RESEARCH PAPER

Role of *Rhizobia* Bacteria and Nitrogen Fertilizer Levels in Enhancing Nutrient Uptake, Growth, and Yield of Cowpea (*Vigna unguiculata*) Under Saline Irrigation Conditions

Alaa M. Khairallah[1], Rashad A. Imran[2], Labeed A. Najm[3]
[1] Department of Soil Science and Water Resources, College of Agriculture, University of Basrah, Iraq, pgs.alaa.maytham@uobasrah.edu.iq
[2] Department of Soil Science and Water Resources, College of Agriculture, University of Basrah, Iraq, rashad.imran@uobasrah.edu.iq
[3] Department of Intelligent Medical Systems, College of Computer Science and IT, University of Basrah, Iraq, labeed.najim@uobasrah.edu.iq
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Abstract
A pot experiment was conducted during the spring season on February 18, 2026, to evaluate the effect of *Rhizobia* strains isolated from root nodules of cowpea plants grown in a saline environment, combined with varying levels of nitrogen fertilizer, on enhancing cowpea growth, yield, and nutrient uptake under irrigation with water of differing salinity levels. The experiment comprised three irrigation salinity levels (2, 4,8) dsm^{-1}, three inoculation treatments with molecularly identified *Rhizobia* strains uninoculated control (R0), inoculation with *Brady rhizobium japonicum* (Rj), and inoculation with *Brady rhizobium* sp. (Rbr) and three nitrogen fertilizer levels (40%, 60%,80% of the recommended rate of 60 kg H $^{1-}$ applied as urea, (46% N). The results revealed that inoculation with Rj and Rbr combined with the 80% nitrogen fertilization level under 8 dsm-1 saline irrigation significantly increased total leaf chlorophyll content, leaf nitrogen, phosphorus, and potassium concentrations, total yield, and seed protein percentage compared to the uninoculated control (R0). These findings demonstrate that bio inoculation can reduce production costs by decreasing recommended mineral nitrogen inputs, mitigate environmental pollution associated with excessive synthetic fertilizer application, maintain soil fertility, and support sustainable agricultural practices in freshwater-scarce regions.
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1. Introduction

Modern agriculture currently faces the critical challenge of maintaining or increasing crop productivity under worsening environmental conditions, particularly abiotic stresses such as drought, salinity, and extreme temperatures—phenomena that have become a growing concern for both farmers and researchers (Abd El Baki et al., 2025).
Rhizobia hold substantial ecological and agricultural significance due to their ability to establish symbiotic relationships with leguminous plants and form root nodules, within which atmospheric nitrogen is fixed and converted into a bioavailable form for the plant. Beyond nitrogen fixation, numerous rhizobial strains exhibit plant-growth-promoting traits, including the production of indole-3-acetic acid (IAA), phosphate solubilization, and the synthesis of siderophores, organic acids, and exopolysaccharides (EPS). Furthermore, certain strains possess 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, which can contribute to reducing elevated ethylene levels in plants under salt-stress conditions (Singh et al., 2025).
Certain strains of rhizobia demonstrate a high tolerance to elevated salt concentrations, rendering them valuable targets for isolating and selecting strains adapted to saline environments. Among the primary mechanisms underlying rhizobial salt tolerance are osmotic balance maintenance through the accumulation of compatible solutes, ion transport regulation, enhanced cell membrane stability, and the production of extracellular polysaccharides (EPS) that protect bacterial cells and improve their survival in high-osmotic-potential environments (Gan et al., 2024; Gharbi et al., 2025). Consequently, investigating indigenous rhizobial isolates is of increasing importance, as strains that naturally originate and adapt to salt-affected soils may possess physiological and molecular attributes enabling superior survival

and symbiotic performance compared to those isolated from non-saline environments. Indeed, several recent studies have demonstrated the feasibility of isolating salt-tolerant rhizobial strains from the root nodules of plants growing in diverse habitats, revealing clear inter-strain variability in NaCl tolerance and plant-growth-promoting traits (Loya et al., 2024).

2. Materials and Methods

2.1. Isolation of Rhizobia was isolated from nodules formed on the roots of cowpea plants grown in saline soils. The roots were preserved in plastic bags to prevent desiccation. Large, active, pink-colored nodules were detached, washed with distilled water, and surface-sterilized using 70% ethanol for three minutes. Following three rinses with sterile distilled water (SDW), the nodules were immersed in a sodium hypochlorite (NaClO) solution (3:1 water to NaClO) for 15 minutes, then rinsed again three times with SDW. The nodules were dried by transferring them to a Petri dish containing sterile filter paper. They were subsequently crushed using a sterile glass rod with the addition of one or two drops of SDW to obtain a suspension. This suspension was utilized to inoculate sterile Petri dishes containing Yeast Extract Mannitol Agar (YEMA) supplemented with Congo Red dye (1 ml L-1 of 2.5% preparation) using the streak plate method. The plates were incubated at 30°C for five days until white colonies emerged, indicating the non-absorption of Congo Red. The isolates were then purified and preserved in liquid glycerol for future use (Beck et al., 1993; Abdullah, 2021).

2.2. Agricultural Experiment The agricultural soil was obtained from the Agricultural Research Station of the University of Basrah, Karma Ali. Biological analyses of the soil were conducted immediately after sampling. Subsequently, the soil was air-dried, crushed, and passed through a 2-mm sieve. Initial physical and chemical analyses of the soil were performed prior to planting, as detailed in Table (1), following standard soil analysis protocols (Black, 1965; Page et al., 1982). The soil was placed in sterilized pots at a rate of 10 kg per pot and arranged according to a Completely Randomized Design (CRD). The experiment comprised a factorial arrangement of three factors, with three levels per factor and three replicates per level, totaling 81 treatments as follows: Irrigation water at three salinity levels (2, 4, and 8 dS m-1); three inoculation treatments (R0, Rj, and Rbr); and three nitrogen fertilization levels representing 40%, 60%, and 80% of the recommended nitrogen application rate (60 kg N ha-1) in the form of urea (46% N). The nitrogen fertilizer was applied in three split doses: the first before planting, the second one month after germination, and the third prior to the flowering stage. Additionally, phosphorus fertilizer was applied at 100 kg P ha-1 as concentrated superphosphate, and potassium fertilizer at 100 kg K ha-1 as potassium sulfate, both mixed into the surface soil layer prior to planting. Organic fertilizer, consisting of air-dried and crushed cow manure, was incorporated at a rate of 150 g per 10 kg of soil.

2.3. Preparation of Liquid Rhizobial Inoculum Following the activation of the salt-tolerant rhizobial isolates, 2 ml of SDW was added to each culture. The microbial growth was harvested and transferred to sterile, transparent plastic tubes to standardize their concentrations against the McFarland standard turbidity. Subsequently, 1 ml of each culture inoculum was added to 0.5 L of liquid Nutrient Broth, and the inoculated cultures were incubated at 28°C for 72 hours (Beck et al., 1993).

Once the liquid inoculum of the efficient, cowpea-specific rhizobia reached a population density of 10^8 CFU ml-1, each type of inoculum was placed in a sterile container. Seeds of a local cowpea cultivar were soaked in the inoculum, with Arabic gum added to enhance adhesion. The inoculated seeds were left for 1.5 hours at room temperature to allow the inoculum to adhere thoroughly. For the control treatment, seeds were soaked in SDW without inoculation. Both inoculated and uninoculated seeds were sown in their respective pots.

Following germination, all treatments were irrigated with RO water up to field capacity. Two weeks post-germination, irrigation commenced using water with different salinity levels, accounting for the leaching requirement. Upon reaching the flowering stage, total leaf chlorophyll content was determined according to Goodwen (1976). Leaf nitrogen concentration was estimated using the method described by Nelson and Sommers (1973), phosphorus concentration via the ascorbic acid method (Murphy and Riley, 1962), and potassium concentration according to Cresser and Parsons (1979). At complete maturity, 90 days after planting, the crop was harvested to record the total yield weight. Seed protein percentage was calculated using the following formula: Seed Protein % = Nitrogen % × 6.25.

Table (1) Selected physical, chemical, and biological properties of the soil prior to planting

Value	Unit	property
7.40	------	PH soil PH
2.25	dSm^{-1}	EC electrical conductivity
13.32	$Cmol_{(+)}kg^{-1}$	**CEC:** Cation Exchange Capacity
1.12	$g\ kg^{-1}$	Total Nitrogen
0.0442		Available Nitrogen
0.0272		Available Phosphorus

0.0884		Available Potassium	
1.96		Organic Matter	
130.00		Total Solid Carbonates	
2.45	mmol L^{-1}	Calcium	Soluble Cations
1.10		Magnesium	
25.33		Sodium	
0.88		Potassium	
0.00		Carbonate	Soluble Anions
1.12		Bicarbonate	
11.22		Sulfates	
10.00		Chloride	
		: ESP Exchangeable Sodium Percentage (ESP)	
396.0	g kg^{-1}	sand	Soil Separates (%)
398.0		silt	
255.0		clay	
Loam		Texture class	
10^3×44.22	CFU kg^{-1}	Total Bacterial Population	
10^4× 2.74		Fungal Population	
10^3×0.45		Rhizobial Population	

3. Results and Discussion

3.1. Total Leaf Chlorophyll Content mg g-1

The results in Table (2) indicate that increasing the salinity of irrigation water significantly affected the total chlorophyll content in cowpea leaves. Mean chlorophyll content significantly decreased to 1.987, 1.573, and 1.281 mg g-1at salinity levels S2, S4, and S8, respectively. This significant reduction is attributed to salt-induced osmotic and ionic disturbances that impair physiological processes responsible for chlorophyll biosynthesis in leaves. Accumulation of sodium (Na+ and chloride (Cl-) ions impedes the uptake of essential nutrients required for chlorophyll synthesis, particularly nitrogen (N), magnesium (Mg^2+, and iron (Fe2+), all of which are structural or catalytic components in chlorophyll molecule formation. Furthermore, salinity enhances the generation of reactive oxygen species (ROS), which induce membrane lipid peroxidation and protein oxidation, accelerate chlorophyll pigment degradation, inhibit key biosynthetic enzymes, and disrupt chloroplast structural integrity and photosynthetic reaction centers, thereby lowering photosynthetic efficiency and total chlorophyll accumulation (Nabi et al., 2024).

Inoculation with rhizobial strains also exerted a significant positive effect on leaf chlorophyll content, increasing from a mean of 1.229 mg g^-1 in the uninoculated control (R0) to 1.739 and 1.873 mg g^-1 in plants inoculated with rhizobial strains Rj and Rbr, respectively. This enhancement is ascribed to the capacity of rhizobia to improve plant nitrogen nutrition through symbiotic atmospheric nitrogen fixation within root nodules. This increases the availability and uptake of N a critical constituent for synthesizing chlorophyll molecules, photosynthetic proteins, and enzymes. Moreover, rhizobial inoculation promotes the uptake of other vital nutrients, notably Mg^2+ and Fe^2+, which are essential for chlorophyll synthesis. Additionally, rhizobia produce plant growth regulators, including auxins, cytokinins, and gibberellins, which stimulate shoot development, enhance chloroplast functional efficiency, and consequently elevate photosynthetic performance and leaf chlorophyll accumulation (Prathima et al., 2024).

Nitrogen fertilizer application also had a significant effect on chlorophyll content, which rose from 1.450 mg g^-1 at the N 40level to 1.601 and 1.789 mg g^-1at N 60 and N 80, respectively. This significant increase occurs because nitrogen is a fundamental structural component of the chlorophyll molecule, as well as an essential constituent of amino acids, proteins, enzymes, and nucleic acids required for vegetative growth and photosynthesis. Adequate nitrogen supply enhances chlorophyll synthesis and chloroplast development efficiency while delaying leaf senescence and maintaining chlorophyll pigment stability over extended periods, leading to higher total chlorophyll levels compared to non-fertilized or low-nitrogen controls (Musa et al., 2021).

A significant two-way interaction was observed between irrigation water salinity and bioinoculation. The highest mean chlorophyll content (2.314 mg g^-1 was recorded under the S2 salinity level combined with the Rbr inoculation treatment, whereas the lowest mean 1.022 mg g^-1 occurred under S8 salinity in the uninoculated control (R0). This response is attributed to the protective role of salt-tolerant rhizobial strains in mitigating the adverse impacts of salt stress. Bioinoculation with rhizobia enhances symbiotic N2 fixation, increases nutrient bioavailability, stimulates endogenous phytohormone secretion, activates enzymatic antioxidant systems, and promotes the accumulation of compatible osmolytes such as proline. Together, these physiological responses protect chloroplast membranes, preserve chlorophyll stability, and sustain photosynthetic activity under saline irrigation conditions (Kibret et al., 2024).

Likewise, the two-way interaction between irrigation water salinity and nitrogen fertilization levels was significant. The maximum mean chlorophyll content 2.102 mg g^-1 was obtained under the S2 level combined with N 80 fertilization, while the minimum value 1.128 mg g^-1 was observed under S8 salinity with N 40 fertilization. This is explained by the fact that adequate nitrogen availability provides the required substrate for chlorophyll assembly and the synthesis of proteins and photosynthetic enzymes under moderate stress conditions (Chaudhary et al., 2025).

The two-way interaction between rhizobial bioinoculation and nitrogen fertilizer levels also proved significant. The highest chlorophyll content 2.079 mg g^-1 was recorded with Rbr inoculation at the N 80 level, compared to the lowest value 1.123 mg g^-1 in the uninoculated R0 times N 40 treatment. This synergistic effect stems from the complementary action of symbiotic nitrogen fixation by specialized rhizobia and mineral nitrogen supplementation, which optimizes plant nutritional status and maximizes nitrogen availability for chlorophyll formation (Gautam et al., 2024).

Finally, the three-way interaction among irrigation salinity, bioinoculation, and nitrogen fertilization levels significantly affected total chlorophyll content. The highest value overall (2.443 mg g^-1 was achieved under S2 salinity (noted as S2/N2 in text), Rbr inoculation, and N80 fertilization, whereas the lowest value (0.857 mg g^-1 occurred under S8 salinity, without inoculation (R0), and at the lowest nitrogen level (N40). This interactive enhancement reflects integrated physiological mechanisms wherein rhizobial symbiosis and optimal nitrogen supply act synergistically to alleviate salt-induced stress and maintain photosynthetic apparatus functionality (Farhan et al., 2024).

Table (2) Effect of rhizobial inoculation, nitrogen fertilization levels, and irrigation water salinity on total leaf chlorophyll content of cowpea mg g^-1

Inoculation×nitrogen level	**Salinity Level**			**Nitrogen level**	Inoculation
	8S	**4S**	**2S**		
1.123	**0.857**	**1.163**	**1.350**	**40 N**	**R0**
1.220	**1.037**	**1.233**	**1.390**	**60 N**	
1.344	**1.173**	**1.360**	**1.500**	**80 N**	
1.564	**1.207**	**1.367**	**2.120**	**40 N**	**Rj**
1.708	**1.347**	**1.563**	**2.213**	**60 N**	
1.943	**1.550**	**1.917**	**2.363**	**80 N**	
1.662	**1.320**	**1.513**	**2.153**	**40 N**	**Rbr**
1.877	**1.420**	**1.863**	**2.347**	**60 N**	
2.079	**1.620**	**2.173**	**2.443**	**80 N**	
	1.281	**1.573**	**1.987**	**S Mean**	
SRN = 0.0518, RN = 0.0299 , S = 0.0173				**L.S.D (0.05)**	
معدل N	**8S**	**4S**	**2S**		
1.450	**1.128**	**1.348**	**1.874**	**40 N**	**Salinity× Nitrogen**
1.601	**1.268**	**1.553**	**1.983**	**60 N**	
1.789	**1.448**	**1.817**	**2.102**	**80 N**	
0.0173	**0.0299**			**L.S.D (0.05)**	
R Mean	**8S**	**4S**	**2S**		

1.229	**1.022**	**1.252**	**1.413**	**R0**	**Salinity×Pollination**
1.739	**1.368**	**1.616**	**2.232**	**Rj**	
1.873	**1.453**	**1.850**	**2.314**	**Rbr**	
0.0173	**0.0299**			**L.S.D (0.05)**	

3.2. Leaf Nitrogen Concentration g kg^-1

The results presented in Table (3) indicate that increasing the salinity of irrigation water significantly affected the nitrogen concentration in cowpea leaves. Mean nitrogen concentrations significantly decreased to 42.881, 38.410, and 25.843 g kg^-1 at salinity levels S2, S4, and S8, respectively. This significant reduction is attributed to salt-induced osmotic stress and ionic toxicity, which adversely impair nitrogen uptake, translocation, and assimilation within the plant. High concentrations of sodium Na^ and chloride Cl^ ions diminish the ability of roots to absorb water, nitrate NO3^-, and ammonium NH4^+ due to ionic competition and disrupted cell membrane permeability. Consequently, the quantity of nitrogen absorbed and translocated to the leaves declines. Furthermore, salinity inhibits key enzymes responsible for nitrogen assimilation, thereby suppressing the reduction of nitrate to ammonia and its subsequent conversion into amino acids and proteins, which lowers leaf nitrogen levels (Gao et al., 2024).

Inoculation with rhizobial strains also exerted a significant positive effect on leaf nitrogen concentration, increasing from a mean of 29.126 g kg^-1 in the uninoculated control R0 to 38.093 and 39.914 g kg^-1 in plants inoculated with rhizobial strains Rj and Rbr, respectively. This significant increase is attributed to the high capacity of efficient rhizobial strains to form functional root nodules and fix atmospheric nitrogen via nitrogenase enzyme activity. This raises bioavailable nitrogen levels relative to uninoculated plants. The fixed nitrogen is translocated to the leaves as organic compounds, such as amino acids and amides, where it is utilized in synthesizing proteins, chlorophyll, and enzymes, ultimately elevating nitrogen concentration in leaf tissues and promoting vegetative growth findings that align with Ayalew et al. (2024).

Nitrogen fertilizer application also had a significant effect on leaf nitrogen concentration, which increased from a mean of 33.270 g kg^-1 at N40 to 35.444 and 38.419 g kg^-1 at N60and N80, respectively. This significant elevation occurs because nitrogen supplementation enhances nitrogen availability in the rhizosphere, increasing its uptake rate as nitrate or ammonium and its subsequent transport and accumulation in leaf tissues (Musa et al., 2021).

A significant two-way interaction was observed between irrigation water salinity and bioinoculation. The highest mean nitrogen concentration (47.658 g kg^-1 was recorded under S2 salinity combined with Rbr inoculation, whereas the lowest mean (22.731 g kg^-1 occurred under S8 salinity in the uninoculated control R0. This increase is ascribed to inoculation with efficient, salt-tolerant *Bradyrhizobium* strains, which mitigate the negative impacts of salinity on biological nitrogen fixation. Although salinity reduces nodule formation and nitrogenase activity, efficient strains maintain high symbiotic activity, increasing the amount of fixed nitrogen translocated to the leaves compared to uninoculated controls under identical salinity levels (Bouzroud et al., 2023).

Similarly, the two-way interaction between irrigation water salinity and nitrogen fertilization levels was significant. The maximum mean nitrogen concentration (46.471 g kg^-1 was achieved under S2 salinity combined with N80fertilization, while the minimum value (24.641 g kg^-1 was observed under S8 salinity with N40 fertilization. This significant increase occurs because adequate nitrogen nutrition alleviates the detrimental effects of salt stress by enabling the plant to maintain osmotic balance through increased accumulation of proline, soluble amino acids, and other nitrogenous compounds that protect cells from salinity-induced physiological drought (Kumar et al., 2023).

The two-way interaction between rhizobial bioinoculation and nitrogen fertilizer levels also proved significant. The highest leaf nitrogen concentration (42.466 g kg^-1 was recorded with Rbr inoculation at N80, compared to the lowest value (26.300 g kg^-1 in the uninoculated R0 N40 control. This enhancement stems from the synergy between biological nitrogen fixation by specialized rhizobia and mineral nitrogen supplied to the soil, providing a sustained nitrogen supply throughout growth stages and increasing leaf nitrogen concentrations beyond those achieved by individual treatments (Costa et al., 2023).

Conversely, the three-way interaction among irrigation water salinity, bioinoculation, and nitrogen fertilization levels showed no significant effect p >0.05 on the leaf nitrogen concentration of cowpea plants.

Table (3) Effect of rhizobial inoculation, nitrogen fertilization levels, and irrigation water salinity on leaf nitrogen concentration of cowpea g kg^-1

Fertilization×nitrogen level	**Salinity Level**			**Nitrogen Level**	Inoculation
	8S	**4S**	**2S**		
26.300	**21.143**	**25.887**	**31.870**	**40 N**	**R0**
28.526	**22.697**	**27.950**	**34.930**	**60 N**	
32.553	**24.353**	**32.943**	**40.363**	**80 N**	
35.851	**26.240**	**39.187**	**42.127**	**40 N**	**Rj**
38.191	**27.427**	**41.377**	**45.770**	**60 N**	
40.237	**28.080**	**44.737**	**47.893**	**80 N**	
37.660	**26.540**	**41.827**	**44.613**	**40 N**	**Rbr**
39.617	**27.880**	**43.767**	**47.203**	**60 N**	
42.466	**28.227**	**48.013**	**51.157**	**80 N**	
	25.843	**38.410**	**42.881**	**S mean**	
SRN = NS , RN = 1.008 , S = 0.582				**L.S.D** (0.05)	
N mean	**8S**	**4S**	**2S**		
33.270	**24.641**	**35.633**	**39.537**	**40 N**	**Salinity× Nitrogen**
35.444	**26.001**	**37.698**	**42.634**	**60 N**	
38.419	**26.887**	**41.898**	**46.471**	**80 N**	
0.582	**1.008**			**L.S.D** (0.05)	
R mean	**8S**	**4S**	**2S**		
29.126	**22.731**	**28.927**	**35.721**	**R0**	**Salinity× Pollination**
38.093	**27.249**	**41.767**	**45.263**	**Rj**	
39.914	**27.549**	**44.536**	**47.658**	**Rbr**	
0.582	**1.008**			**L.S.D** (0.05)	

3.3. Leaf Phosphorus Concentration g kg^-1

The results presented in Table (4) indicate that increasing the salinity of irrigation water significantly affected the phosphorus concentration in cowpea leaves. Mean phosphorus concentration significantly decreased to 4.864, 3.714, and 2.969 g kg^-1at salinity levels S2, S4, and S8, respectively. This significant reduction is attributed to salt-induced impairments in phosphorus absorption, translocation, and utilization through interactive physiological and chemical mechanisms. High concentrations of sodium Na^+ and chloride Cl^- salts in the soil solution lower osmotic potential, restricting root water and nutrient uptake. Consequently, root capacity to absorb phosphate ions H2PO4^- and HPO4^2-and translocate them to the leaf tissue is severely compromised (Chaudhary et al., 2024).

Inoculation with rhizobial strains exerted a significant positive influence on leaf phosphorus concentration, increasing from a mean of 2.930 g kg^-1 in the uninoculated control R0 to 4.176 and 4.441 g kg^-1 in plants inoculated with rhizobial strains Rj and Rbr, respectively. This increase stems from the capacity of rhizobial inoculation to enhance root system architecture by proliferating root hair density and functional root nodule development, thereby expanding root surface area and nutrient absorption efficiency. Furthermore, effective rhizobial strains secrete organic acids and hydrolytic enzymes such as phosphatases, which solubilize insoluble phosphate complexes into bioavailable forms readily absorbed by the host plant (Afkairin et al., 2024).

Nitrogen fertilization levels also significantly influenced phosphorus concentration, which increased from a mean of 3.707 g kg^-1at N40to 3.827 and 4.013 g kg^-1 at N60 and N80, respectively. This progressive elevation is explained by optimal nitrogen supplementation stimulating root growth and hair density, thereby maximizing the root-soil contact area for phosphate intercepting. In addition, nitrogen application enhances root physiological activity, promoting proton and organic acid exudation into the rhizosphere. This rhizosphere acidification solubilizes fixed phosphorus minerals, facilitating higher phosphorus accumulation in leaf tissues (Yang et al., 2024).

A significant two-way interaction was observed between irrigation water salinity and bioinoculation. The highest mean phosphorus concentration (5.668 g kg^-1 was recorded under S2 salinity combined with Rbr inoculation, whereas the lowest mean (2.462 g kg^-1 occurred under S8 salinity in the uninoculated control R0. Inoculation with salt-tolerant rhizobial strains alleviates the adverse effects of saline irrigation on phosphorus uptake and vascular transport by preserving active nodulation and supporting root growth under salinity stress (Chaudhary et al., 2024; Pang et al., 2024).

The two-way interaction between irrigation water salinity and nitrogen fertilization levels was also significant. The maximum mean phosphorus concentration (5.198 g kg^-1 was obtained under S2 salinity combined with N80 fertilization, while the minimum value (2.942 g kg^-1 was recorded under S8 salinity with N40 fertilization. Balanced mineral nitrogen nutrition under moderate stress maintains osmotic adjustment via accumulation of solutes like proline and soluble amino acids, preserving root functional integrity for phosphate uptake (Yang et al., 2024).

The two-way interaction between rhizobial bioinoculation and nitrogen fertilization levels proved significant as well. The highest phosphorus concentration (4.573 g kg^-1 was observed with Rbr inoculation at N80, compared to the minimum value (2.769 g kg^-1 in the R0 N40 control. This interaction highlights a synergistic interplay between biological nitrogen fixation and mineral nitrogen nutrition, which enhances root physiological capacity and phosphate translocation to the leaves (Costa et al., 2023).

Finally, the three-way interaction among irrigation water salinity, bioinoculation, and nitrogen fertilization levels significantly affected leaf phosphorus concentration. The highest overall mean (5.923 g kg^-1 was achieved under S2 salinity (noted as S2/N2 in text), Rbr inoculation, and N80 fertilization, whereas the lowest value (2.413 g kg^-1 was recorded under S8 salinity in the uninoculated R0 \times N40 treatment. This interactive response underscores an integrated physiological synergy wherein stress-tolerant rhizobia and adequate mineral nitrogen collectively mitigate salinity damage, improve root growth and bio-N2 fixation, and maximize phosphorus bio-availability and leaf accumulation.

Table (4) Effect of rhizobial inoculation, nitrogen fertilization levels, and irrigation water salinity on leaf phosphorus concentration of cowpea g kg^-1

Fertilization×nitrogen level	Salinity Level			Nitrogen Level	Inoculation
	8S	4S	2S		
2.769	2.413	2.790	3.103	40 N	R0
2.910	2.477	2.883	3.370	60 N	
3.111	2.497	3.003	3.833	80 N	
4.018	3.203	3.657	5.193	40 N	Rj
4.154	3.227	3.803	5.433	60 N	
4.354	3.260	3.967	5.837	80 N	
4.333	3.210	4.363	5.427	40 N	Rbr
4.418	3.217	4.383	5.653	60 N	
4.573	3.220	4.577	5.923	80 N	
	2.969	3.714	4.864	S mean	
SRN = 0.052 , RN = 0.030 , S = 0.017				L.S.D (0.05)	
N معدل	8S	4S	2S		
3.707	2.942	3.603	4.574	40 N	Salinity× Nitrogen
3.827	2.973	3.690	4.819	60 N	
4.013	2.992	3.849	5.198	80 N	
0.017	0.030			L.S.D (0.05)	

R mean	8S	4S	2S		
2.930	2.462	2.892	3.436	R0	Salinity×Pollination
4.176	3.230	3.809	5.488	Rj	
4.441	3.216	4.441	5.668	Rbr	
0.017	0.030			L.S.D $_{(0.05)}$	

3.4. Leaf Potassium Concentration g kg^-1

The results presented in Table (5) indicate that increasing the salinity of irrigation water significantly affected the potassium concentration in cowpea leaves. Mean potassium concentration significantly decreased to 33.523, 29.682, and 19.066 g kg^-1at salinity levels S2, S4, and S8, respectively. This significant reduction is attributed to high salt concentrations in irrigation water particularly sodium chloride NaCl leading to an accumulation of sodium ions Na^+ in the rhizosphere. This causes direct ionic competition with potassium ions K^+ for binding sites on root cell membranes, diminishing root water and nutrient uptake capacity, impairing high-affinity K^+transporters, and accelerating Na^+ nflux into root cells (Chaudhary et al., 2024).

Inoculation with rhizobial strains exerted a significant positive influence on leaf potassium concentration, increasing from a mean of 20.039 g kg^-1in the uninoculated control R0 to 29.733 and 32.499 g kg^-1 in plants inoculated with rhizobial strains Rj and Rbr, respectively. This elevation stems from the ability of these strains to promote root growth and architecture, increase root hair density, and establish functional root nodules, thereby enhancing the overall efficiency of water and nutrient absorption, including K^+ (Lima et al., 2024).

Nitrogen fertilization levels also significantly influenced leaf potassium concentration, which increased from a mean of 24.696 g kg^-1 at N40 to 27.314 and 30.261 g kg^-1 at N60and N80, respectively. Nitrogen stimulates root growth and root hair proliferation, expanding the active absorbing surface area. Under optimal nitrogen availability, accelerated vegetative growth and protein synthesis elevate the plant's physiological demand for K^+ to maintain ionic equilibrium and regulate water and solute transport within tissues, thus facilitating K^+ uptake and leaf accumulation (El-Hadidi et al., 2024).

A significant two-way interaction was observed between irrigation water salinity and bioinoculation. The highest mean potassium concentration (41.058 g kg^-1 was recorded under S2 salinity combined with Rbr inoculation, whereas the lowest mean (16.602 g kg^-1 occurred under S8 salinity in the uninoculated control R0. Efficient, salt-tolerant rhizobial strains promote root growth and enhance K^+ uptake and vascular transport under saline conditions. Furthermore, these strains regulate the expression of ion transporter proteins, restricting Na^+ entry while favoring K^+retention under salt stress (Khantsi and Babalola, 2026).

The two-way interaction between irrigation water salinity and nitrogen fertilization levels was also significant. The maximum mean potassium concentration (37.123 g kg^-1 was obtained under S2 salinity combined with N80 fertilization, while the minimum value (18.092 g kg^-1 was recorded under S8 salinity with N40 fertilization. Appropriate nitrogen application counteracts salinity stress by expanding root absorbing surfaces, sustaining root physiological activity, and preserving the functional integrity of K^+ transport systems (Sulaman et al., 2025).

Conversely, the two-way interaction between rhizobial bioinoculation and nitrogen fertilization levels showed no significant effect $p > 0.05$ on leaf potassium concentration.

Finally, the three-way interaction among irrigation water salinity, bioinoculation, and nitrogen fertilization levels significantly affected leaf potassium concentration. The highest overall mean (43.547 g kg^-1 was achieved under S2 salinity (noted as S2/N2 in text), Rbr inoculation, and N80 fertilization, whereas the lowest value (15.893 g kg^-1 was recorded under S8 salinity in the uninoculated R0 times N40treatment. This interactive enhancement reflects an integrated synergy that optimizes nutrient uptake efficiency and alleviates the physiological constraints of salinity stress (Al-Maskari and Yaish, 2025).

Table (5) Effect of rhizobial inoculation, nitrogen fertilization levels, and irrigation water salinity on leaf potassium concentration of cowpea g kg^-1

Fertilization×nitrogen level	**Salinity Level**			**Nitrogen level**	Inoculation
	8S	**4S**	**2S**		
17.534	**15.893**	**17.590**	**19.120**	**40 N**	**R0**
19.581	**16.417**	**19.850**	**22.477**	**60 N**	

23.000	**17.497**	**22.980**	**28.523**	**80 N**	
26.732	**18.853**	**28.797**	**32.547**	**40 N**	**Rj**
29.931	**19.580**	**33.650**	**36.563**	**60 N**	
32.534	**21.273**	**37.030**	**39.300**	**80 N**	
29.820	**19.530**	**31.480**	**38.450**	**40 N**	**Rbr**
32.429	**20.240**	**35.870**	**41.177**	**60 N**	
35.248	**22.307**	**39.890**	**43.547**	**80 N**	
	19.066	**29.682**	**33.523**	**S mean**	
SRN = 1.277 , RN = NS , S = 0.425				**L.S.D (0.05)**	
N mean	**8S**	**4S**	**2S**		
24.696	**18.092**	**25.956**	**30.039**	**40 N**	**Salinity× Nitrogen**
27.314	**18.746**	**29.790**	**33.406**	**60 N**	
30.261	**20.359**	**33.300**	**37.123**	**80 N**	
0.425	**0.732**			**L.S.D (0.05)**	

R mean	**8S**	**4S**	**2S**		
20.039	**16.602**	**20.140**	**23.373**	**R0**	**Salinity× Pollination**
29.733	**19.902**	**33.159**	**36.137**	**Rj**	
32.499	**20.692**	**35.747**	**41.058**	**Rbr**	
0.425	**0.732**			**L.S.D (0.05)**	

3.5. Total Yield g pot^-1

The results in Table (6) indicate that increasing irrigation water salinity significantly reduced the total yield of cowpea plants. Mean total yield significantly decreased to 107.815, 92.166, and 30.579 g pot-1 at salinity levels S2, S4, and S8, respectively. This decline occurs because salinity subjects cowpea plants to osmotic stress and ionic toxicity, restricting vegetative and reproductive growth, which ultimately compromises yield components and total yield (Akter et al., 2022).

Inoculation with rhizobial strains exerted a significant positive effect on total yield, increasing mean yield from 46.786 g pot^-1 in the uninoculated control R0 to 88.096 and 95.677 g pot^-1 with rhizobial strains Rj and Rbr, respectively. This enhancement is attributed to improved symbiotic efficiency, increased effective root nodule formation, and enhanced biological nitrogen fixation, which elevated plant nutritional status and enhanced overall yield components (Dhami et al., 2025).

Nitrogen fertilization levels also significantly affected total yield, increasing mean yield from 67.601 g pot^-1 at N40 to 76.897 and 86.061 g pot}^-1 at N60 and N80, respectively. Optimal nitrogen supply improves plant nutritional status, boosts vegetative growth, and elevates photosynthetic efficiency, positively influencing yield components (Danso et al., 2025).

A significant two-way interaction was observed between irrigation water salinity and bioinoculation. The highest mean total yield 130.539 g pot^-1 was recorded under S2 salinity combined with Rbr inoculation, whereas the lowest mean (20.028 g pot^-1 occurred under S8 salinity in the uninoculated control R0. Bioinoculation with beneficial microorganisms such as rhizobia mitigates salinity-induced stress by improving biological nitrogen fixation, promoting nutrient uptake, and maintaining ionic balance, thereby enhancing growth, pod development, and seed filling (Kumawat et al., 2025).

Similarly, the two-way interaction between irrigation water salinity and nitrogen fertilization levels was significant. The maximum mean total yield (116.309 g pot^-1 was achieved under S2 salinity with N80 fertilization, while the minimum (24.039 g pot^-1 occurred under S8 salinity with N40 fertilization. Adequate nitrogen fertilization supports chlorophyll, protein, and enzyme synthesis, sustaining physiological activity, pod formation, and grain filling under saline irrigation conditions (Yadav et al., 2024), consistent with findings by Danso et al. (2025) showing that nitrogen application enhances cowpea grain yield and nitrogen fixation.

The two-way interaction between rhizobial bioinoculation and nitrogen fertilization levels also proved significant. The highest yield (103.626 g pot^-1 was recorded with Rbr inoculation at N80, compared to 41.283 g pot^-1 in the uninoculated R0 times N40 control. This response reflects the synergistic interplay between rhizobial bio-N2 fixation and soil-available mineral nitrogen, supporting nodulation, growth, and yield development (Danso et al., 2025).

Finally, the three-way interaction among irrigation water salinity, bioinoculation, and nitrogen fertilization levels significantly affected total yield. The highest mean yield overall (138.173 g pot^-1 was recorded under S2 salinity (noted as S2/N2 in text), Rbr inoculation, and N80 fertilization, whereas the lowest (16.587 g pot^-1 occurred under S8 salinity without inoculation R0 at N40. This synergistic effect demonstrates that combining rhizobial bioinoculation with optimal nitrogen supply alleviates salinity stress and maintains physiological function, thereby maximizing total cowpea yield (Danso et al., 2025).

Table (6) Effect of rhizobial inoculation, nitrogen fertilization levels, and irrigation water salinity on total yield of cowpea g pot^-1

Fertilization×nitrogen level	Salinity Level			Nitrogen level	Inoculation
	8S	4S	2S		
41.283	**16.587**	**42.177**	**65.087**	**40 N**	**R0**
45.533	**17.620**	**49.330**	**69.650**	**60 N**	
53.541	**25.877**	**58.333**	**76.413**	**80 N**	
76.146	**27.593**	**86.290**	**114.553**	**40 N**	**Rj**
87.127	**28.963**	**113.743**	**118.673**	**60 N**	
101.017	**42.863**	**125.847**	**134.340**	**80 N**	
85.376	**27.937**	**106.773**	**121.417**	**40 N**	**Rbr**
98.030	**42.013**	**120.050**	**132.027**	**60 N**	
103.626	**45.753**	**126.950**	**138.173**	**80 N**	
	30.579	**92.166**	**107.815**	**S mean**	
SRN = 6.41 , RN = 3.70 , S = 2.14				**L.S.D (0.05)**	
N mean	**8S**	**4S**	**2S**		
67.601	**24.039**	**78.413**	**100.352**	**40 N**	**Salinity× Nitrogen**
76.897	**29.532**	**94.374**	**106.783**	**60 N**	
86.061	**38.164**	**103.710**	**116.309**	**80 N**	
2.14	**3.70**			**L.S.D (0.05)**	
R mean	**8S**	**4S**	**2S**		

46.786	**20.028**	**49.947**	**70.383**	**R0**	**Salinity× Pollination**
88.096	**33.140**	**108.627**	**122.522**	**Rj**	
95.677	**38.568**	**117.924**	**130.539**	**Rbr**	
2.14	**3.70**			**L.S.D (0.05)**	

3.6. Seed Protein Content (%)

The results presented in Table (7) indicate that increasing irrigation water salinity significantly reduced the seed protein content of cowpea plants. Mean seed protein percentage significantly decreased to 25.663%, 23.610%, and 21.073% at salinity levels S2, S4, and S8, respectively. This decline occurs because salt stress adversely affects nitrogen uptake, biological fixation, assimilation, and translocation to the seeds, while simultaneously impairing photosynthetic efficiency and dry matter accumulation. Since proteins are primarily composed of nitrogenous compounds, a reduced nitrogen supply to the plant directly depresses seed protein accumulation (Praxedes et al., 2022).

Inoculation with rhizobial strains exerted a significant positive effect on seed protein content, increasing mean values from 20.751% in the uninoculated control R0 to 24.226% and 25.369% in plants inoculated with rhizobial strains Rj and Rbr, respectively. This enhancement stems from the high efficiency of rhizobial strains in biological nitrogen fixation, which increases nitrogen availability to the host plant and subsequently promotes higher nitrogen accumulation in developing seeds, thereby elevating total protein content (Sufar et al., 2024).

Nitrogen fertilization levels also significantly influenced seed protein content, which increased from a mean of 22.573% at N40 to 23.306% and 24.466% at N60 and N80, respectively. Enhanced mineral nitrogen availability in the rhizosphere promotes root nitrogen absorption and metabolic assimilation. This facilitates greater translocation of nitrogenous compounds to the seeds during the pod filling stage, directly enhancing amino acid and protein synthesis (Musa and Yusuf, 2026).

A significant two-way interaction was observed between irrigation water salinity and bio inoculation. The highest mean seed protein content (27.919%) was recorded under S2 salinity combined with Rbr inoculation, whereas the lowest mean (19.302%) occurred under S8 salinity in the uninoculated control R0. Bio inoculation with beneficial microorganisms enhances nodule formation and biological nitrogen fixation while improving nutrient uptake and maintaining ionic balance under saline conditions, thereby supplying essential nitrogen for seed protein synthesis (Suriya and Selvaraj, 2026).

Similarly, the two-way interaction between irrigation water salinity and nitrogen fertilization levels was significant. The maximum mean protein content (26.677%) was achieved under S2 salinity with N80 fertilization, while the minimum (20.392%) occurred under S8 salinity with N40 fertilization. Adequate nitrogen supply improves plant nutritional status and provides the nitrogen required for amino acid and protein synthesis, facilitating the translocation and accumulation of nitrogenous compounds in seeds while partially mitigating salinity-induced physiological stress (Alves et al., 2024).

The two-way interaction between rhizobial bio inoculation and nitrogen fertilization levels was also significant. The highest protein content (26.510%) was recorded with Rbr inoculation at N80, compared to 20.163% in the uninoculated R0 \times N40 control. Combining mineral and biological nitrogen sources optimizes overall plant nitrogen status, enhancing dry matter accumulation and the mobilization of nitrogenous compounds from vegetative tissues to developing seeds (Musa and Yusuf, 2026).

Finally, the statistical analysis revealed a significant three-way interaction among irrigation water salinity, bio inoculation, and nitrogen fertilization levels on seed protein percentage. The highest mean protein content overall (29.390%) was recorded under S2 salinity (noted as S2/N2 in , Rbr inoculation, and N80 fertilization, whereas the lowest (19.183%) occurred under S8 salinity without inoculation R0 at N40. This synergistic enhancement reflects the combined benefits of mineral nitrogen fertilization and biological nitrogen fixation, along with the stress-mitigating capacity of rhizobial inoculation under saline irrigation (Musa and Yusuf, 2026).

Table (7) Effect of rhizobial inoculation, nitrogen fertilization levels, and irrigation water salinity on seed protein content of cowpea (%)

Fertilization×nitrogen level	**Salinity Level**			**Nitrogen level**	Inoculation
	8S	**4S**	**2S**		

20.163	**19.183**	**19.810**	**21.497**	**40 N**	**R0**
20.601	**19.227**	**20.330**	**22.247**	**60 N**	
21.490	**19.497**	**22.080**	**22.893**	**80 N**	
23.240	**20.227**	**23.247**	**26.247**	**40 N**	**Rj**
24.038	**21.473**	**24.060**	**26.580**	**60 N**	
25.399	**22.290**	**26.160**	**27.747**	**80 N**	
24.316	**21.767**	**24.413**	**26.767**	**40 N**	**Rbr**
25.280	**22.640**	**25.600**	**27.600**	**60 N**	
26.510	**23.350**	**26.790**	**29.390**	**80 N**	
	21.073	**23.610**	**25.663**	**S mean**	
SRN =0.408 , RN = 0.235 , S =0.136				**L.S.D (0.05)**	
N mean	**8S**	**4S**	**2S**		
22.573	**20.392**	**22.490**	**24.837**	**40 N**	**Salinity× Nitrogen**
23.306	**21.113**	**23.330**	**25.476**	**60 N**	
24.466	**21.712**	**25.010**	**26.677**	**80 N**	
0.136	**0.235**			**L.S.D (0.05)**	
R mean	**8S**	**4S**	**2S**		
20.751	**19.302**	**20.740**	**22.212**	**R0**	**Salinity× Pollination**
24.226	**21.330**	**24.489**	**26.858**	**Rj**	
25.369	**22.586**	**25.601**	**27.919**	**Rbr**	
0.136	**0.235**			**L.S.D (0.05)**	

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