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Pseudomonas and Trichoderma's Combined Impact on Mungbean Yield and Growth under Salinity Stress

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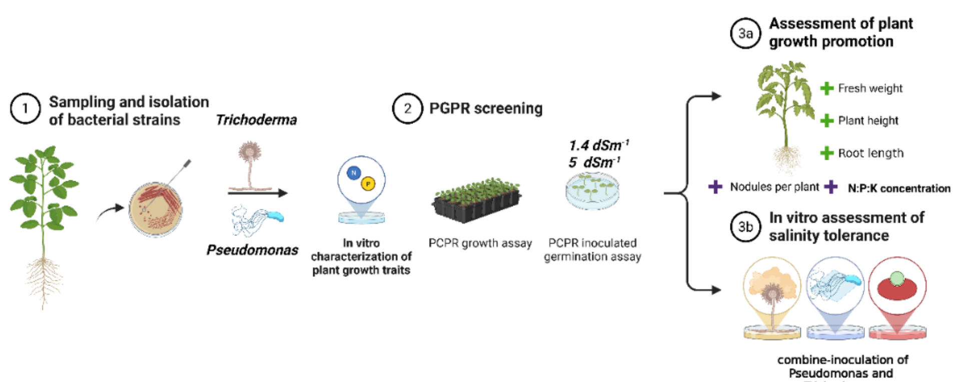
Abstract

Farming systems are now facing serious difficulties due to salinity which is mostly caused by climate change. A variety of methods which incorporate mungbean, are in use to mitigate the effects of salinity and increase plant growth, crop yields and make soils richer by fixing nitrogen. Separately and together, identified Pseudomonas bacterial and Trichoderma fungal strains were used in this research to investigate their impact on mungbean growing under saline conditions. Pot experiments were carried out in nature at salinities 1.4 and 5.0 dS m-1, respectively, after a random arrangement of treatments. Results from the data indicate that salinity stress affects the development, nodulation and production of mungbeans. These different plant parts increased under salinity stress when Pseudomonas and Trichoderma were used, both singly and together, in mungbean. In this study, using Pseudomonas and Trichoderma together led to improved growth, more nodules and greater grain output under salt conditions.

Graphical Abstract: Plant Growth-Promoting Rhizobacteria (PGPR) in Salinity Stress Tolerance



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1. Introduction

Salinity has recently emerged as a daunting challenge in modern commercial-oriented agriculture (Mogal *et al.*, 2020; Harman *et al.*, 2000). A significant portion of agricultural land worldwide, particularly in South Asia, is affected by salinity, mainly in the South Asia regions. Millions of hectares of agricultural land have been converted into barren land, reducing the production capacity of these regions (Peoples *et al.*, 2009). The exporting countries have been changed into importing countries, especially in agronomic crops (i.e., oilseed crops, cereal, pulses, and grains) and products prepared from these agronomic crops like sugar and oils (Manasa *et al.*, 2017). This era needs to develop and produce such varieties of agronomic crops that are strong enough to grow in saline conditions. In Pakistan, pulses and cereals play a crucial role in the population's diets, supplying essential amino acids, fatty acids, carbohydrates, vitamins, and minerals that meet approximately 80% of the inhabitants' nutritional needs (Howieson *et al.*, 2007). Various strategies are employed to sustain the production levels of these crops.

Various ways can be applied to address salinity stress so that Plant development and productivity increase. Plant growth-promoting rhizobacteria (PGR) are now getting special attention compared to others (Mogal *et al.*, 2020). Plant Growth Promoting Rhizobacteria are unique group of bacteria that benefits the plant's root zone. Symbiosis with the soil obtained from PGPR enhances both the growth and yield of mungbean when under stress conditions. PGPR build their osmolyte storage, protect themselves from osmotic pressure, enable N_2 fixation, enhance the uptake of NPK and other key nutrients and potentially solubilize phosphatic minerals, help with ISR and IST, synthesize plant hormones, produce ACC and achieve ROS scavenging. All these functions assist in reducing agricultural stresses (Howieson *et al.*, 2000). The benefits of PGPR-based mechanics include an optimum comprehension of production in plant genetics and microbial community function as a complex product, as well as a designed increase in salinity tolerance. Plant growth-promoting rhizobacteria (PGPR) greatly increases root development, biofertilization, and the remediation of saline rhizosphere samples. It also plays a crucial role in nutrient competition and plant antibiotic tests. These processes, along with nutrient competition, contribute to healthier plant growth and increased yield, as highlighted by Singh *et al.* (2017). PGPR bacteria naturally occur in soil and can accelerate the growth of their host plants when in symbiotic relationships (Alotaibi *et al.*,2023). Rhizobia and legumes are vital components of many agricultural systems (Howieson *et al.*, 2007). Symbiotic legumes and crop rotations not only improve soil composition and nutrient status but also help control illnesses, weeds, and pests (Howieson et al., 2000). Legumes are important contributors to the sustainable nitrogen economy of soils and are great sources of high-protein fodder crops (Peoples et al., 2009). PGPR can be chosen according to how well they produce hormones that support crop development and growth. Furthermore, PGPR promotes plant growth by reducing ethylene production under biotic and abiotic stress conditions (Belimov et al., 2009).

Because of its antagonistic effects on a number of plant pathogens, the genus Trichoderma, which belongs to the Deuteromycetes class of fungus, is frequently utilised as a biocontrol agent against several pathogenic fungi (Chet et al., 1987). Trichoderma strains are also known for their ability to promote plant growth (Harman *et al.*, 2000; Rabeendran *et al.*, 2000; Ikram *et al.*, 2025). These fungi can mitigate salinity stress in seedlings was mitigated through modulation of their physiological processes (Mastouri *et al.*, 2010; Ikram *et al.*, 2025). The observed effect may result from the ability of Trichoderma to produce antioxidants that reduce ROS-induced damage during salinity stress.. Mastouri *et al.* (2010) reported resembling findings, noting a reduction in seedling damage from ROS accumulation in stressed plants inoculated with Trichoderma. Beneficial fungi such as Trichoderma species have also been shown to modify plant transcriptional responses to biotic and abiotic stresses(Mastouri *et al.*, 2010; Ikram *et al.*, 2025). Ahmad *et al.* (2015) suggested that Trichoderma species increase the activities of enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

Mungbean is a major traditional pulse crop in Pakistan, typically a green crop. It stands out among other pulses due to its excellent nutritionally rich , easily digestible , and non-gas formin. Mungbean is highly nutritious and plays a crucial role in improving soil productivity (Ikram *et al.*, 2025; Tagore *et al.*, 2013). This is achieved through the fixation of atmospheric nitrogen into an accessible form, facilitated by Rhizobacteria species in the root nodules. Mungbean can grow in arid or semi-arid areas of Pakistan, contributing significantly. Mungbean is a vital pulse crop in Pakistan

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Table 1: Major morpho-physio-chemical properties of the study area and soil

Morphological properties

Properties	Information
Latitude/Longitude	31⁰ 71⁰
Elevation (above mean sea level) (m)	423 ft (129m)
Mean annual max. temperature (°C)	41°C
Mean annual min. temperature (°C)	6°C
Maen annual rainfall/ Precipitation (mm)	187 mm
Primary soil type	Clay loam to sandy clay loam
Major Cops grown preceding crop in the experimental area	Wheat, cotton, sugarcane, rice, maize, sunflower, fodder crops

Physio-Chemical properties of soil

Soil properties	Information
Texture	Clay loam
pH	8.312
EC (dSm^{-1})	3.61
Organic carbon (%)	0.65
Available N (kg/ha)	0.40
Available P (kg/ha)	11.4
Exchangeable K (kg/ha)	124

which is mainly grown on dry land. During 2015-2016, it was grown on 146.3 million hectares of land, producing 98.0 million tons (Ikram *et al.*, 2024a; Ikram *et al.*, 2024b). From 2014 to 2015, it grew to 127.4 million hectares, producing 98.8 million tons. Mungbean is nutritious and plays a key economic role among legumes, but it struggles with salinity especially as a young seedling. The most important phase of a crop plant's life, when seedlings grow, is also the part most susceptible to salt stress (Parida *et al.*, 2005; Ikram *et al.*, 2018). Mungbean production has been reduced in recent decades because of stress from biotic and abiotic sources such as salinity. Salinity stress is one of the major abiotic stresses that trouble plant systems around the globe. Many mungbean fields are being threatened by salinity stress. This stress is not simple and there are not many good techniques for introducing important characteristics into crops to manage it. So far, less research work has looked into different salt-tolerant types, especially varieties of mungbean (Manasa *et al.*, 2017; Naz *et al.*, 2025).

The effectiveness of various microbial strains in promoting plant development in saline environments was examined. Studies examining the combined impact of two or more strains in saline environments, however, are less common. The current investigation was carried out to assess the contribution of Pseudomonas and Trichoderma to salt stress reduction and improved growth and nodulation of mungbean.

2. Materials and Methods

2.1. Preparation of bacterial and fungal cultures for inoculation

Lauribruni (LB) and Potato Dextrose (PD) media were used to create an inoculum of Pseudomonas bacteria and Trichoderma fungi in flasks, respectively. Pseudomonas and Trichoderma stains were added to each LB and PD broth medium flask, respectively. For 72 hours, it was shaken at 100 revolutions per minute while being incubated at 28˚C. Prior to seed inoculation, a consistent population density was maintained and optical density was assessed after incubation.

2.2. Pot trial

The strains of Trichoderma and Pseudomonas were inoculated separately, and their combination was evaluated to evaluate their potential to increase growth, nodulation, and yield of mungbean under salinity stress conditions in a pot trial. The treatments of the study were (i) control, i.e., no inoculation (T1), (ii) inoculation with PGPR (Pseudomonas Spp) (T2), (iii) inoculation with Trichoderma (T3) and (iv) inoculation with PGPR (Pseudomonas Spp) + Trichoderma (T4). The morpho-physico-chemical characteristics of the experimental soil were evaluated and presented in Table1. Based on the treatment plan, the team inoculated mungbean seeds either with Trichoderma, Pseudomonas or both bacteria separately. To inoculate the plants, sterilized peat was incorporated into the carrier, along with a ten % disinfected sugar solution to help them stick.

Inoculum to peat ratio is 1:1 v/w. Mixing autoclaved peat with uninoculated broth and sugar allowed the researchers to preserve uninoculated control. Each pot was given 12 kilograms of ground and sieved soil. I formed Normal (1.4 dSm^{-1}) and saline (5 $dS\ m^{-1}$) pots by adding NaCl water to each set. Five seeds were planted in each pot and only one was left per pot as they germinated. We did each treatment six times over. Plots of pots were made completely at random under typical indoor light and room temperature. The pots received irrigation with top-quality water. We removed plants from three replications after they had flowered so we could obtain nodule data, while the other replications grew until they reached maturity.

3. Results and Discussion

The study evaluated the impact of different treatments on plant growth parameters, including plant height, fresh weight of shoots, dry weight of shoots, and root length. The treatments consisted of control conditions and applications of Pseudomonas, Trichoderma, and their combination at two electrical conductivity (EC) levels: 1.4 EC $dS\ m^{-1}$ and 5 EC $dS\ m^{-1}$. The results indicated that the combined treatment of Pseudomonas and Trichoderma significantly enhanced all measured plant growth parameters compared to the control and single treatments ($p < 0.05$) (Fig. 1). Specifically, plant height, fresh weight of shoots, dry weight of shoots, and root length were all highest in the combined treatment groups. The control treatment consistently showed the lowest values for these parameters, with no significant differences observed between the two EC levels within the control group. These findings suggest that the synergistic effect of Pseudomonas and Trichoderma applications can significantly improve plant growth under the tested 33conditions, regardless of the EC level.

In this study, we assessed the impact of various treatments-control, Pseudomonas, Trichoderma, and a combination of Pseudomonas and Trichoderma-on key plant parameters at two different electrical conductivity (EC) levels: 1.4 EC $dS\ m^{-1}$ and 5 EC $dS\ m^{-1}$. The parameters evaluated included root biomass (fresh and dry), number of roots, and total fresh weight The results demonstrated that the combined treatment of Pseudomonas and Trichoderma significantly enhanced all measured parameters compared to the control and single treatments ($p < 0.05$). Specifically, the root fresh weight, dry weight, and root number were highest in the combined treatment groups at both EC levels. Similarly, the total fresh weight was significantly higher in the combined treatment, indicating a robust growth response (Fig. 2). The control treatment consistently showed the lowest values across all parameters, with no significant differences observed between the two EC levels within the control group. These findings suggest that the synergistic application of Pseudomonas and Trichoderma can substantially improve plant growth and development under the tested conditions (Ikram *et al.*, 2025; Prasad *et al.*, 2017).

The study assessed the impact of various microbial treatments-Pseudomonas, Trichoderma, and their combination on key agricultural parameters under two different electrical conductivity (EC) levels, 1.4 EC $dS\ m^{-1}$ and 5 EC $dS\ m^{-1}$. The parameters measured included grain yield, nodule number, potassium concentration in leaves, and sodium concentration in leaves. The findings revealed that combining Pseudomonas and Trichoderma treatments significantly enhanced grain yield and nodule number at both EC levels, compared to the control and single treatments. Additionally, this combined treatment led to a higher potassium concentration and a lower sodium concentration in leaves, indicating improved nutrient uptake and potentially greater stress tolerance (Balasubramanian *et al.*, 1976). The control treatment consistently demonstrated the least favorable outcomes across all measured parameters (Fig. 3.). These results suggest that the synergistic application of Pseudomonas and Trichoderma can lead to substantial improvements in plant performance, particularly under the tested EC conditions.

The study evaluated the effects of different treatments, including control, *Pseudomonas*, Trichoderma, and a combination of *Pseudomonas* and Trichoderma, on various physiological parameters of plants under two electrical conductivity (EC) levels: 1.4 EC $dS\ m^{-1}$ and 5 EC $dS\ m^{-1}$. The parameters assessed included the potassium/sodium ratio in leaves, phosphorus concentration in grains, nitrogen level in grains, and crude protein content in grains. The potassium/sodium ratio in leaves (Figure 4A) was significantly higher in the combined treatment of *Pseudomonas* and Trichoderma at both EC levels relative to the control and single treatments ($p < 0.05$). The control treatment showed the lowest potassium/sodium ratio, with no significant difference between the two EC levels. Phosphorus concentration in grains (Figure 4B) was significantly higher in the combined treatment at both EC levels ($p < 0.05$). The control treatment had the lowest phosphorus concentration, with no significant difference between the two EC levels. Nitrogen concentration in grains (Figure 4C) was significantly higher in the combined treatment at both EC levels compared to the control and single treatments ($p < 0.05$). The control treatment had the lowest nitrogen concentration, with no significant difference between the two EC levels. Crude protein content in grains (Figure 4D) was significantly higher in the combined treatment at both EC levels compared to the control and single treatments ($p < 0.05$). The control treatment had the lowest crude protein content, with no significant difference between the two EC levels (Chebotar *et al.*, 2001; Epstein *et al.*, 1980). These results suggest that the combined application of *Pseudomonas* and Trichoderma can significantly improve key physiological parameters in plants, enhancing nutrient uptake and protein content, particularly under the EC conditions tested.

PGPR are valuable bacterium that lives in the plant root zone (Paul *et al.*,2014; Iqbal *et al.*,2017; Ikram *et al.*, 2018). In several regions of the world, PGPR has been used more frequently in recent years to encourage plant growth (Zahir et al., 2004; Haider et al., 2024). Our findings showed that salt stress had a detrimental effect on all aspects of mungbean development, nodulation, and yield. Pseudomonas and Trichoderma both improved growth, nodulation, and yield in mungbean grown in salty circumstances (Kaur et al., 2015; Saleem et al., 2017). The findings suggest that Pseudomonas and Trichoderma applied together improved mungbean growth, yield, and nodulation.

In the present research, P. (PGPR) and T. were studied separately and as a mix to check if they can enhance mungbean growth under saline conditions. Based on the findings, mungbean plant height, shoot weight in both fresh and dry form, root weight in both fresh and dry form, root length and dry matter production were all significantly reduced by salinity stress. It might be inferred that salinity stress hampered mungbean growth most probably by restricting legume-rhizobium symbioses, which reduced N supply as demonstrated by a significant reduction in growth parameters (Guetsky *et al.*, 2002; Korir *et al.*, 2017). Our findings showed that combined inoculation of *Pseudomonas* and *Trichoderma* promoted mungbean growth under salinity stress, such responses might be due to osmolyte buildup, enhanced uptake of NPK, solubilization of mineral phosphates, provision of essential nutrients, and production of phytohormones.. The finding of Singh *et al.* (Singh *et al.*, 2017) supports these results, who reported that PGPR recorded taller plants and effectively enhanced growth and yield of legumes by increasing N_2 fixation rate, which supplied more fixed N_2.

It promoted above and below ground vegetative growth and boosted carbohydrate portioning to sinks, and ultimately higher shoot and root growth was witnessed. Due to an extra strain of Trichoderma atroviride, the dry weight of the shoot and root improved threefold (Gravel et al., 2006; Ikram et al., 2024), and the amount of fruit that could be sold increased dramatically (Gravel et al., 2007; Mehran et al., 2023). According to (Kumari et al., 2018), PGPR-based approaches offer benefits such as improved salinity tolerance and conceptual comprehension underlying mechanisms of various PGPR strains, which boost crop productivity, along with the role of plant genetics and microbial community function in alleviating adverse effects of abiotic stresses.

As per our findings, PGPR applied in conjunction (*Pseudomonas* and *Trichoderma*) promoted the number and their fresh weights in mung bean compared to their solo application. It might be inferred that PGPR formed a symbiotic association with plant roots, and excessive N2 supply resulted in higher biomass accumulation of roots, shoots, and pods. Similar to our findings, it has been reported that legume-rhizobium symbiotic association can potentially and complementarily add over 20-40 kg N ha^{-1}, leading to higher root and shoot growth even under saline environment (Das *et al.*, 2014; Murtaza *et al.*, 2022). In addition, residual organic matter and total N_2 in the soil were positively affected by rhizobium inoculation in legumes (Ikram *et al.*, 2024). PGPR enhances salt tolerance and improves the yield of crops as the outcome of genetic features and microbial efforts (Singh *et al.*, 2017; Mishra *et al.*, 2009; Mudgal *et al.*, 2010).

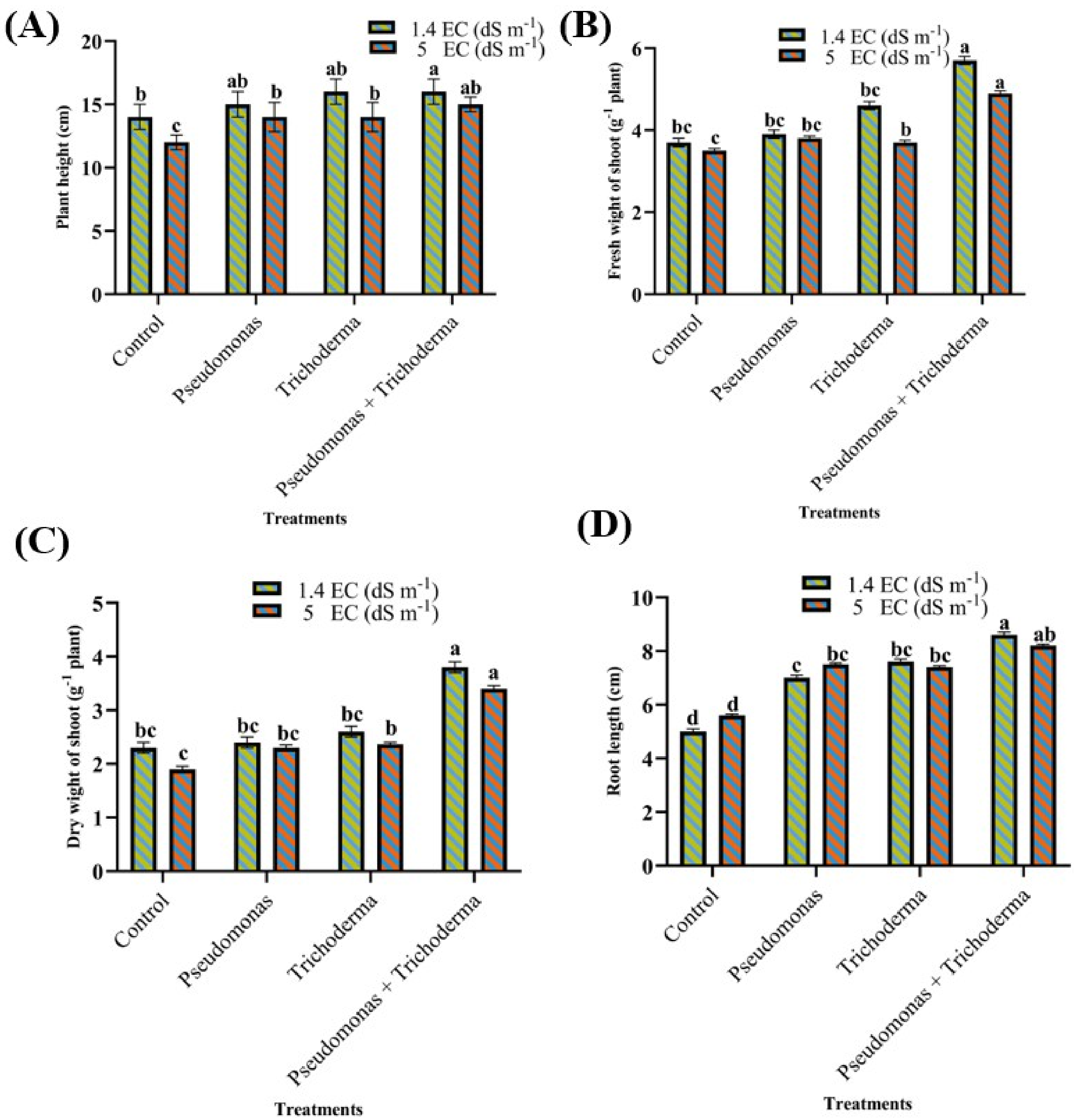

Figure 1. Effects of Different Treatments on Plant Growth Parameters (A) Plant height, (B) Fresh weight of shoots, (C) Dry weight of shoots, and (D) Root length under control conditions and treatments with Pseudomonas, Trichoderma, and their combination at two different EC levels.

Error bars represent standard deviation (n = 3). Different letters above the bars indicate significant treatment differences (p < 0.05).

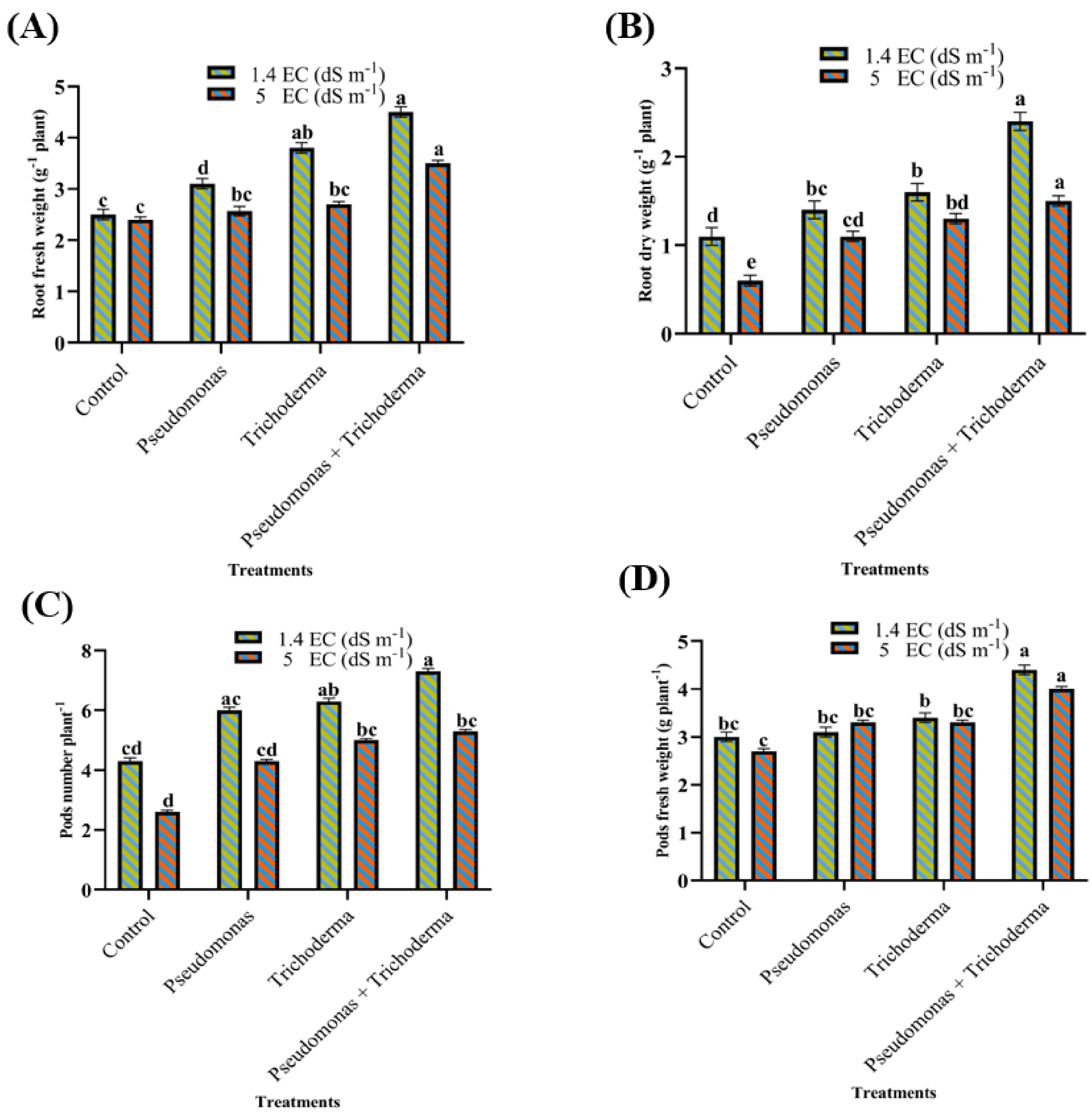

Figure 2. Effects of Different Treatments on Plant Parameters (A) Root fresh weight, (B) Root dry weight, (C) Root number, and (D) Total fresh weight under control conditions and treatments with Pseudomonas, Trichoderma, and their combination at two different EC levels (1.4 EC dS m^{-1} and 5 EC dS m^{-1}).
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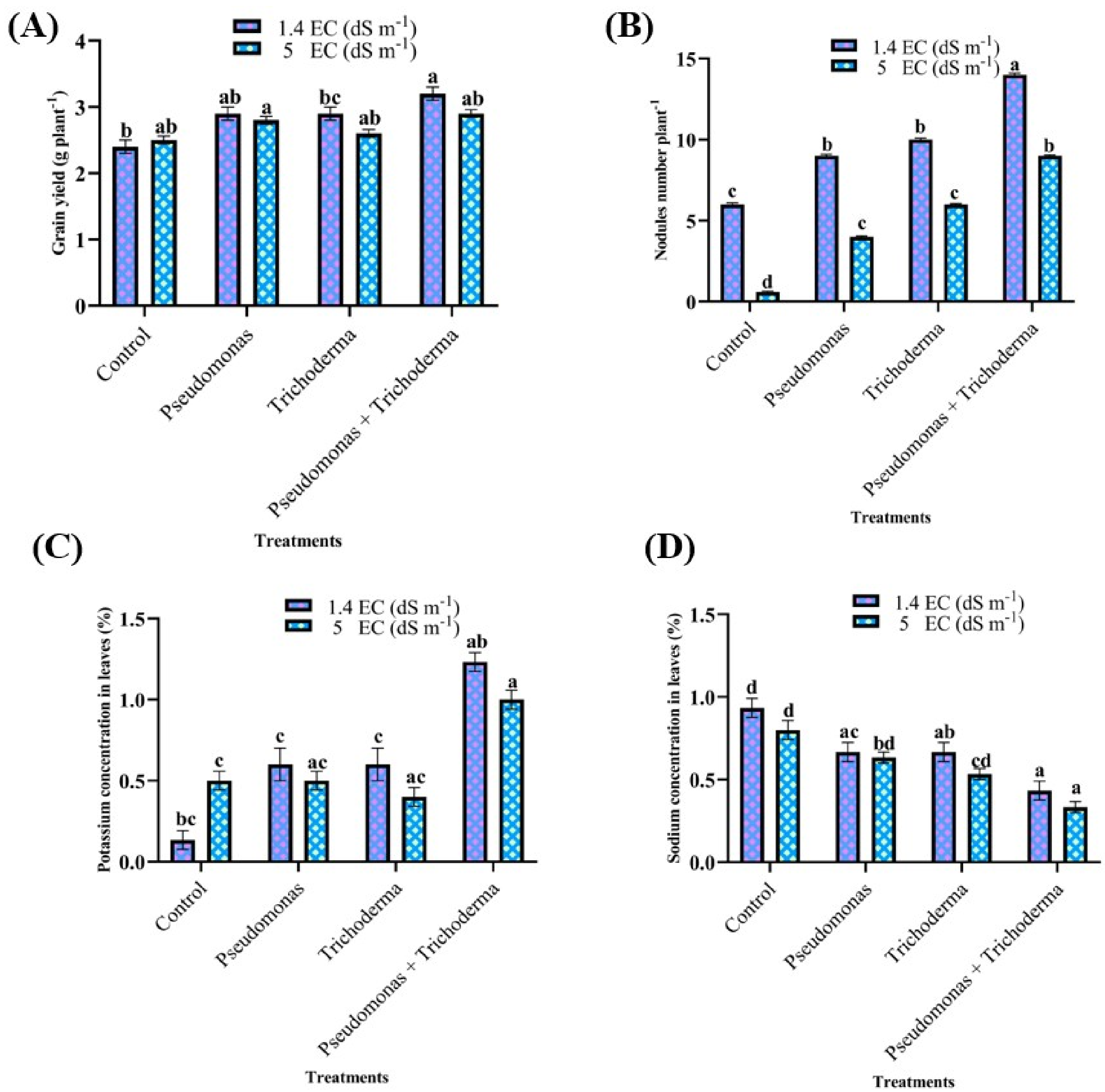

Figure3. Effects of Different Treatments on Plant Parameters (A) Grain yield, (B) Nodule number, (C) Potassium concentration in leaves, and (D) Sodium concentration in leaves under control conditions and treatments with Pseudomonas, Trichoderma, and their combination at two different EC levels (1.4 EC dS m^{-1} and 5 EC dS m^{-1})

Error bars represent standard deviation (n = 3). Different letters above the bars indicate significant treatment differences ($p < 0.05$).

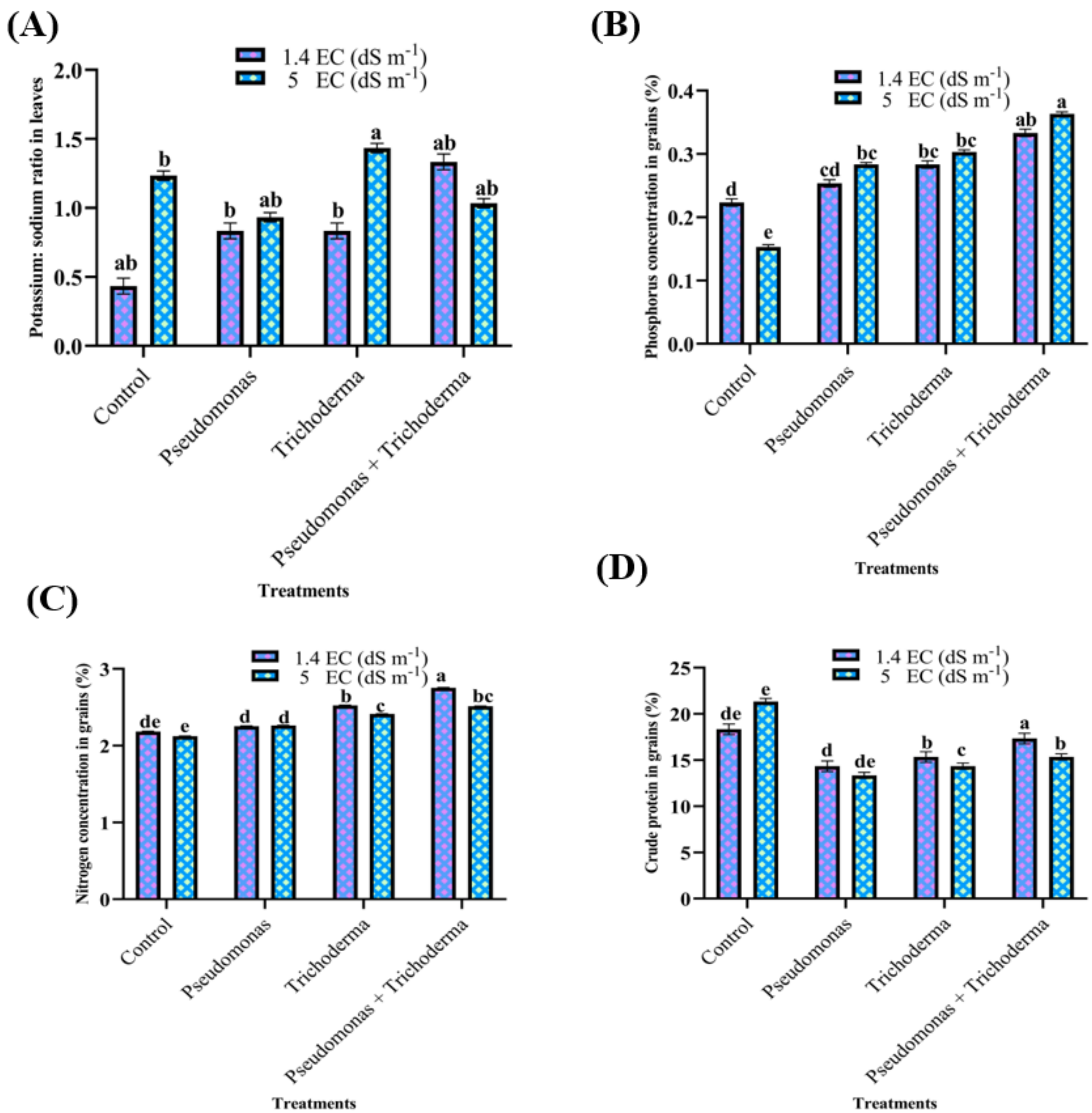

Figure 4. Impact of Microbial Treatments on Plant Physiological Parameters (A) Potassium to sodium ratio in leaves, (B) Phosphorus concentration in grains, (C) Grain nitrogen concentration and (D) crude protein content under both control and Pseudomonas, Trichoderma, and their combination treatments at two different electrical conductivity (EC) levels (1.4 EC dS m−1 and 5 EC dS m−1). *Error bars represent standard deviation (n = 3). Different letters above the bars indicate significant treatment differences ($p < 0.05$).*

Different *Trichoderma strains* were revealed to play a role in boosting fresh shoot weight, root weight or root length in peas (Naseby *et al.*, 2000; Ashraf *et al.*, 2022) and corn (Vasquez *et al.*, 2000). Yedidia *et al.*(2001) reported that *Trichoderma* inoculant in cucumber increased the root area, length, and fresh and dry weights, leading to significantly higher yield.

Our study reports that the combined application of PGPR boosted yield attributes and the number that multiplied grain yield of mungbean. It might be inferred that improved rhizobacteria colonization ability increased N_2 fixation by producing more excellent nodules, solubilization of phosphorus, biosynthesis of phytohormones, activation of chitinase, and biosynthesis of numerous biologically active substances, causing yield increment (Aamir *et al.*, 2013; Rawat *et al.*, 2011). Using *Pseudomonas* and *Trichoderma* during inoculation made the plants resilient against high salinity and lowered salinity-related ionic changes, water potential, the harmful effects of certain ions and ill effects on physiological processes (Mogal *et al.*, 2020; Minhas *et al.*, 2025; Ikram *et al.*, 2024). Furthermore,The synergistic use of Trichoderma and Pseudomonas improved growth, nodulation activity, and grain yield in pulses and cereals subjected to abiotic stresses, notably salinity. (Egamberdieva *et al.*, 2017; Paul *et al.*, 2014; Haseeb-ur-Rehman *et al.*, Nadeem *et al.*, 2018).

Moreover, Sabagh *et al.* (2021) attributed the root colonization of rhizobacteria to the higher grain yield of mungbean in a saline environment. Regarding the acquisition and concentration of nutrients, cumulative inoculation of *Trichoderma* and *Pseudomonas* resulted in the highest content of potassium (K) and sodium (Na) along with their K: Na ratio in the leaves of mungbean. In addition, the maximum phosphorus, nitrogen, and protein concentrations in mungbean were recorded by the combined application of *Trichoderma* and *Pseudomonas* (Shaharoona *et al.*, 2006; Zahran *et al.*, 1999). It might be interpreted that root colonization of PGPR improved nutrient uptake, boosting nutrients and protein concentration in mungbean grains. These findings are supported by (Erman *et al.*, 2021; Alotaibi *et al.*, 2023; El Sabagh *et al.*, 2020); who concluded that higher nutrient acquisition led to greater protein contents of pulses like mungbean and groundnuts.

PGPR are valuable bacterium that lives in the plant root zone (Paul *et al.*,2014; Iqbal *et al.*,2017; Ikram *et al.*, 2018).In Globally, PGPR has been used more frequently in recent years to encourage plant growth (Zahir et al., 2004;Haider *et al.*, 2024). All parameters of mungbean growth, nodulation and yield were negatively impacted by salinity stress in our results. Applying both Pseudomonas and Trichoderma to mungbean growing in salty conditions resulted in better growth, nodulation and a higher yield (Kaur *et al.*, 2015; Saleem *et al.*, 2017). The findings suggest that Pseudomonas and Trichoderma applied together improved mungbean growth, yield, and nodulation.

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Moreover, Sabagh *et al.* (2021) attributed the root colonization of rhizobacteria to the higher grain yield of mungbean in a saline environment. Regarding the acquisition and concentration of nutrients, cumulative inoculation of *Trichoderma* and *Pseudomonas* resulted in the highest content of potassium (K) and sodium (Na) along with their K: Na ratio in the leaves of mungbean. In addition, the maximum phosphorus, nitrogen, and protein concentrations in mungbean were recorded by the combined application of *Trichoderma* and *Pseudomonas* (Shaharoona *et al.*, 2006; Zahran *et al.*, 1999). It might be interpreted that root colonization of PGPR improved nutrient uptake, boosting nutrients and protein concentration in mungbean grains. These findings are supported by (Erman *et al.*, 2021; Alotaibi *et al.*, 2023; El Sabagh *et al.*, 2020); who concluded that higher nutrient acquisition led to greater protein contents of pulses like mungbean and groundnuts.

4. Conclusion

The research findings aligned with the postulated hypothesis as the combined application of PGPR (*Pseudomonas*) and *Trichoderma*. They remained superior in recording the highest growth parameters, such as plant height, root and shoot weights, pod number and weight, which boosted the grain yield of mungbean under a saline environment. The same inoculation gave the maximum acquisition of nutrients in leaves and grains of mungbean exposed to salinity. It can be inferred that the combined application of PGPR and *Trichoderma* strains can be used as a potential candidate to boost the productivity of pulses. The co-inoculation with Rhizobium and PGPR could be explored further to develop it as a practical approach for inducing tolerance against salinity by improving mung bean's nodulation, growth, and yield under salt-affected conditions.

5. Funding

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6. Acknowledgment

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7. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

8. Authors' Contribution

Muhammad Faizan Khuram: Conceptualization, Formal analysis, Methodology, Figures preparation, Writing - original draft, Writing - review & editing. Burhan Khalid: Methodology, Figures preparation, Formal analysis, Writing - original draft, Writing - review & editing. Maryam Tahira; Muhammad Akif; Muhammad Zeeshan Mola Bakhsh: Figures preparation, Formal analysis, Writing - review & editing. Muhammad Abu Bakar Hayat: Writing - review & editing. Haseeb ur Rehman, Nazim Hussain: Funding acquisition, Writing - review & editing.

9. SDGs Addressed

This study addresses SDG 2: Zero Hunger by enhancing mungbean productivity under salinity stress and SDG 13: Climate Action by promoting sustainable, climate-resilient farming practices.

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