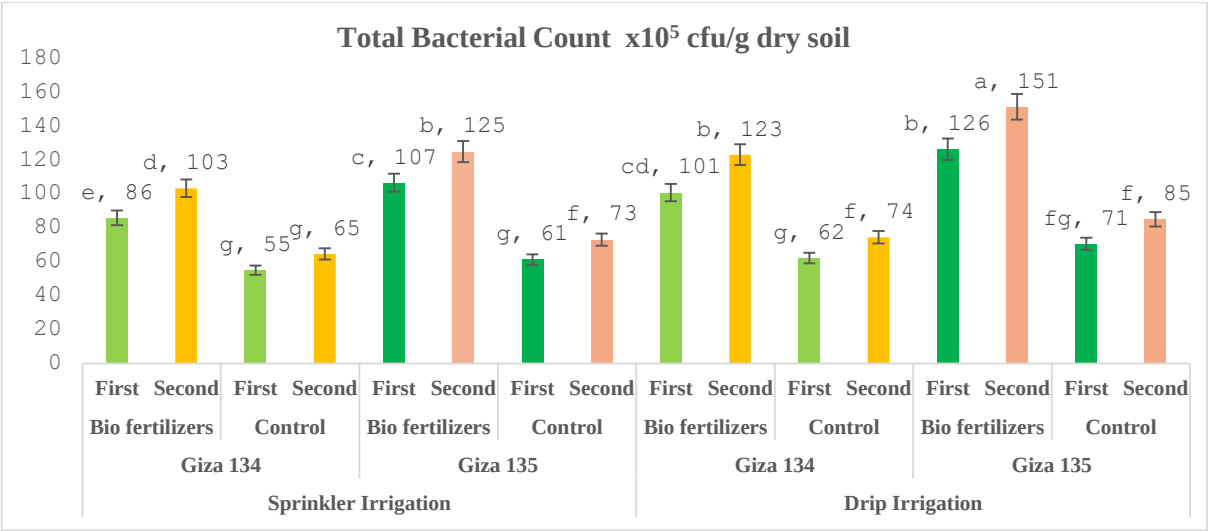


Fig. (7). Effect of biofertilizer *(A. brasilense and P. mucilaginosus)* and irrigation system on total bacterial Count (TBC) in soil cultivated with two barley varieties during the two growing seasons

Nitrogen-Fixing Bacteria

As shown in Figure (8) Nitrogen-fixing bacterial populations peaked under biofertilizers *(A. brasilense and P. mucilaginosus)* treatments combined with drip irrigation system in Giza 135 variety (258 x10^3 CFU/g dry soil), showing progressive accumulation from the first to the second season. Controls recorded substantially lower populations across all irrigation systems (65 x10^3 CFU/g dry soil). Inoculation directly boosts diazotrophic populations, which counteract salt-induced osmotic stress by producing protective exopolysaccharides and osmo-protectants. Drip irrigation system maintains optimal soil air-water balance in hyper-saline South Sinai soils, supporting higher nitrogenase efficiency and bacterial survivability compared to surface-wetting sprinkler systems (**Alotaibi *et al.*, 2024**).

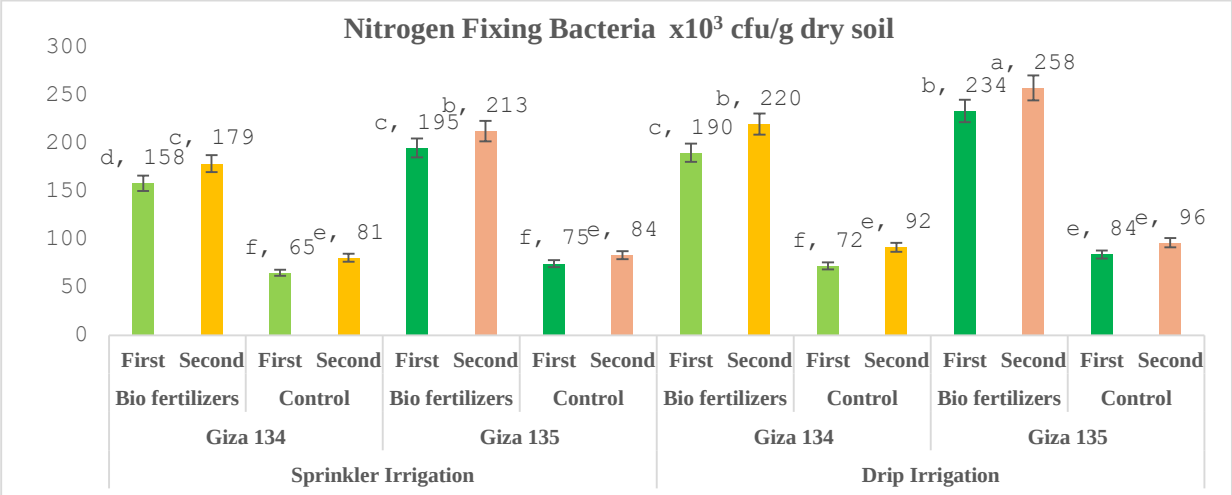


Fig. (8). Effect of biofertilizers *(A. brasilense and P. mucilaginosus)* and irrigation system on nitrogen fixing bacterial count in soil cultivated with two barley varieties during the two growing seasons

Phosphate-Dissolving Bacteria

Phosphate-solubilizing bacteria reached maximum counts under biofertilization *(A. brasilense and P. mucilaginosus)* with drip irrigation in Giza 135 variety (102 x 10^3 CFU/g dry soil in season 2), whereas control values remained low (34 x10^3 CFU/g dry soil) (Fig. 9). Giza 135 variety demonstrated superior rhizospheric compatibility with phosphate-dissolving strains under both irrigation systems. Phosphate-dissolving microflora secretes organic acids that solubilize fixed phosphate ions, overcoming phosphorus immobilization typical of calcareous and saline arid soils. The micro-environment created by drip irrigation preserves rhizospheric organic acids and bacterial density far more effectively than sprinkler irrigation under arid stress (**Alsudays *et al.*, 2024; Machado & Serralheiro, 2017**).

Potassium-Solubilizing Bacteria

Inoculated treatment with biofertilizers *(A. brasilense and P. mucilaginosus)* combined with drip irrigation system, as depicted in Figure (10) produced the highest potassium-solubilizing bacterial population (37 x 10^3 CFU/g dry soil in Giza 135 variety, second season), significantly higher than controls (14 x 10^3 CFU/g dry soil). Sprinkler irrigation system resulted in lower bacterial survival across all treatments. Potassium-solubilizing bacteria release organic acids and chelate silicate minerals to mobilize structural soil potassium, mitigating sodium toxicity in barley roots. Drip irrigation system reduces salt crust formation in the topsoil, maintaining favorable soil osmotic potential required for microbial potassium mobilization in saline environments (**Alotaibi *et al.*, 2024; Machado & Serralheiro, 2017**).

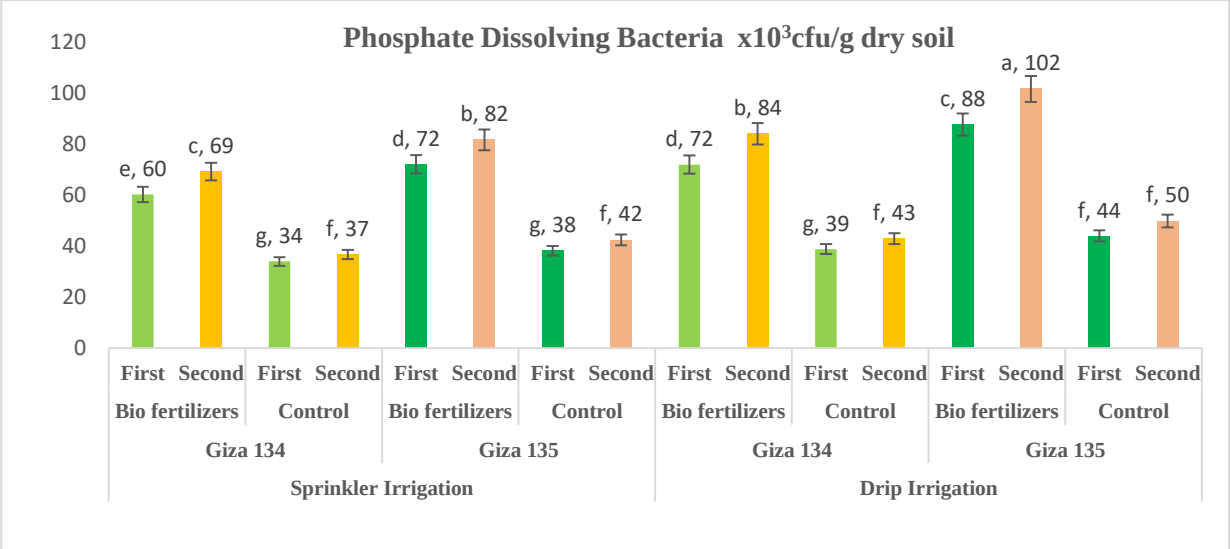


Fig. (9). Effect of biofertilizers *(A. brasilense and P. mucilaginosus)* and irrigation system on phosphate dissolving bacterial count in soil cultivated with two barley varieties during the two growing seasons

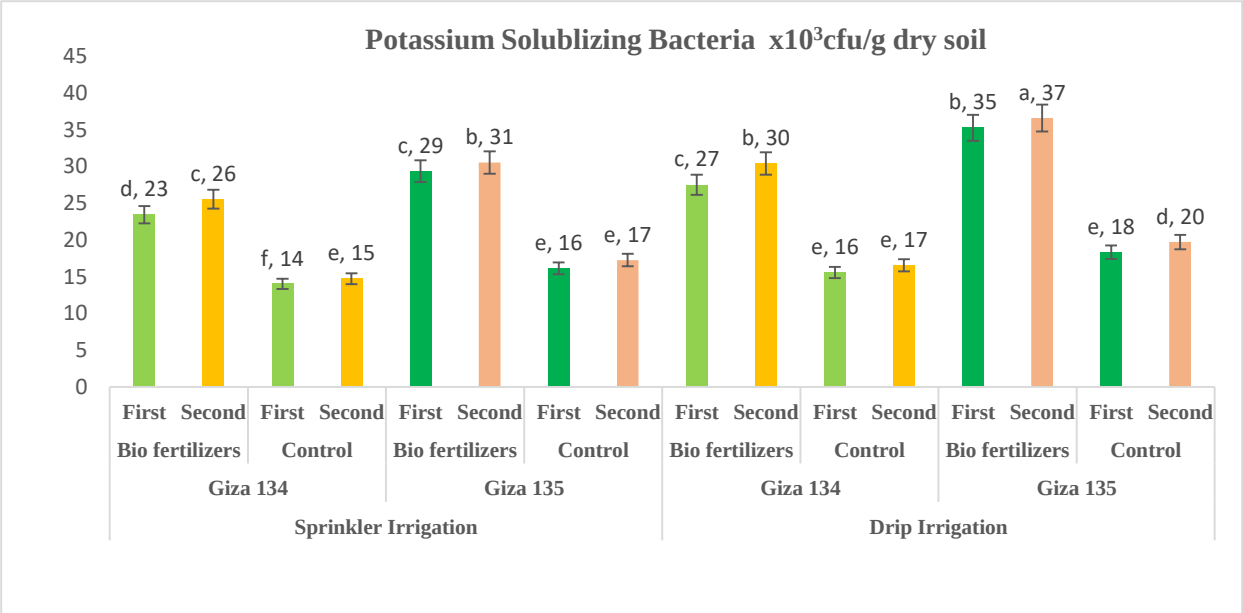


Fig. (10). Effect of biofertilizers *(A. brasilense and P. mucilaginosus)* and irrigation system on potassium solubilizing bacterial count in soil cultivated with two barley varieties during the two growing seasons

Dehydrogenase Activity

According to the data shown in Figure (11), soil dehydrogenase activity reached its highest level in biofertilization *(A. brasilense and P. mucilaginosus)* drip-irrigated Giza 135 variety (207 µg TPF/g dry soil/24h), compared to low baseline levels in controls (96 µg TPF/g dry soil/24h). Activity was consistently higher in the second season across all treatments. Dehydrogenase activity serves as a direct indicator of total viable microbial respiration and metabolic status in saline soils. Bio-fertilization revitalizes soil biological function under salinity stress, while drip irrigation prevents enzyme denaturation by stabilizing rhizosphere moisture and osmotic pressure (**Ilakiya *et al.*, 2023; Manda *et al.*, 2023**).

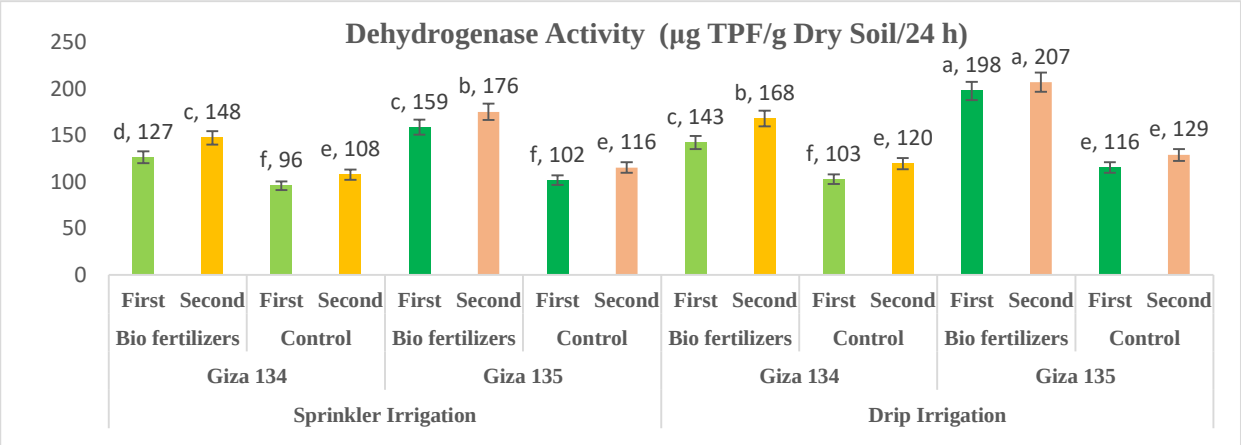


Fig. (11). Effect of biofertilizers *(A. brasilense and P. mucilaginosus)* and irrigation system on dehydrogenase Activity in soil cultivated with two barley varieties during the two growing seasons.

Available Nitrogen in Soil

Soil available nitrogen peaked at (86 ppm) under biofertilization *(A. brasilense and P. mucilaginosus)* drip irrigation system in Giza 135 variety, whereas control plots recorded minimum concentrations (54 ppm) as detailed in Figure (12). The combination of biofertilizers and drip irrigation system maintained higher available N levels in both seasons compared to sprinkler irrigation system. Biological nitrogen fixation by introduced bio-inoculants enriches available soil ammonium and nitrate fractions, counteracting salinity-induced inhibition of mineral N uptake. Drip irrigation system minimizes nitrate leaching losses that otherwise occur under sprinkler irrigation system in light-textured desert soils (**Alotaibi *et al.*, 2024; Machado & Serralheiro, 2017**).

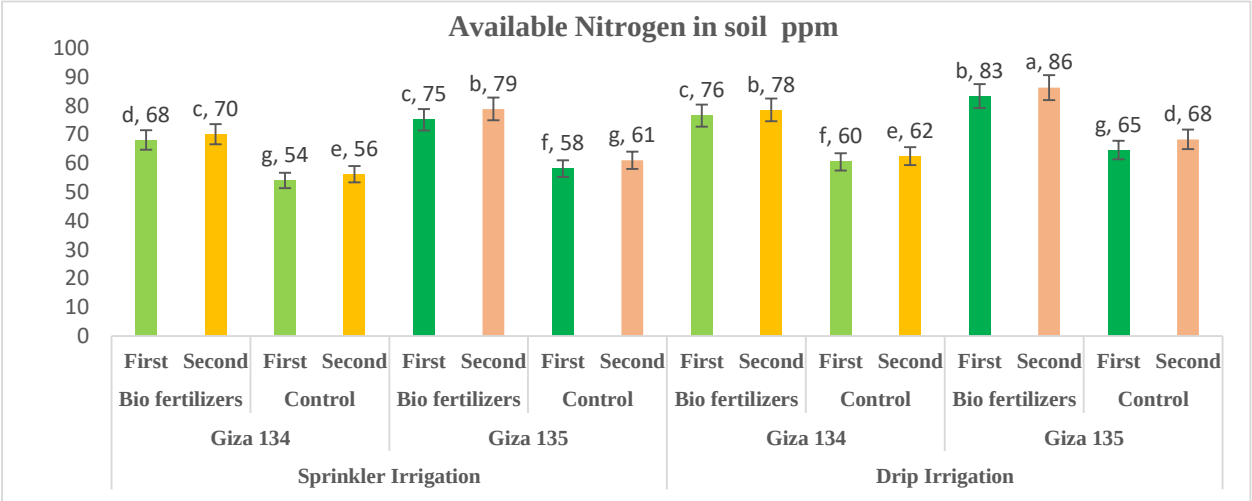


Fig. (12). Effect of biofertilizers *(A. brasilense and P. mucilaginosus)* and irrigation system on available nitrogen in soil cultivated with two barley varieties during the two growing seasons

Available phosphorus in soil

Available soil phosphorus was highest under bio-fertilized *(A. brasilense and P. mucilaginosus)* drip irrigation with Giza 135 variety (11.8 ppm in season 2), compared to uninoculated control (6.8 ppm) (Fig. 13). Drip irrigation system yielded higher available P contents than sprinkler irrigation system across all cultivar-inoculation combinations. Phosphate-dissolving bacteria convert insoluble calcium phosphate into bio-available forms through proton and organic acid extrusion. Localized drip irrigation maintains lower soil pH around the dripper zone, preventing re-precipitation of phosphorus in saline-alkaline soils (**Alsudays *et al.*, 2024; Machado & Serralheiro, 2017**).

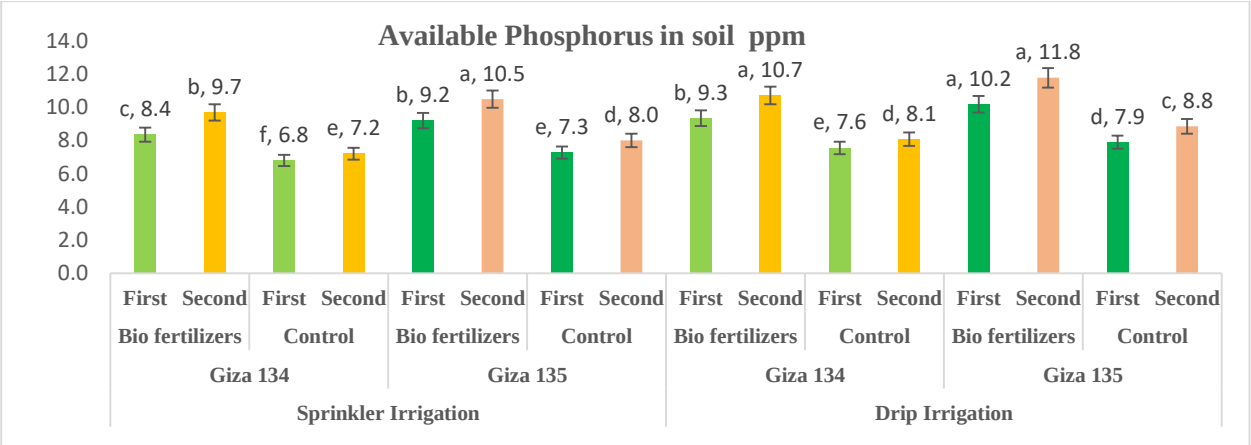


Fig. (13). Effect of biofertilizers *(A. brasilense and P. mucilaginosus)* and irrigation system on available phosphorus in soil cultivated with two barley varieties during the two growing seasons.

Available potassium in soil

As illustrated in Figure (14), available soil potassium reached a maximum of (287 ppm) under bio-fertilized *(A. brasilense and P. mucilaginosus)* drip irrigation in Giza 135 variety during the second season, compared to (153 ppm) in control treatment. Drip irrigation consistently maintained higher K availability than sprinkler irrigation. Bio-fertilizer strains mobilize mineral potassium while enhancing the root zone's K\Na ratio, which is crucial for barley salt tolerance. Drip irrigation delivers water directly to the root zone, reducing surface salt accumulation and keeping bio-mobilized potassium in available form for crop uptake (**Alotaibi *et al.*, 2024; Machado & Serralheiro, 2017**).

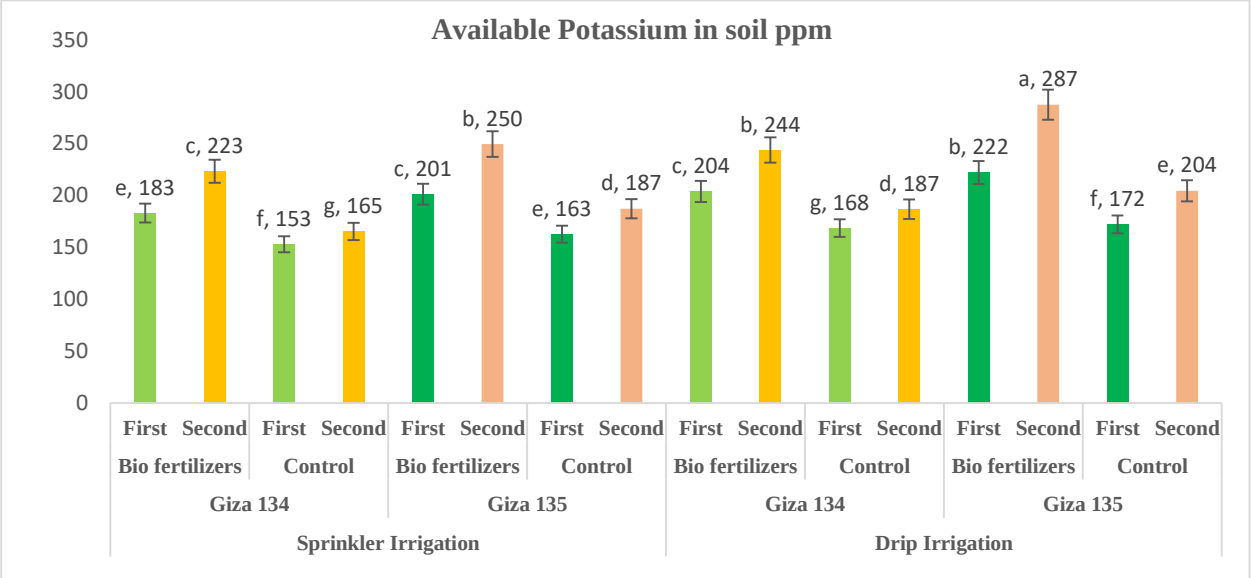


Fig. (14). Effect of biofertilizers *(A. brasilense and P. mucilaginosus)* and irrigation system on available potassium in soil cultivated with two barley varieties during the two growing seasons

Interactive Effects and Practical Implications

The data reveals important interactions among the three experimental factors. The three-way interaction among irrigation system × variety × biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) was significant for most parameters, indicating that the response to biofertilizers depended on both the variety grown and the irrigation method used.

For Giza 134 under drip irrigation, bio-fertilizer application increased yield by approximately 1.7 t/ha compared to the control. For the same variety under sprinkler irrigation, the bio-fertilizer benefit

was about 1.3 t/ha. For Giza 135, the corresponding benefits were roughly 1.2 t/ha under drip and 0.8 t/ha under sprinkler. Thus, both the absolute and relative benefits of biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) were largest when combined with the more efficient irrigation system and the more responsive variety.

From a practical perspective, these findings suggest that farmers in South Sinai and similar environments should consider the following:

- Drip irrigation provides clear advantages over sprinkler systems for barley production under saline conditions, improving all measured parameters and enhancing the response to biofertilizers.
- biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) application is a low-cost, environmentally friendly intervention that consistently improves yield and water use efficiency. The benefits are substantial enough to justify routine use, particularly when combined with drip irrigation.
- Variety selection matters. Giza 134 consistently outperformed Giza 135, suggesting it carries traits that confer better adaptation to the prevailing conditions or greater responsiveness to PGPR.
- The combination of drip irrigation, Giza 134, and bio-fertilizer treatment represent the optimal management package identified in this study, producing the highest yields and water use efficiency.

The mechanisms underlying these responses, while not directly measured in this field study, can be inferred from the extensive literature on PGPR. As reviewed by **Hayat *et al.* (2010)**, beneficial rhizobacteria enhance plant performance through multiple pathways: phytohormone production, nutrient solubilization, induced systemic resistance, and osmotic adjustment. Under saline conditions, these mechanisms are likely to help plants maintain better water status, continue nutrient uptake despite high osmotic pressure, and protect photosynthetic machinery from salt-induced damage.

4. Conclusion

This study demonstrates that barley production in the saline, arid conditions of South Sinai can be substantially improved through integrated management combining efficient irrigation, adapted varieties, and bio-fertilizer application. The key findings can be summarized as follows:

1. Drip irrigation consistently outperformed sprinkler irrigation across all measured parameters, producing taller plants, higher tillering rates, heavier grains, better water use efficiency, and greater yields. The superiority of drip systems likely reflects both reduced evaporative losses and avoidance of foliar salt accumulation.
2. Bio-fertilizer application significantly enhanced all parameters under both irrigation systems, though the magnitude of benefit was larger under drip irrigation. Yield increases of 1.5-1.7 t/ha from bio-fertilizer treatment under drip irrigation represent economically meaningful improvements that justify routine adoption.
3. Varietal differences were apparent, with Giza 134 showing superior performance across most treatment combinations. This suggests that genetic improvement programs have successfully developed materials with better adaptation to the prevailing constraints.
4. The optimal treatment combination identified was drip irrigation with Giza 134 and bio-fertilizer application, producing yields approaching 6 t/ha with water use efficiency exceeding 2 kg/m³. This represents a practical recommendation for farmers in similar environments.
5. Water use efficiency more than doubled between the least and most effective treatment combinations, highlighting the critical importance of integrated management when water is the primary limiting factor.

These findings contribute to the growing evidence that plant growth-promoting rhizobacteria offer practical, sustainable options for maintaining crop production in marginal environments. They also underscore the importance of matching biological amendments with appropriate irrigation methods and adapted varieties to maximize benefits. For the farming communities of South Sinai and similar arid regions facing soil salinity and water scarcity, the combination of drip irrigation, improved varieties,

and biofertilizers (*Azospirillum brasilense* & *Paenibacillus mucilaginosus*) represents a viable pathway toward more resilient and productive cereal cultivation.

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