



Potential of PGPRS for Managing Bacterial Wilt Disease and to Enhance Growth and Biochemical Attributes of Tomato

Shabana Ehsan^{*1,2}, Muhammad Ehetisham ul Haq^{3,4}, Muhammad Kamran^{3,5}, Muhammad Umar Shahbaz³, Muhammad Faraz Anwar², Zain Mushtaq⁶, Muhammad Usman⁷

¹Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad

²Soil and Water Testing Laboratory, Faisalabad, Pakistan

³Plant Pathology Research Institute, Faisalabad, Pakistan

⁴Oilseeds Research Institute, Faisalabad, Pakistan

⁵Sugarcane Research Institute, Faisalabad, Pakistan

⁶Department of Soil Science, University of the Punjab, Lahore

⁷Department of Plant Pathology, University of Agriculture, Faisalabad

***Corresponding author email: Shabana Ehsan; shabanaehsans@gmail.com**

Article Received 12-01-2026, Article Revised 13-02-2026, Article Accepted 15-02-2026

ABSTRACT

Pathogenic stress causes severe cellular damage and leads to plant diseases. Hydrogen cyanide (HCN)-producing plant growth-promoting rhizobacteria (PGPR) may help to manage disease and significantly improve plant growth and physiological attributes in arid and semiarid environments. This study aimed to evaluate PGPRs against pathogen *Ralstonia solanacearum* (the real cause of bacterial wilt disease) as biocontrol agents and their role in improving tomato (*Solanum lycopersicum*) yield during 2023. Twenty PGPR strains were isolated from the root rhizosphere and assessed for plant growth-promoting traits i.e. indole-3-acetic acid (IAA), phosphate, potassium and zinc solubilization, as well as HCN and siderophore production. Seven PGPR strains were analyzed using 16S rRNA gene sequencing. The disc sensitivity technique was used to evaluate PGPRs against *R. solanacearum* under laboratory conditions with three replicates. Completely Randomized Design (CRD) was used to evaluate the PGPRs under greenhouse conditions with 3 replicates (5 plants per replicate). *Pseudomonas aeruginosa* was the most effective strain, inhibiting pathogen growth by 56% and reducing disease incidence. PGPR-treated tomato seedlings showed improvement in plant growth through increased plant height, root, shoot length and fruit production. HCN-producing PGPR (*P. aeruginosa* and *Bacillus megaterium*) enhanced plant immunity by increasing superoxide dismutase, peroxidase, and catalase enzyme levels. Mineral nutrition improved through enhanced solubilization of potassium, phosphorus and zinc under biotic stress. HCN-producing PGPRs enhance tomato yield by improving nutrient availability and inducing plant defense mechanisms. These findings highlight their potential as biocontrol agents for sustainable tomato production in arid and semiarid regions.

KEYWORDS: Antioxidant enzymes, bacterial wilt, hydrogen cyanide production, mineral solubilization, plant growth-promoting rhizobacteria, *Solanum lycopersicum*

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is a widespread, virulent tomato plant disease (Popoola *et al.*, 2015). It causes yield losses in the field ranging from 10.8 to 90.6% depending on prevailing environmental conditions and the growth stage of crop. The wilt disease can appear at any growth stage of the plant; the symptoms are seen usually on foliar parts. At the earlier stages of the disease, wilting of the youngest leaves at the top of branches is observed. Before wilting, the lower leaves droop. As the disease develops, the entire plant wilts quickly. The bacterial ooze is seen when the lower stem is cut and squeezed. The sudden drooping of leaves without yellowing may be found if wet weather prolongs with high temperature (Singh, 2001).

The pathogen (*Ralstonia solanacearum*) has wide ecological fitness and has the ability to infect more than 200 plant species in over 50 families including a wide range of crop plants, ornamentals and weeds around the world. The pathogen is both soil-borne (Coutinho, 2005) and water-borne (Pradhanang & Momol, 2001), infested soil and water bodies acts as reservoirs for primary inoculum. *R. solanacearum* survives from months to years depending upon the physico-chemical property and microflora of the soil (Yadessa *et al.*, 2010). Several sustainable multidirectional approaches from the last couple of decades were adopted to minimize the defilement of the ecosystem and to use eco-friendly practices to encounter the food security challenge and control disease outbreaks (Gomiero, 2019; Rehman *et al.*, 2022). Remarkable advancements

have been made to decipher the effective capacity of different beneficial microorganisms to enhance crop production and ensure food security ultimately (Daniel *et al.*, 2022).

Plant growth promoting rhizobacteria (PGPRs) are a beneficial group of microorganisms that resides in the root rhizosphere and promote plant growth (Podile & Kishore, 2006; Saharan & Nehra, 2011; Verma *et al.*, 2010). Numerous studies have revealed a significant positive impact of PGPRs on the growth of vegetables (Gupta *et al.*, 2018; Mekonnen & Kibret, 2021). PGPRs have shown significant potential in suppressing soil-borne pathogens and improving plant growth and crop productivity. Integrated disease management strategies involving PGPR, plant extracts and bactericides have also demonstrated effective pathogen suppression and enhanced plant performance under both axenic and field conditions (Usman *et al.*, 2025). PGPRs are versatile in their actions and significantly impact on plant growth through a variety of mechanisms, both directly and indirectly (Dinesh *et al.*, 2015). Direct mechanisms involve root growth stimulation, biofertilization, rhizoremediation and plant stress control (Lugtenberg & Kamilova, 2009). Indirectly, reducing the disease severity and incidence through antibiosis (Beneduzi *et al.*, 2012), nutritional competition (Saharan & Nehra, 2011), and induction of systemic resistance (Pieterse *et al.*, 2003); that is the uniqueness of these microbes to enhance crop yield without disturbing the environment (Rawat *et al.*, 2020). The cost-effective, accurate and reliable genome sequencing helped greatly to identify these microbes (Olusanya, 2020). Mostly, the PGPRs strains having ability to inhibit the phytopathogen, are identified from *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* genera (Parthasarathy, 2008).

The advancement in molecular and biochemical techniques facilitated our understanding of the underlying biological mechanisms adapted by PGPRs for disease suppression (Priya *et al.*, 2021; Rai *et al.*, 2023). Surprisingly, the disease suppression mechanisms vary with the specific pathogen-plant interaction and even with the particular PGPR. PGPRs release antibacterial compounds to combat soil-borne pathogenic diseases caused by bacteria (Kenawy *et al.*, 2019; Xie *et al.*, 2018), thus, having a direct impact on inhibiting the bacterial pathogen. The competition for nutrient(s) in the presence of PGPRs is challenging for the pathogen's survival in the plant roots rhizosphere (Prasad *et al.*, 2015). The siderophores producing PGPRs scavenge the iron (Fe) in the environment and hence the pathogen fails to continue Fe-dependent biological functions in its cell (Sayyed *et al.*, 2012). The biofilm formed by *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Paenibacillus polymyxa* and competes for dominance in the rhizosphere arena, produce extra polysaccharides

(EPS) as a barrier against pathogens diffusion through cell wall (Rafique *et al.*, 2015). Furthermore, the PGPRs stimulate the growth of mycorrhizal fungi and other antagonistic microbes to suppress the pathogens population (Prasad *et al.*, 2015; Siddiqui & Sayeed Akhtar, 2009). Interestingly, the unique ability to induce the jasmonic acid and ethylene hormones in plants confers systemic resistance against both soil and air-borne pathogens (Beneduzi *et al.*, 2012).

Since the *R. solanacearum* is a soil-borne pathogen also called "cancer of tomato" is difficult to control as it is systemic in its induction and also resistance by host plant is limited. The concept of biocontrol of plant disease is through induction of systemic resistance in plants by other biological means. PGPRs are known to exhibit bio-control of parasite pathogens through antagonistic behavior for nutrient scavenging and boost plant metabolism through increased antioxidant enzymes. Fluorescent producing PGPR (*P. aeruginosa*) has broad spectrum control against plant pathogens. Similarly, sporulating gram positive bacteria like *B. subtilis* has also been used successfully for control of bacterial wilt disease through increased phosphate solubilization, IAA, HCN and siderophore production that ultimately enhanced induced systemic resistance in host plants (Mekonnen *et al.*, 2022; Sun *et al.*, 2023).

Although *P. aeruginosa* isolated from plant rhizosphere demonstrated strong plant growth-promoting and antagonistic properties in agriculture, exhibit significant genetic and functional differences from clinical isolates which is recognized as an opportunistic human pathogen (Moradali *et al.*, 2017; Klockgether & Tümmeler, 2017). Several researches have also revealed the plant growth-development and biocontrol potential of *Pseudomonas* species through production of antimicrobial metabolites and nutrient solubilization (Raaijmakers *et al.*, 2010; Vessey, 2003). As an endophytic bacterium, *P. aeruginosa* serves as non-pathogenic biocontrol agent that inhibits the *R. solanacearum* by producing phenazines (antimicrobial compounds) and enhancing systemic resistance through HCN (by targeting electron transport chain of pathogen) and siderophore production (Suansia & Senapati, 2022).

Although several studies report PGPR-mediated suppression of bacterial wilt, limited information exists regarding the combined role of indigenous HCN-producing rhizobacteria in modulating antioxidant defense and mineral uptake in tomato. Keeping in view the above facts, the present study lies in the integrated evaluation of indigenous PGPR isolates based on hydrogen cyanide production and mineral solubilization to enhance the potential of tomato plants to combat bacterial wilt stress under semi-arid environmental conditions through boosting antioxidant defense mechanism. This combined functional assessment under pathogen stress provides a comprehensive understanding of beneficial bacterial-mediated disease repression leading to tomato growth.

MATERIALS AND METHODS

Soil sampling and analysis: Rhizospheric soils were collected from chickpea, lentil, soybean, sorghum, wheat, maize and rice crops grown in Faisalabad district of Punjab, Pakistan. The collected soils were immediately transferred to an icebox and taken to the laboratory. Soil samples for glasshouse experiment were collected and analyzed for physico- chemical properties of soil. Soil pH was measured in 1:5 suspensions in water and electrical conductivity (EC) of soil after extraction through vacuum pump (McLean

& Page, 1983). Available potassium determined using 1M NH₄OAC extractant solution and analyzed on flame photometer (Rowell, 1994). Available P measured through Olsen P method after extraction with 0.5M NaHCO₃ with pH 8.5 (Olsen, 1954) and organic matter contents using method of Walkley (1947). Both the collected rhizospheric soil and experimental soil were alkaline in nature, free from salinity and sodicity with adequate potassium and phosphorus contents and lower in organic matter (Table 1).

Table 1. Physico-chemical properties of rhizospheric and experimental soils

Parameters	Unit	Rhizospheric soil	Experimental soil
Texture	Class	Clay loam	Clay loam
pH		8.01 ± 0.24	7.99 ± 0.51
EC	dS m ⁻¹	1.72 ± 0.30	1.86 ± 0.04
Organic matter	g kg ⁻¹	0.63 ± 0.20	0.58 ± 0.004
Available P	mg kg ⁻¹	8.13 ± 0.13	8.25 ± 0.10
Extractable K	mg kg ⁻¹	180 ± 15.8	182 ± 14.9

Values represent mean ± SE (n = 3). Means followed by different letters differ significantly at $p \leq 0.05$ according to LSD test.

Separation of PGPR: Almost 10 g moist soil was taken in 90 mL sterilized water to prepare 10⁻¹ dilution after shaking for 10 min. Then serial dilutions were prepared up-to 10⁻¹⁰, followed by pouring of 10 µL of each dilution on petri plates containing King's B (KB) agar medium having glucose, yeast, potassium and magnesium sources. The petri plates were transferred to the incubator at 28 °C (favorable for PGPRs) for 72 hours. Diverse growth of rhizobacteria was produced on KB. The single colonies were picked and further purified on NA/nutrient agar medium. Approximately 20 bacterial bodies were selected and given specific identity strain codes for further biochemical analysis. Pure isolates were also preserved at -80 °C in cryovials in an ultra-low temperature freezer having 50% glycerol for inoculation purposes.

Indole acetic acid/IAA and hydrogen cyanide/HCN production: The isolates were scanned for IAA production ability by the prescribed method of Bric *et al.* (1991). The 10 mL of sterilized peptone broth prepared using L-tryptophan (0.204 g), peptone (10 g), NaCl (5 g), beef extract (3 g) in deionized water (1 L) with pH 7 was injected with 100 µL of cell suspension into 25 mL cultural tubes and later kept in incubator for 72 h at 28 °C. Subsequently 1.5 milli-Liter of this inoculated broth was processed through centrifugation at 12,000 rpm for 10 min. Later on, 1 mL of Salkowski color developing reagent (50 mL of 35% of perchloric acid, 1 mL of 0.5 M FeCl₃ solution) added to 2 mL eppendorf tube having 1mL of the supernatant solution. The eppendorf tubes were then placed in dark under incubation at 37 °C for 1 hour. Uninoculated broth was used as negative control and standard IAA curve was prepared for quantification. Development of a pink color considered positive for hormone (IAA) production, and the bacterial growth density was measured through OD 520 nm using a

spectrophotometer and interpreted from a standard graph curve to measure the IAA concentration in mL⁻¹ basis according to Sarwar and Kremer, 1995.

The production of HCN by selected strains was assessed through modified method of Kloepper *et al.* (2004). Almost 25 µL bacterial cultures were used for inoculation of 100 mL KB broth spiked with of glycine (4.4 g L⁻¹) in 250 mL of glass jars. Strips of sterilized filter paper first soaked in picric acid medium (12.5 g Na₂CO₃ + 2.5 g picric acid in 1 L deionized water) then hanged in the half of glass jars and tighten with screwcap. Parafilm sealed jars were kept under incubation for 72 hours in a mechanical shaker. The indication of strip color change from yellow to brown red was semi-quantitative positive sign for HCN production. For quantitative HCN measurement, the color was extracted by placing filter paper in distilled water (10mL) and read its absorbance at 625 nm on spectrophotometer to check the intensity of color (Reetha *et al.*, 2014).

Solubilization of minerals P, K, Zn: Dissolution test for P and K was done by using medium of Pikovskaya's agar containing inorganic calcium phosphate (Pikovskaya, 1948) and Alexandrov agar medium containing mica (Hu *et al.*, 2006). One drop of the 24-hour fresh bacterial culture was picked with loop and spotted at four equidistant spaces on the respective medium and placed under incubation at 28 °C for 96 hours. Similarly zinc solubilization was measured on zinc solubilizing media amended with 0.1% ZnO (Venkatakrishnan *et al.*, 2003). The solubilization of phosphorus, zinc and potassium minerals was calculated by clearance zones around colonies. The solubilization index (SI) and solubilization efficiency (SE) were calculated using formula

$$SI = \frac{\text{Colony diameter (cm)} + \text{halozone diameter (cm)}}{\text{Colony diameter}}$$

$$SE (\%) = \frac{\text{Halozone diameter (cm)}}{\text{Colony diameter (cm)}} \times 100$$

Quantitative phosphate solubilization was done by growing pure cultures of phosphate solubilizing microbes in liquid Pikovskaya's (PVK) medium spiked with tricalcium phosphate; 80 mL of each broth was transferred to 100 mL conical flask. The broth was autoclaved at 121 °C for 20 min after maintaining pH 7.0, after cooling add 1 mL of pure culture to broth and incubate for 5 days under 30 °C with continuous shaking at 100 rpm. After that centrifuged at 5000 rpm, collect supernatant, filter and measure pH. Solubilization of phosphorus was measured at absorbance 880 nm and compute values by using KH₂PO₄ standards curve (Olsen, 1954). K-feldspar powder was added to liquid Alexandrov medium to assess the K-releasing ability of isolates. After maintaining pH 7.0 of Alexandrov liquid broth, autoclaved it at 121 °C for 20 min, inoculated with pure isolates and then kept under incubation for 7 days at 28 ± 2°C at 130 rpm shaking. The available K contents measured after centrifuged at 5000 rpm at 7th days for 10 min in the supernatant using flame photometer. Calculate values of solubilized K concentrations using standards curve graph prepared with KCl salt. Drop in pH of broth was also measured on pH meter. Similarly, Burnt and Rovira's 1 L broth spiked with 0.1% ZnO prepared for zinc solubilization, after maintaining pH 7.0, broth was divided in 150 mL conical flasks

containing 50 mL broth in each. Each flask was inoculated separately with each strain. After 10 days of incubation cells were removed by centrifugation. The collected supernatant was digested with di-acid mixture (HNO₃ and HClO₄) to measure quantity of soluble zinc on atomic absorption spectrophotometer.

Siderophore production: Chrome Azurol S (CAS) assay was used for qualitative measurements of siderophores (Milagres *et al.*, 1999). For spot inoculation for clearing zones around bacterial colonies was done at five equidistance places on the CAS-agar medium plate under incubation of 48 hours at (28 ± 2 °C). Quantitative measurement of siderophore production was done following the prescribed method of the Chaiarn *et al.* (2009) and Kotasthane *et al.* (2017). For this purpose, about 0.5 mL of aliquots of culture (6 × 10⁶ cfu/mL) grown on LB medium was added to 2 mL eppendorfs after 7 days of incubation and later centrifuged for 10 min at 10,000 rpm to get supernatant. After that, 0.5 mL of CAS reagent was added for color development in 0.5 mL of suspension samples and in blank. Finally, a color change was noticed and measured on colorimetry at 600 nm wavelength. The quantitative siderophore production in percentage (%) was calculated by using calculations as given below:

$$\% \text{ Siderophore unit} = \frac{Ar - As}{Ar} \times 100$$

As= Absorbance of sample;

Ar= Absorbance of Reference/blank

Recognition of bacterial isolates: The identity of promising strains was checked through PCR analysis, later on confirmed through 16S rRNA analysis. For genomic DNA analysis of selected PGPR, protocol of Sambrook and David (2000) was used. The 16S rRNA gene was amplified using universal primer set 785F (5'-GCATTAGATACCCTGGTA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') in a 25 µL reaction mixture containing 2.5 µL of 10× PCR buffer, 0.5 µL of 10 mM dNTP mix, 0.5 µL of each primer (10 µM), 1.5 µL MgCl₂ (25 mM), 0.1 µL Taq DNA polymerase (5 U µL⁻¹), 1 µL template DNA (100 ng µL⁻¹), and nuclease-free water added to make the final volume of 25 µL. The thermocycling conditions included an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 2 min 10 s, and a final extension at 72 °C for 10 min using an Eppendorf Master cycler thermocycler.

The final PCR product was recovered through 0.8% agarose gel prepared in TAE/TBE buffer and after that purification with the elution kit (Sigma, India). The DNA sequencing was done through

Macrogen Lab, Korea. Bacterial recognition/identification was done through comparison of the obtained sequences from Macrogen Lab. against those already deposited in the following public databases: Ribosomal Database Project II (RDPII) (<https://rdp.cme.msu.edu/>) using the Classifier program with 95% confidence (Wang *et al.*, 2007) and GenBank from the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) by using the BLAST tool (Altschul *et al.*, 1997). The phylogenetic tree of bacterial isolate showing similarity to sequences available in public databases was constructed using MEGA (Molecular evolutionary genetic analysis) XI software (Figure 1). Although 16S rRNA sequencing confirmed genus-level identity, additional molecular approaches are required for precise species confirmation.

Isolation of the pathogen (R. solanacearum): A small portion of stem from infected plant was cut and macerated by pestle and mortar. The pathogen was isolated using serial dilution technique on Triphenyl Tetrazolium Chloride (TTC) culture media (casein hydrolysate 1 g, peptone 10 g, glucose 5 g, agar 15 g, and 2, 3, 5-triphenyl tetrazolium chloride -1% 5 mL)

(Kelman, 1954). The plates were wrapped with parafilm and kept under incubation at 28 °C for one day and night. For further purification, single bacterial colony was picked and streaked on fresh TTC containing petri plates. The isolated purified bacteria were later inoculated in test tubes containing LB media broth and then preserved in 50% sterilized glycerol in 2 mL of eppendorfs for further studies. Pathogenicity of *R. solanacearum* was confirmed through re-inoculation and symptom reproduction on healthy plants.

In-vitro antagonistic potential of PGPRs: In-vitro antagonistic potential of PGPRs against *R. solanacearum* was checked through inhibition zone. For this purpose, fresh liquid culture of each PGPR and the *R. solanacearum* was prepared in Luria Bertani media and was incubated at 25 °C for 48 hours. The disc sensitivity technique (Bell, 1975) was used to evaluate the antibiotic potential of PGPRs against the *R. solanacearum*. The discs were dipped in PGPRs liquid cultures (6×10^6 cfu/mL) and positioned at the center of the petri plates containing Nutrient Glucose media (peptone salt 5 g, beef extract 3 g, NaCl 5 g, glucose 1 g, agar 15 g and water 1 L) medium inoculated with *R. solanacearum*. The plates were wrapped and incubated at 28 °C. The experiment was

$$\text{Disease Incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

Potential of PGPRs on physiological attributes: The experiment was executed in a greenhouse of Soil Bacteriology Section during 2023 to measure the potential of PGPR on physiological attributes of tomato. The 5 kg soil was sterilized with formalin (to lessen the effect of native microbes, to minimize the competition for added resources in soil and to see full potential of added inoculum) and added in the 30-40 cm diameter and depth pots and moistened with sterilized water. 50 ml culture of the PGPR (6×10^6 cfu/mL) was added in pots with measuring cylinder prior to seven days of tomato seedling transplantation. Sterilized broth was applied in control (uninoculated) pots. Healthy tomato seedlings of Nadir variety were treated with PGPRs liquid culture (6×10^6 cfu/mL) for half an hour before sowing. Experimental units were replicated thrice and CRD was adopted to analyze the data. The agronomic practices were carried out on all 24 experimental units accordingly. At maturity of tomato plants, shoot height was taken from the collar portion to plant tip using a measuring scale. The shoot diameter was measured at three different locations of the stem using a Vernier caliper. The root fresh weight was recorded by cutting the roots from plants and weighed on digital weight balance. Fruits per plant were recorded by counting the number of fruits on each plant in the growing season.

Biochemical Parameters of Plants: For analysis of biochemical parameters, 200 mg plant leaf extract was homogenized in 2 mL of 0.2 M potassium phosphate buffer (pH 7.8) and centrifuged at 15000 g and 4 °C for

executed in Completely Randomized Design (CRD) with three replicates. The data was recorded by measuring the inhibition zones after 24 hours.

Later on, the experiment was conducted at greenhouse of Plant Pathology Research Institute, Faisalabad in 2023 for measuring disease incidence percentage. The potting soil was sterilized by treating with 5 % formalin (formaldehyde-37%) at the rate 1L/sq. ft and covered with polythene sheet. After a week, polythene sheet was removed and 12" pots were filled with sterilized soil. Prior to seven days of tomato seedling transplantation, 50 mL culture of the PGPR (10^8 CFU mL⁻¹) and *R. solanacearum* was added in pots with measuring cylinder. In control, only sterilized broth was applied. The pots' soil was adequately moistened with sterilized water. Healthy tomato seedlings of Nadir variety were taken from Vegetable Research Institute, Faisalabad and roots were dipped in PGPRs liquid culture (6×10^6 CFU mL⁻¹) for half an hour before sowing. Each experimental treatment was replicated three times with five plants per experimental unit. Pots were arranged in CRD and repositioned weekly to minimize environmental variation. The pots were arranged and all the agronomic cultural practices were carried out. The disease data were recorded based on disease incidence (%).

20 minutes. Then, the tissue extract was kept at -20 °C and antioxidant enzyme activities were measured within 48 hours of storage. Superoxide dismutase (SOD) activity was measured by the modified NBT (nitro blue tetrazolium) method (Giannopolitis & Ries, 1977). The catalase (CAT) and peroxidase (POD) activity was measured by the method of (Chance & Maehly, 1955).

Mineral analysis of plant materials: For mineral analysis, plant leaves were ground using a pestle and mortar. The samples were digested in HNO₃, HClO₄, H₂SO₄ at 10:4:1 ratio respectively (Wolf, 1982). The K and Fe concentrations were measured through an atomic absorption spectrophotometer (Soltanpour *et al.*, 1976). To measure the P in tomato plant leaves, chlorostannous molybdophosphoric blue color technique in H₂SO₄ was used (Chapman & Pratt, 1961).

Statistical analysis: All experimental values were analyzed through analysis of variance (ANOVA) using Statistix 8.1 software. Experimental application means were contrasted using Least Significant Difference (LSD) test at $p \leq 0.05$ significance level. Data are presented as mean $\pm$ standard error (SE) and statistical significance levels are indicated in tables and figures.

RESULTS

Molecular identification of bacterial strains: The seven efficient strains were identified through 16S rRNA sequencing. The results revealed that isolate coded 2.3 showed 100% sequence similarity with *P. aeruginosa* strain VSS6 (accession # KJ528948.1), isolate coded as 3.2 showed 99% sequence similarities

with *Agrobacterium fabrum* strain SLAJ731 (accession # KX500242.1), isolate coded as 6.2 showed 100% similarities with *Pseudomonas putida* strain RC5.2 (accession # PP905304.1), isolate coded as 9.2 showed 99.79% similarities with *B. megaterium* strain DSM32 (accession # KJ476721.1), isolate coded as 10 showed 100% similarities with *Cytobacillus firmus* strain NBRC (accession # OK083589.1), isolate coded as 11 showed 99% sequence similarities with *Enterobacter cloacae* (accession # KX242267.1) and isolate coded as 12 showed 99% similarities with *Delftia acidovorans* BGW1 (accession # EU195867.1). All bacterial sequences were submitted to GenBank on NCBI website after downloading FASTA files, which are accessible through accession numbers OR741775.1 (*P. aeruginosa*), OR553483.1 (*A. fabrum*), OR553480.1 (*B. megaterium*), PX496465.1 (*P. putida*), OR741774.1 (*C. firmus*), OR559983.1 (*Enterobacter cloacae*) and OR741773.1 (*Delftia acidovorans*) respectively (Table 2). Later on,

phylogenetic tree was constructed using molecular techniques of MEGA XI software (Figure 1).

Characterization of bacterial isolate for plant growth promoting trait: Twenty different gram positive and gram-negative bacterial strains were isolated from rhizosphere soils adhering to plant roots. All the 20 bacterial strains were characterized on the basis of plant growth promoting ability and only 7 were highly potent PGPR strains based on phytohormones, siderophore and hydrogen cyanide production. High IAA contents were produced by *P. aeruginosa* (2.3), followed by *B. megaterium* (9.2) and *P. putida* (6.2). Similarly, high siderophore contents were produced by *B. megaterium* (9.2) followed by *P. aeruginosa* (2.3) and *Delftia acidovorans* (12). *P. aeruginosa* (2.3), *B. megaterium* (9.2), *C. firmus* (10), *E. cloacae* (11) and *D. acidovorans* (12) showed maximum HCN production with OD_{620nm} values of: 0.095, 0.088, 0.066, 0.079 and 0.089, respectively (Table 2). All strains found positive for P solubilization with *P. aeruginosa* maximum solubilizer of insoluble P mineral (Table 3).

Table 2. Plant growth promoting traits of different microbial isolates

Isolate	IAA (mg L ⁻¹)	Siderophore SU (%)	HCN OD 625 nm	GenBank ID #
<i>P. aeruginosa</i>	2.18 ± 0.07 ^a	38.9 ± 0.88 ^b	0.095 ± 0.0015 ^a	OR741775.1
<i>A. fabrum</i>	2.01 ± 0.04 ^a	24.4 ± 0.49 ^f	0.015 ± 0.0012 ^d	OR553483.1
<i>P. putida</i>	2.11 ± 0.05 ^a	31.6 ± 0.46 ^d	0.012 ± 0.0009 ^d	PX496465.1
<i>B. megaterium</i>	2.15 ± 0.01 ^a	41.7 ± 0.55 ^a	0.088 ± 0.0035 ^{ab}	OR553480.1
<i>C. firmus</i>	2.09 ± 0.04 ^a	26.7 ± 0.46 ^e	0.066 ± 0.0026 ^c	OR741774.1
<i>E. cloacae</i>	1.18 ± 0.05 ^b	22.5 ± 0.55 ^g	0.079 ± 0.0059 ^{bc}	OR559983.1
<i>D. acidovorans</i>	1.08 ± 0.06 ^b	35.0 ± 0.64 ^c	0.089 ± 0.0031 ^{ab}	OR741773.1

IAA: Indole acetic acid equivalents (mg L⁻¹), SU: siderophore unit percentage, OD: optical density of different coded isolates measured at 625nm for Hydrogen Cyanide production, ID: Identity number of GenBank on NCBI website. Values represent mean ± SE (n = 3). Means followed by different letters differ significantly at p ≤ 0.05 according to LSD test.

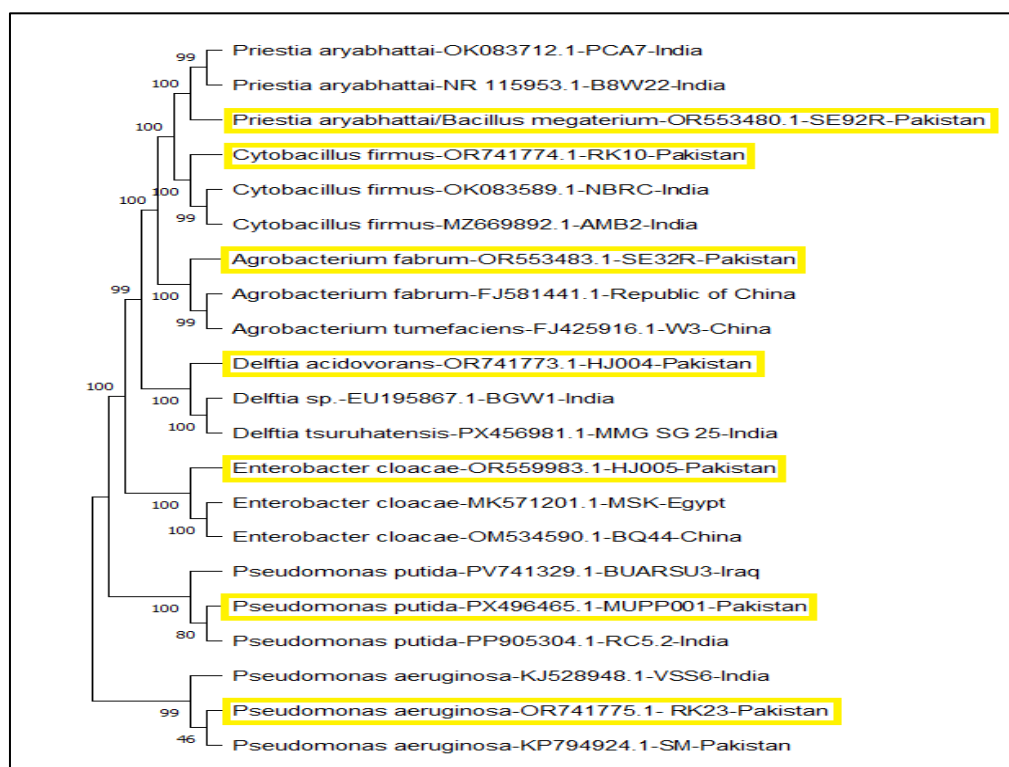


Figure 1. Phylogenetic tree constructed using the Neighbor-Joining method based on 16S rRNA gene sequences showing evolutionary relationships among different isolates and their reference strains.

Data in table 3 showed maximum phosphorus solubilization index and efficiency was shown by *P. aeruginosa* and *B. megaterium*. Similarly, maximum (655 mg L⁻¹) solubilized p was found in *P. aeruginosa* broth due to drop in pH from 7 to 5.4. Potassium solubilization index (KSI), potassium efficiency (KE) and quantity of solubilized K concentrations are given in Table 4. Data revealed that as the pH of broth drop from 7 due to microbial activities, the KSI and KSE of insoluble K by microbes increases with increase in

available K contents. *P. aeruginosa*, *B. megaterium* and *C. firmus* statistically at par in case of KSI. Similarly, maximum K contents (21.6 mg L⁻¹) were solubilized by *P. aeruginosa* followed by *C. firmus* (15.8 mg L⁻¹) as pH drop was same in both cases (Table 4). Qualitative and quantitative estimation of zinc solubilization showed that maximum zinc concentration, drop in pH, solubilization index and solubilizing efficiency were shown by *P. aeruginosa* (Table 5).

Table 3. Qualitative and quantitative test for insoluble phosphorus solubilization

Isolate	Colony diameter (cm)	Halozone diameter (cm)	PSI	PSE (%)	P Conc. (mg L ⁻¹)	pH
<i>P. aeruginosa</i>	0.51 ± 0.03 ^d	2.6 ± 0.05 ^d	6.2 ± 0.11 ^a	5.2 ± 0.10 ^{ab}	655 ± 50.7 ^a	5.4 ± 0.43 ^b
<i>A. fabrum</i>	3.80 ± 0.05 ^a	6.8 ± 0.10 ^a	2.8 ± 0.04 ^d	1.8 ± 0.72 ^c	427 ± 15.5 ^c	6.5 ± 0.45 ^a
<i>P. putida</i>	1.00 ± 0.08 ^c	1.1 ± 0.06 ^g	2.1 ± 0.12 ^e	1.1 ± 0.06 ^f	410 ± 12.7 ^c	6.1 ± 0.97 ^{ab}
<i>B. megaterium</i>	0.50 ± 0.03 ^d	2.9 ± 0.04 ^c	6.5 ± 0.47 ^a	5.5 ± 0.13 ^a	559 ± 48.0 ^b	5.8 ± 0.10 ^{ab}
<i>C. firmus</i>	0.50 ± 0.03 ^d	2.3 ± 0.08 ^f	5.5 ± 0.11 ^{bc}	4.5 ± 0.31 ^{cd}	630 ± 16.6 ^a	5.5 ± 0.13 ^b
<i>E. cloacae</i>	1.20 ± 0.04 ^b	4.9 ± 0.07 ^{ab}	5.1 ± 0.11 ^c	4.0 ± 0.21 ^d	510 ± 10.0 ^b	6.5 ± 0.58 ^a
<i>D. acidovorans</i>	0.50 ± 0.03 ^d	2.4 ± 0.05 ^e	5.8 ± 0.08 ^b	4.9 ± 0.25 ^{bc}	620 ± 31.2 ^a	5.6 ± 0.51 ^b

Colony and halozone diameter, PSI: phosphorus solubilizing Index, KSE: potassium solubilizing efficiency, available phosphorus concentration (mgL⁻¹), and drop in pH of liquid medium by different isolates. Values represent mean ± SE (n = 3). Means followed by different letters differ significantly at p ≤ 0.05 according to LSD test.

Table 4: Qualitative and quantitative test for insoluble potassium solubilization

Isolate	Colony diameter (cm)	Halozone diameter (cm)	KSI	KSE (%)	K Conc. (mg L ⁻¹)	pH
<i>P. aeruginosa</i>	0.6 ± 0.03 ^c	0.1 ± 0.38 ^{cde}	6.8 ± 0.31 ^a	5.7 ± 0.37 ^a	21.6 ± 1.02 ^a	5.4 ± 0.35 ^b
<i>A. fabrum</i>	0.8 ± 0.09 ^{ab}	3.6 ± 0.31 ^{bc}	5.4 ± 0.22 ^b	4.4 ± 0.36 ^{bc}	9.2 ± 0.57 ^d	6.6 ± 0.49 ^a
<i>P. putida</i>	0.7 ± 0.12 ^{bc}	2.5 ± 0.31 ^e	4.7 ± 0.26 ^c	3.7 ± 0.38 ^c	8.5 ± 0.64 ^d	6.2 ± 0.61 ^{ab}
<i>B. megaterium</i>	0.8 ± 0.06 ^{ab}	4.7 ± 0.17 ^a	6.7 ± 0.20 ^a	5.7 ± 0.39 ^a	12.5 ± 1.51 ^c	5.7 ± 0.05 ^{ab}
<i>C. firmus</i>	0.5 ± 0.02 ^c	2.9 ± 0.06 ^{de}	6.6 ± 0.45 ^a	5.6 ± 0.27 ^a	15.8 ± 0.82 ^b	5.4 ± 0.35 ^b
<i>E. cloacae</i>	0.8 ± 0.12 ^{ab}	3.4 ± 0.13 ^{bcd}	5.2 ± 0.08 ^{bc}	4.2 ± 0.12 ^{bc}	12.4 ± 0.86 ^c	5.5 ± 0.11 ^b
<i>D. acidovorans</i>	0.9 ± 0.04 ^a	3.9 ± 0.18 ^b	5.6 ± 0.16 ^b	4.6 ± 0.57 ^b	10.1 ± 1.02 ^d	6.1 ± 0.79 ^{ab}

Colony and halozone diameter, KSI: potassium solubilizing Index, KSE: potassium solubilizing efficiency, available potassium concentration (mgL⁻¹), and drop in pH of liquid medium by different isolates. Values represent mean ± SE (n = 3). Means followed by different letters differ significantly at p ≤ 0.05 according to LSD test.

Table 5: Qualitative and quantitative test for insoluble zinc solubilization

Isolate	Colony Diameter (cm)	Halozone Diameter (cm)	ZSI	ZSE (%)	Zn conc. (mg L ⁻¹)	pH
<i>P. aeruginosa</i>	0.53 ± 0.02 ^a	2.3 ± 0.20 ^a	5.6 ± 0.06 ^a	4.6 ± 0.03 ^a	62 ± 1.23 ^a	5.4 ± 0.11 ^d
<i>A. fabrum</i>	0.46 ± 0.03 ^c	1.2 ± 0.01 ^d	3.2 ± 0.03 ^e	4.4 ± 0.03 ^b	42 ± 1.52 ^d	6.4 ± 0.36 ^{ab}
<i>P. putida</i>	0.41 ± 0.01 ^d	0.9 ± 0.03 ^e	3.2 ± 0.03 ^e	4.1 ± 0.03 ^c	37 ± 0.65 ^c	6.9 ± 0.29 ^a
<i>B. megaterium</i>	0.51 ± 0.03 ^{ab}	2.2 ± 0.10 ^a	5.4 ± 0.06 ^b	4.1 ± 0.03 ^c	46 ± 1.36 ^c	5.8 ± 0.08 ^{cd}
<i>C. firmus</i>	0.46 ± 0.02 ^c	1.9 ± 0.05 ^b	5.1 ± 0.04 ^c	3.8 ± 0.06 ^d	45 ± 1.02 ^{cd}	5.6 ± 0.12 ^d
<i>E. cloacae</i>	0.41 ± 0.01 ^d	1.6 ± 0.02 ^c	4.8 ± 0.06 ^d	2.2 ± 0.03 ^e	43 ± 1.59 ^d	6.3 ± 0.46 ^{a-c}
<i>D. acidovorans</i>	0.36 ± 0.03 ^e	1.4 ± 0.01 ^c	5.0 ± 0.02 ^c	2.1 ± 0.03 ^e	52 ± 1.04 ^b	5.8 ± 0.49 ^{b-d}

Colony and halozone diameter, ZSI: zinc solubilizing Index, ZSE: zinc solubilizing efficiency, available zinc concentration (mgL⁻¹), and drop in pH of liquid medium by different isolates. Values represent mean ± SE (n = 3). Means followed by different letters differ significantly at p ≤ 0.05 according to LSD test.

Relative efficacy of PGPRs: A significant difference in PGPRs efficacy was seen to inhibit the *R. solanacearum*. After 24 hours, *P. aeruginosa* was the most significant to inhibit the pathogen's growth as compared to the others. *A. fabrum* was the second most effective PGPR in inhibiting the pathogen's growth. No significant difference in antibacterial efficacy was observed between *P. putida* and *B. megaterium*. The inhibiting efficacy of these PGPRs (*P. putida* and *B. megaterium*) against *R. solanacearum* was less

compared to *P. aeruginosa* and *A. fabrum* however was more as compared to the other ones. *D. acidovorans* was found least effective PGPR to inhibit bacterial growth of *R. solanacearum* but was effective as compared to the control (Table 6).

In greenhouse, the efficacy of PGPRs differed significantly against bacterial wilt disease caused by *R. solanacearum*. *P. aeruginosa* was found most effective in lowering the disease incidence as compared to the other PGPRs and control treatments. *A. fabrum* was the

second one most effective for disease management. *C. firmus* was found less effective as compared to *P. aeruginosa*, *A. fabrum*, *P. putida* and *B. megaterium*.

No difference in between the efficacy of *E. cloacae* and *D. acidovorans* was recorded, however, were found more effective as compared to the control (Figure 2).

Table 6. Relative efficacy of different PGPRs against *R. solanacearum* under lab conditions

PGPRs	Code	Inhibition zone (cm)
<i>P. aeruginosa</i>	2.3	2.4 ± 0.31 ^a
<i>A. fabrum</i>	3.2	2.1 ± 0.41 ^b
<i>P. putida</i>	6.2	1.7 ± 0.22 ^c
<i>B. megaterium</i>	9.2	1.7 ± 0.26 ^c
<i>C. firmus</i>	10.2	1.5 ± 0.14 ^d
<i>E. cloacae</i>	11	1.2 ± 0.26 ^e
<i>D. acidovorans</i>	12	0.7 ± 0.19 ^f
Control	0	0.0 ± 0.00 ^g

Inhibition zone produced by PGPR against *R. solanacearum* after 24 hours of incubation. Values represent mean ± SE (n = 3). Means followed by different letters differ significantly at $p \leq 0.05$ according to LSD test

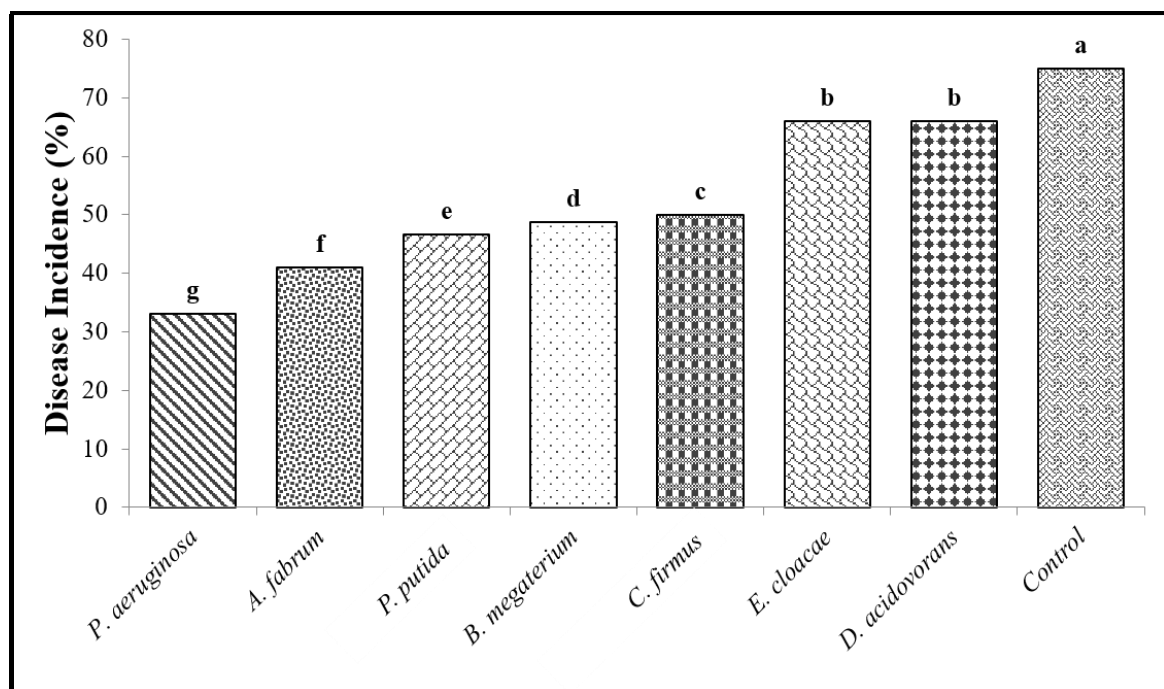


Figure 2: Relative potential of different PGPRs to manage bacterial wilt disease in greenhouse conditions with LSD: 1.73 at $\alpha=0.05$.

Effect of PGPR on physiological parameters of plant: A significant difference in shoot length was observed in PGPRs applied tomato plants. Maximum shoot length (152.6 cm) was recorded where *P. aeruginosa* was applied. *A. fabrum* applied tomato plants were less in shoot length as compared to *P. aeruginosa* applied plants but were more efficient as compared to the other PGPR treated plants in increasing shoot length of tomato. No significant difference in shoot length (142 cm) was recorded between *P. putida*, and *B. megaterium* treated plants, however, their shoot length was more as compared to *C. firmus*, *B. megaterium* and *E. cloacae* applied tomato plants. *C. firmus* applied tomato plants' shoot length was more from *E. cloacae* and *D. acidovorans*. *E. cloacae* was found second least effective in enhancing the shoot length of the tomato plants. *D. acidovorans* applied plants' shoot length were found least as compared to the others PGPRs applied plants

but was more as compared to the control (Table 7). *P. aeruginosa* was found the only one to increase the shoot diameter of the tomato plants. No difference in shoot diameter was recorded in the remaining PGPRs applied plants (Table 7).

Root fresh weight of tomato plants significantly increased with the application of PGPRs. *P. aeruginosa* application was found most effective in enhancing the root fresh weight (57 g) of the tomato plants. *A. fabrum* was the second most effective PGPR to increase the root fresh weight (48.33 g). No statistically significant difference in root fresh weight of tomato plant was recorded where *P. putida*, and *B. megaterium* were applied. However, their efficacy in enhancing root fresh weight was more as compared to *D. acidovorans*. *D. acidovorans* applied tomato plant roots were found least efficient in increasing fresh weight (41.37 g) as compared to the other PGPRs

applied tomato plants, however, were more when compared to control plants (Table 7).

A significant difference in fruit production was recorded among PGPR-applied plants. The maximum number of tomatoes (26) were counted where *P. aeruginosa* was applied. *A. fabrum* applied plants produced a smaller number of fruits (24) as compared to *P. aeruginosa* applied plants, however, were more as compared to other PGPRs applied plants. *P. putida*

was found third most effective in enhancing fruit production (22) in tomato plants. No significant difference in number of tomatoes were seen between *C. firmus* and *E. cloacae* applied plants. *D. acidovorans* applied plants produced the least number of fruits as compared to the other PGPRs, however, were more in numbers when compared with the control (Table 7).

Table 7: Comparative potential of different PGPRs to enhance the physiological parameters of tomato plants

Treatments	Shoot Length (cm)	Shoot diameter (cm)	Root Fresh Weight (g)	Fruits per plant
<i>P. aeruginosa</i>	152.7 ± 3.35 ^a	1.64 ± 0.03 ^a	57.0 ± 3.4 ^a	26.0 ± 0.8 ^a
<i>A. fabrum</i>	148.0 ± 4.65 ^b	1.45 ± 0.03 ^b	48.3 ± 1.9 ^b	24.0 ± 1.2 ^b
<i>P. putida</i>	142.6 ± 1.25 ^c	1.34 ± 0.04 ^b	43.0 ± 1.4 ^e	22.0 ± 1.2 ^c
<i>B. megaterium</i>	149.3 ± 2.84 ^c	1.34 ± 0.01 ^b	43.2 ± 2.4 ^e	20.0 ± 1.9 ^d
<i>C. firmus</i>	139.3 ± 3.84 ^d	1.35 ± 0.02 ^b	43.8 ± 2.7 ^d	18.0 ± 0.9 ^e
<i>E. cloacae</i>	131.1 ± 1.52 ^e	1.33 ± 0.04 ^b	45.2 ± 1.5 ^c	18.0 ± 1.2 ^e
<i>D. acidovorans</i>	129.12 ± 1.50 ^f	1.34 ± 0.05 ^b	41.4 ± 1.4 ^f	17.0 ± 1.1 ^f
Control	112.3 ± 2.18 ^g	1.31 ± 0.03 ^b	37.3 ± 0.9 ^g	15.0 ± 0.8 ^g
LSD	1.4	0.17	0.73	0.52

Values represent mean ± SE (n = 3). Means followed by different letters differ significantly at $p \leq 0.05$ according to LSD test.

Effect of PGPR on the activity of antioxidant enzymes under stress: Superoxide dismutase (SOD), Peroxidase (POD) and catalase (CAT) concentrations significantly differed in tomato plants' leaves where PGPRs applied. Maximum SOD, POD and CAT concentrations were measured in *P. aeruginosa* applied tomato plant leaves as compared to other PGPRs and control. *A. fabrum* applied tomato plant leaves were less in SOD, POD and CAT concentrations as compared to *P. aeruginosa* applied tomato plant leaves, however, were more in concentrations as compared to other PGPRs applied plant leaves. No significant difference in SOD, POD and CAT concentrations were recorded among *P. putida*, *B. megaterium* and *C. firmus* applied plant leaves however their concentrations were more as compared to *E. cloacae* and *D. acidovorans* applied plants' leaves. *E. cloacae* and *D. acidovorans* applied plants' leaves were statistically the same in SOD and POD concentration, however, *E. cloacae* plants' leaves were more CAT concentration compared to *D. acidovorans* applied plants. In control, where no PGPR was added, the concentrations of SOD, POD and CAT concentrations were the lowest as compared to the PGPRs applied plants (Figure 3).

Effect of PGPR on the mineral analysis of plant material under stress: Phosphorus (P) concentration in tomato leaves significantly varied where PGPRs were applied. The maximum concentration of Fe was measured in *P. aeruginosa* applied tomato plants. *A. fabrum* was the second most effective in enhancing P concentration in tomato leaves. *P. putida* applied tomato plant leaves were more in concentration as compared to *B. megaterium*, *C. firmus*, *E. cloacae* and

D. acidovorans treatment plants. No significant difference in P was recorded among *C. firmus*, *E. cloacae* and *D. acidovorans* applied tomato leaves.

A significant difference in Iron (Fe) concentration was recorded in PGPRs applied to tomato leaves. *P. aeruginosa* applied tomato leaves were highest in Fe concentration as compared to other PGPRs applied plant leaves. *A. fabrum* was the second most effective PGPR to enhance the Fe contents in tomato leaves. *P. putida* and *B. megaterium* ranked 3rd and 4th respectively among tested PGPRs to increase the iron contents in tomato plants. No significant difference in Fe concentration was recorded in tomato leaves where *C. firmus* and *E. cloacae* was applied. *D. acidovorans* was the least effective in enhancing the Fe contents in leaves, however, were more to control treatment.

Potassium (K) contents significantly increased in tomato plants where PGPRs were applied. Maximum K concentration was found in *P. aeruginosa* applied tomato plant leaves. *A. fabrum* applied tomato plant leaves were less in concentration as compared *P. aeruginosa* applied plant leaves. *P. putida* was found third most effective PGPRs to enhance K concentration in tomato leaves as compared to other ones. *B. megaterium* was at 4th rank among tested PGPRs to enhance the K contents in tomato leaves. The K contents in *C. firmus* applied tomato leaves were lower in concentration as compared to *P. aeruginosa*, *A. fabrum*, *P. putida* and *B. megaterium* applied tomato leaves, however, were more with respect to *E. cloacae*, *D. acidovorans* and control treatments tomato plants. *E. cloacae* were the second least effective and *D. acidovorans* was the least one among evaluated PGPRs to enhance K contents in tomato leaves (Figure 4).

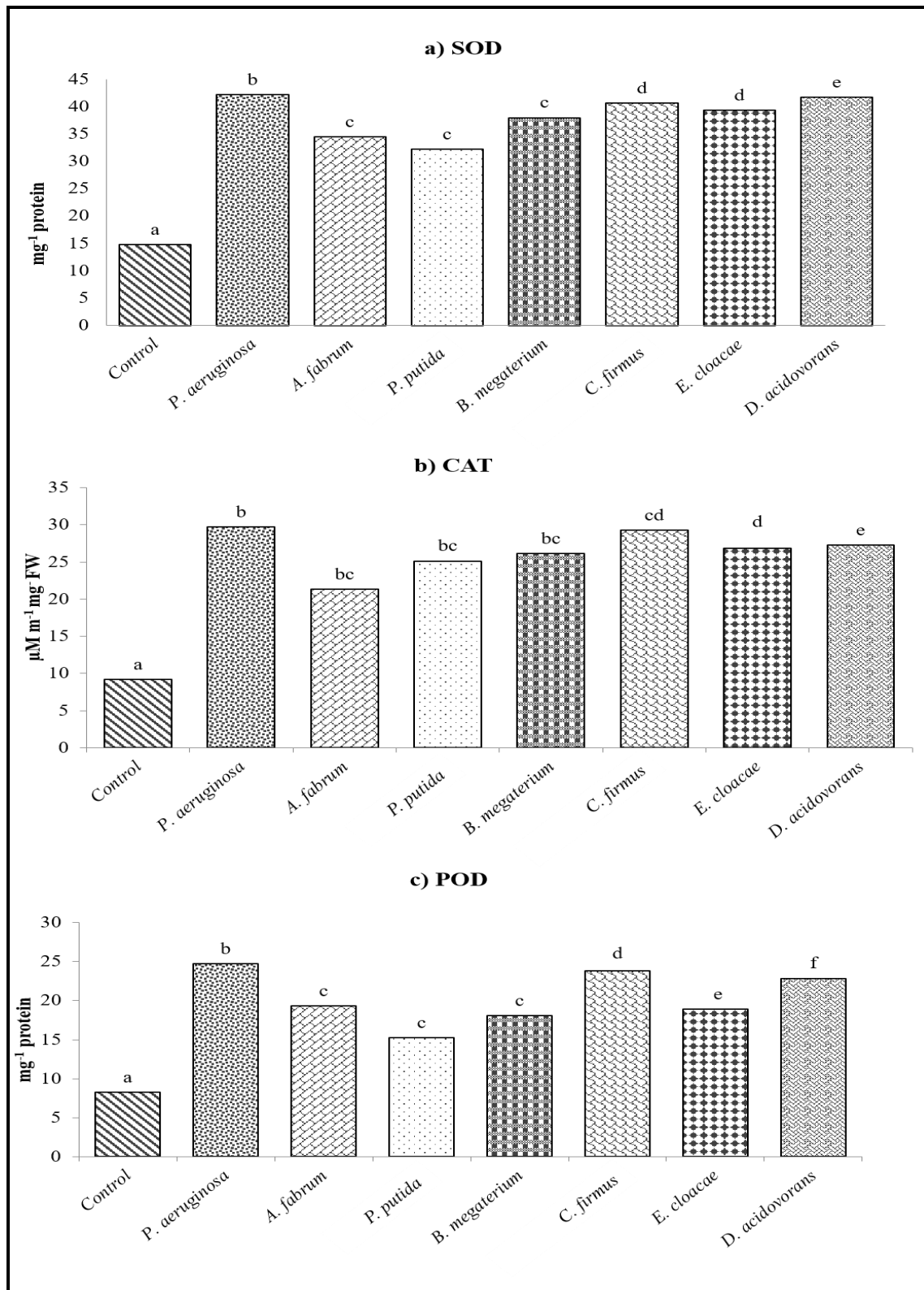


Figure 3: Comparative efficacy of different PGPRs to enhance antioxidants; **a)** *Superoxide dismutase* (SOD) LSD: 1.07, **b)** *Catalase* (CAT) LSD: 0.88, **c)** *Peroxidase* (POD) LSD: 1.42 at $\alpha=0.05$.

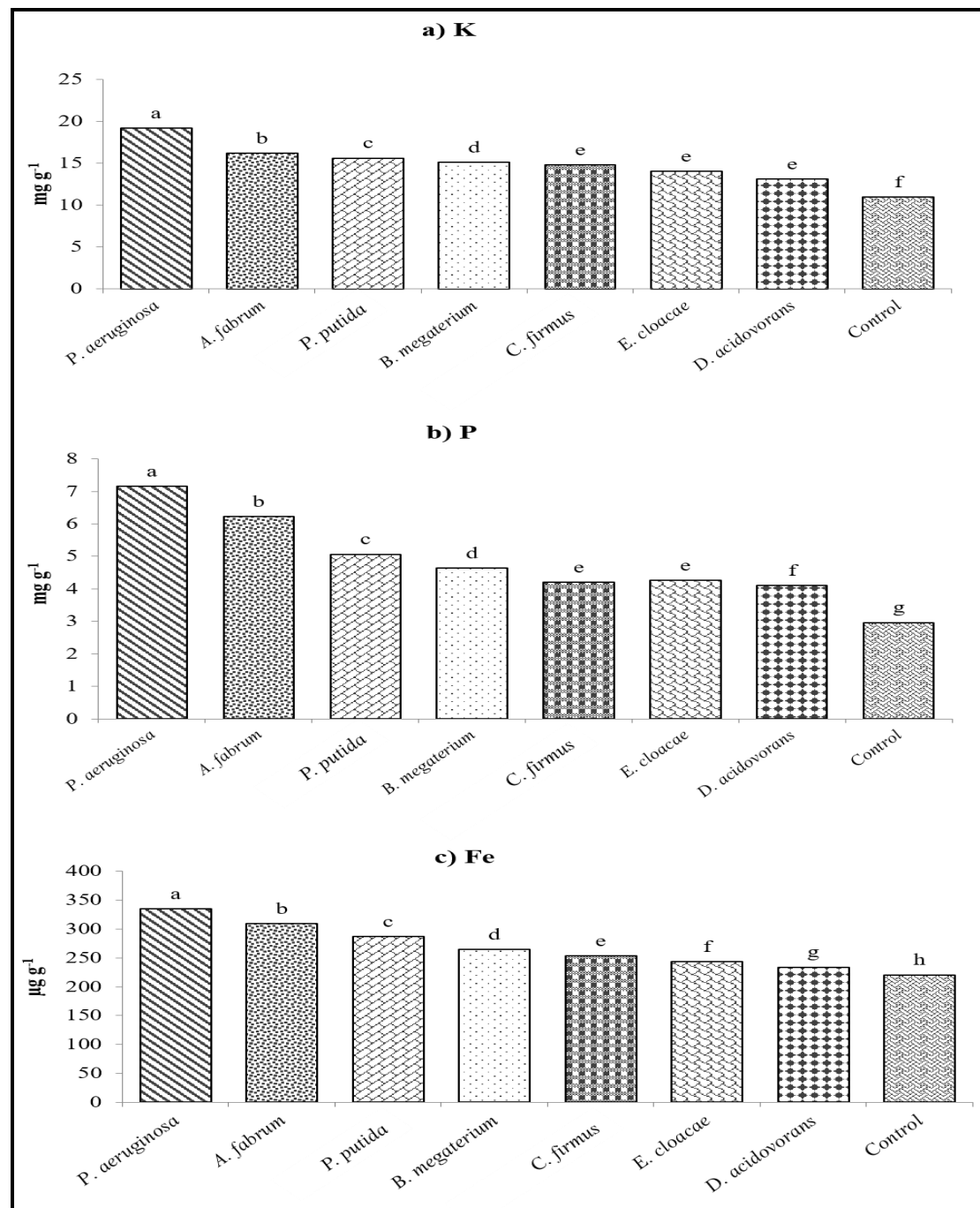


Figure 4: Potential of different PGPRs to enhance different element concentration in tomato plants; **a) K:** Potassium contents, **b) P:** Phosphorus contents, **c) Fe:** iron contents with 0.32, 0.26 and 10.07 LSD values respectively at $\alpha=0.05$.

DISCUSSION

The ailment of terrestrial and aquatic ecosystems by the use of chemicals around the globe has compelled us to find eco-friendly approaches to manage plant diseases. The HCN production is a novel ability of the PGPRs which increases the in biotic and abiotic environments (Ahl *et al.*, 1986; Flury *et al.*, 2017; Siddiqui *et al.*, 2006). These secondary metabolite production initiates at the end of the exponential phase and the start of the stationary phase, the synthesis starts by the oxidation of the glycine (Laville *et al.*, 1998). From the present study, we found that all the PGPRs had the potential to inhibit bacterial pathogen in lab

conditions. The strong bactericidal ability of HCN produced by PGPRs against plant pathogens has been revealed in the last few decades (Michavila *et al.*, 2017). All the PGPRs used in this study were found to produce the HCN, however, their concentrations varied. *P. aeruginosa* was found most prominent among the other PGPRs to inhibit the pathogen's colony growth and its HCN production was also high. A positive correlation was observed between the HCN production and the inhibition efficacy from this study. For instance, *C. firmus* was ranked among the highest HCN produced PGPRs and was more to *P. putida*, however, the efficacy to inhibit the pathogen's growth

was less as to *P. putida*. The speculates that the other secondary metabolites despite HCN production may contribute to disease suppression along with multiple PGPR-mediated mechanisms.

In greenhouse conditions, in our study, all the PGPRs were found effective in lowering the disease incidence. *R. solanacearum* is a soil-borne plant pathogens (Wang *et al.*, 2023), the survival time directly relates with the physicochemical properties of the soil (Messiha *et al.*, 2009). The PGPRs are versatile microbes, a variety of mechanisms have been deciphered of these microbes to manage the plant pathogens in vivo (Beneduzi *et al.*, 2012). PGPRs are ectophytic and endophytic and manage the plant diseases directly and indirectly (Koul *et al.*, 2019). Pseudomonads are unique, most commonly produce compounds which are essential in biocontrol mechanisms including pyrrolnitrin, pyoluteorin, and phenazine-1-carboxylic acid (PCA), 2, 4-diacetylphloroglucinol (DAPG), phenazine-1-carboxamide, lipopeptides, (Dimkić *et al.*, 2022). Furthermore, PGPRs compete for the nutrients i.e. siderophore that deprive the pathogen from the iron, thus, the soil inhibiting *R. solanacearum* fails to get the essential element necessary for metabolic function and population significantly effects (Sayyed *et al.*, 2012). *Bacillus* form the biofilm in around the roots and prevents the pathogen to colonize on plant's roots (Bais *et al.*, 2004). Despite having a direct impact against pathogens, these play a pivotal role to trigger and enhance systemic acquired resistance against plant pathogens (Meena *et al.*, 2020).

From the present study, all the PGPRs significantly enhance the plant growth physiological parameters. *Pseudomonas* and *Bacillus* produce auxins, particularly Indole Acetic Acid (IAA), which directly influences cell elongation and division in plant shoots. Higher auxin levels lead to enhanced shoot growth and elongation. Patani *et al.* (2023) applied *B. firmus* (NCT1), *B. cereus* (LAT3), *B. pumilus* (NCT4), *B. licheniformis* (LCT4) and *B. safensis* (LBM4) and found significant increase in tomato plant's physiological parameters like seedling growth and plant height. Furthermore, it also plays a significant role in stimulating root elongation, lateral root formation, and root hair development (Wang *et al.*, 2020), thus ultimately increase the root fresh weight of the plants (Zafar *et al.*, 2012). Suansia and Senapati (2023) treated tomato seeds with *P. aeruginosa* strain OD13 and found a significant increase in root length, shoot length, fresh weight and dry weight of tomato plants

Bacillus spp. produce cytokinin (Sansinenea, 2019) that promotes cell division and shoot formation and delays leaf senescence which contributes to prolonged shoot growth and root tissues leading to enhanced root fresh weight and biomass (Walia *et al.*, 2014). Sitlaothaworn *et al.* (2023) evaluated fifty endophytic bacteria and found promising results of *P. putida* LSG1 for significant increased root length.

Moreover, PGPRs ameliorate the root's architecture which promote lateral root formation and increase root hair density, this increase the root fresh weight and root surface area (Ehsan *et al.*, 2023; Gamalero *et al.*, 2004; Ijaz *et al.*, 2023; Iqbal *et al.*, 2023).

By the pathogen attack, the oxidative stress in the plant increase tremendously due to increase in superoxide radicals (Larson, 1995). A significant increase in Superoxide dismutase (SOD), Peroxidase (POD) and catalase (CAT) was seen by the PGPRs used in this study when compared to the control treatment. Superoxide dismutase (SOD) converts superoxide to O₂ and hydrogen peroxide (H₂O₂) (Jatoi *et al.*, 2016; Jatoi *et al.*, 2019; Khan *et al.*, 2024; R Buettner, 2011). This is crucial for mitigating the oxidative stress which is caused usually by pathogens attack or drought conditions. Hence, the more the SOD will produce, the more the plant will healthy. From the present study, we found inverse relationship between the disease incidence and the SOD production. *P. aeruginosa* applied plant were most prominent in SOD production and minimum in disease incidence as compared to other PGPRs applied. While, *D. acidovorans* applied plant leaves were less in SOD and maximum disease incidence was observed. PGPRs are novel in enhancement of Peroxidase (POD) enzyme synthesis in plants (Iqbal *et al.*, 2022; Khan *et al.*, 2024; Khatri *et al.*, 2024; Naqqash *et al.*, 2020; Naveed *et al.*, 2022).

POD detoxifies the plants by catalyzing the H₂O₂ and other organic peroxides using electron donors driven from phenolic compounds (De Gara, 2004). The peroxidase (POD) plays important role in plant defense (Almagro *et al.*, 2009; Rahim *et al.*, 2024; Saeed *et al.*, 2021). Despite the catalyzing the H₂O₂ and other organic oxides, POD contributes in the synthesis of lignin which strengthen the cell wall (Quiroga *et al.*, 2000) and increase the resistance against the pathogen's invasion. Furthermore, it plays a significant role in regulation of ROS signaling pathways which are essential for plant responses against biotic (Almagro *et al.*, 2009) and abiotic stress. So, the more POD will produce, more the plant will have the ability of resistance against the pathogen. Catalase enzyme reduces the H₂O₂ into water (H₂O) and oxygen (O₂), prevents the cell components from the oxidative damage. PGPRs boost the CAT production through the stimulation of phytohormones, and inducing the systemic resistance. The same happens in our experimental results, we recorded minimum disease incidence from those tomato leaves where CAT was measured maximum.

In the present study, all the PGPRs significantly enhance the P, K and Fe in tomato plant leaves when compared to control. PGPRs vitally increase nutrient solubilization and uptakes in plants (Hasan *et al.*, 2024; Khatoon *et al.*, 2020). The mineralization process involves several biochemical and physiological mechanisms to convert the insoluble minerals to soluble that a plant can readily absorb (Etesami & Adl,

2020). PGPRs produce gluconic acid, oxalic acid, and lactic acid. These acids lower the pH of soil microenvironment and chelate ions of minerals and convert them into soluble form (Olaniyan *et al.*, 2022; Sharma *et al.*, 2017). For instance, PGPRs produced organic acids convert insoluble tri-calcium phosphate to plant-soluble monobasic and dibasic phosphate (El-Sayed & Hagab, 2020; Kaur *et al.*, 2016). Furthermore, The *Bacillus* and *Pseudomonas* increase P contents in the plant by producing Phosphatases (Kumar *et al.*, 2012; Ramani, 2014) and phytase enzymes (El-Toukhy *et al.*, 2013; Kim *et al.*, 2002). Phosphatases hydrolyze the organic phosphate compounds into inorganic and make them available to plants (Tazisong *et al.*, 2015). The phytase enzymes break down phytate and release inorganic phosphate (Hayes *et al.*, 2000).

The Iron (Fe) solubilization is enhanced by the siderophores (Jin *et al.*, 2010), organic acids (Shen *et al.*, 2002), and other biochemical mechanisms. PGPRs produce siderophores, a high affinity chelating compounds bind to Fe^{+3} ions in soil and form soluble siderophore - Fe^{+3} complexes that a plant can absorb (Kloepper *et al.*, 1980). *Pseudomonas* spp commonly produce Pyoverdine and pyochelin siderophores (Braud *et al.*, 2010) and bacillibactin is produced by *Bacillus* spp. (Miethke *et al.*, 2006). Moreover, the PGPRs secrete oxalic acid, citric acid and gluconic acid which acidify the soil microenvironment and increase the iron and zinc compounds solubility to plants. *Bacillus* enhance the Fe availability by extruding the proton (H^{+}) into the rhizosphere and dissolve iron oxides and hydroxides (Singh *et al.*, 2020). The PGPRs increase K contents in plants in a variety of ways (Singh *et al.*, 2020). The PGPRs like *Pseudomonas* and *Bacillus* secrete tartaric acid, malic acid and gluconic acid which chelate the potassium-associated calcium and magnesium and release soluble K^{+} ions in soil (Olaniyan *et al.*, 2022). Furthermore, remarkable mechanism of PGPRs biofilm formation on root surface creates a conducive environment to solubilize potassium (K) (Gupta *et al.*, 2017). Several enzymes have been isolated for PGPRs especially from *Pseudomonas* and *Bacillus* which break down organic matter or mineral structures into plant soluble K ions (Etesami & Adl, 2020). By providing the plants with the inorganic form of elements PGPR maintains the fertility of the soil and is an important aspect of sustainable agriculture (Goswami *et al.*, 2016). The present study was conducted under controlled greenhouse conditions with limited replication, which may restrict the direct extrapolation of results to field environments. Further multi-location field trials and long-term validation studies are required to confirm the consistency and practical applicability of the findings. The study did not include PGPR-only treatments without pathogen inoculation, which could further differentiate direct plant growth promotion from disease suppression effects. Future studies should incorporate such controls for clearer functional interpretation.

CONCLUSIONS

The study confirmed that indigenous plant growth promoting rhizobacteria containing hydrolytic enzymes have the potential for the management of bacterial wilt diseases in tomato plants and act as biocontrol agents. Besides these promising isolates possess desirable plant growth promoting traits leading to increase in tomato shoot length, shoot diameter, fruits per plant under field condition. It is also obvious that strain *P. aeruginosa* (OR741775.1) proved to have more promising effect in controlling bacterial wilt disease in tomato plant as compared to other strains. Due to its high nutrient solubilization it increased P, K and Fe contents in plants and boost their antioxidant enzymes systems to combat with the disease. The overall result of this study proved that "*P. aeruginosa*" can be used for plant growth promotion and as promising biocontrol for management of bacterial wilt disease of tomato under field conditions. Further biosafety evaluation and multi-location field trials are required before commercialization of these strains.

AUTHORS CONTRIBUTIONS

SE. Writing Conceptualization. ME UH. original draft, Writing review & editing, Data curation, Formal analysis. MK. Greenhouse experiment management, Supervision, Validation, Visualization. MUS. review & editing. MFA. Field Resources, Software. ZM. & MU. Writing review & editing

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGEMENTS

This project was supported by administration team of Ayub Agricultural research Institute, Faisalabad.

REFERENCES

- Ahl, P., Voisard, C., & Défago, G. (1986). Iron bound-siderophores, cyanic acid, and antibiotics involved in suppression of *Thielaviopsis basicola* by a *Pseudomonas fluorescens* strain. *Journal of Phytopathology*, **116**(2), 121-134. <https://doi.org/https://doi.org/10.1111/j.1439-0434.1986.tb00903.x>
- Almagro, L., Gómez Ros, L., Belchi-Navarro, S., Bru, R., Ros Barceló, A., & Pedreño, M. (2009). Class III peroxidases in plant defence reactions. *Journal of Experimental Botany*, **60**(2), 377-390. <https://doi.org/https://doi.org/10.1093/jxb/ern277>
- Bais, H. P., Fall, R., & Vivanco, J. M. (2004). Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiology*, **134**(1), 307-319. <https://doi.org/https://doi.org/10.1104/pp.103.028712>
- Bell, S. M. (1975). The CDS disc method of antibiotic sensitivity testing (calibrated dichotomous sensitivity test). *Pathology*, **7**(sup4), 1-48. <https://doi.org/https://doi.org/10.3109/00313027509082602>

- Beneduzi, A., Ambrosini, A., & Passaglia, L. M. (2012). Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology*, **35**, 1044-1051. <https://doi.org/https://doi.org/10.1590/s1415-47572012000600020>
- Braud, A., Geoffroy, V., Hoegy, F., Mislin, G. L., & Schalk, I. J. (2010). Presence of the siderophores pyoverdine and pyochelin in the extracellular medium reduces toxic metal accumulation in *Pseudomonas aeruginosa* and increases bacterial metal tolerance. *Environmental Microbiology Reports*, **2**(3), 419-425.
- Chaiham, M., Chunhaleuchanon, S., & Lumyong, S. (2009). Screening siderophore producing bacteria as potential biological control agent for fungal rice pathogens in Thailand. *World Journal of Microbiology and Biotechnology*, **25**, 1919-1928. <https://doi.org/https://link.springer.com/article/10.1007/s11274-009-0090-7>
- Chance, B., & Maehly, A. (1955). [136] Assay of catalases and peroxidases.
- Chapman, H., & Pratt, P. (1961). Methods of analysis for soils, plants and waters. Division of Agricultural Sciences. *University of California, Berkeley*, 169-176.
- Coutinho, T. (2005). Introduction and prospectus on the survival of *R. solanacearum*. *Bacterial wilt disease and the Ralstonia solanacearum species complex*, 29-38.
- Daniel, A. I., Fadaka, A. O., Gokul, A., Bakare, O. O., Aina, O., Fisher, S.,...Klein, A. (2022). Biofertilizer: the future of food security and food safety. *Microorganisms*, **10**(6), 1220. <https://doi.org/https://doi.org/10.3390/microorganisms10061220>
- De Gara, L. (2004). Class III peroxidases and ascorbate metabolism in plants. *Phytochemistry Reviews*, **3**(1), 195-205.
- Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., & Fira, D. (2022). Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms-A review. *Physiological and Molecular Plant Pathology*, **117**, 101754. <https://doi.org/https://doi.org/10.1016/j.pmpp.2021.101754>
- Dinesh, R., Anandaraj, M., Kumar, A., Bini, Y. K., Subila, K. P., & Aravind, R. (2015). Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. *Microbiological Research*, **173**, 34-43. <https://doi.org/https://doi.org/10.1016/j.micres.2015.01.014>
- Ehsan, S., Qureshi, A., Chaudhary, N., Ali, A., Niaz, A., Javed, H.,...Anwar, S. A. (2023). Effect of fluorescent-producing rhizobacteria on cereal growth through siderophore exertion. *Journal of Applied Research in Plant Sciences*, **4**(02), 601-611.
- El-Sayed, S. Y., & Hagab, R. H. (2020). Effect of organic acids and plant growth promoting rhizobacteria (PGPR) on biochemical content and productivity of wheat under saline soil conditions. *Middle East J. Agric. Res*, **9**(2), 227-242.
- El-Toukhy, N. M., Youssef, A. S., & Mikhail, M. G. (2013). Isolation, purification and characterization of phytase from *Bacillus subtilis* MJA. *African Journal of Biotechnology*, **12**(20).
- Etesami, H., & Adl, S. M. (2020). Plant growth-promoting rhizobacteria (PGPR) and their action mechanisms in availability of nutrients to plants. *Phyto-Microbiome in Stress Regulation*, 147-203.
- Flury, P., Vesga, P., Péchy-Tarr, M., Aellen, N., Dennert, F., Hofer, N.,...Ma, Z. (2017). Antimicrobial and insecticidal: cyclic lipopeptides and hydrogen cyanide produced by plant-beneficial *Pseudomonas* strains CHA0, CMR12a, and PCL1391 contribute to insect killing. *Frontiers in Microbiology*, **8**, 100. <https://doi.org/https://doi.org/10.3389/fmicb.2017.00100>
- Gamalero, E., Trotta, A., Massa, N., Copetta, A., Martinotti, M. G., & Berta, G. (2004). Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. *Mycorrhiza*, **14**, 185-192. <https://doi.org/https://doi.org/10.1007/s00572-003-0256-3>
- Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, **59**(2), 309-314. <https://doi.org/https://doi.org/10.1104/pp.59.2.309>
- Gomiero, T. (2019). *Soil and crop management to save food and enhance food security*. <https://doi.org/https://doi.org/10.1016/B978-0-12-815357-4.00002-X>
- Gupta, G., Snehi, S. K., & Singh, V. (2017). Role of PGPR in biofilm formations and its importance in plant health. <https://doi.org/https://doi.org/10.1002/9781119246329.ch2>
- Gupta, S., Kaushal, R., & Sood, G. (2018). Impact of plant growth-promoting rhizobacteria on vegetable crop production. *International Journal of Vegetable Science*, **24**(3), 289-300. <https://doi.org/https://doi.org/10.3923/ijar.2010.954.983>
- Hasan, A., Tabassum, B., Hashim, M., & Khan, N. (2024). Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: A review. *Bacteria*, **3**(2), 59-75. <https://doi.org/https://doi.org/10.3390/bacteria3020005>
- Hayes, J., Richardson, A., & Simpson, R. (2000). Components of organic phosphorus in soil extracts that are hydrolysed by phytase and acid phosphatase. *Biology and Fertility of Soils*, **32**, 279-286. <https://doi.org/https://doi.org/10.1007/s003740000249>
- Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., & Xiong, L. (2006). Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proceedings of the National Academy of Sciences*, **103**(35), 12987-12992. <https://doi.org/https://doi.org/10.1073/pnas.0604882103>
- Ijaz, F., Ijaz, M. F., Javed, H., Amin, H. A., Zafar, H., Hamza, A.,...Alvi, A. (2023). Co-inoculation of Bradyrhizobium and phosphate solubilizing microbes on growth promotion of groundnut under rain-fed conditions. *Journal of Applied Research in Plant Sciences*, **4**(01), 348-355.
- Iqbal, M., Naveed, M., Sanaullah, M., Brtnicky, M., Hussain, M. I., Kucerik, J.,...Mustafa, A. (2023). Plant microbe mediated enhancement in growth and yield of canola (*Brassica napus* L.) plant through auxin production and increased nutrient acquisition. *Journal of Soils and Sediments*, **23**(3), 1233-1249.
- Iqbal, Z., Bushra, Hussain, A., Dar, A., Ahmad, M., Wang, X.,...Mustafa, A. (2022). Combined use of novel endophytic and rhizobacterial strains upregulates

- antioxidant enzyme systems and mineral accumulation in wheat. *Agronomy*, **12**(3), 551. <https://doi.org/https://doi.org/10.3390/agronomy12030551>
- Jatoi, G. H., Abro, M. A., Tariq, J. A., Memon, S., Mangi, N., Gadhi, M. A.,...Qiu, D. (2016). Screening of potential bacterial bio-control agents against the Helminthosporium oryzae in-vitro conditions. Caused by brown spot of rice. *Pakistan Journal of Biotechnology*, **13**(2), 95-100.
- Jatoi, G. H., Keerio, A. U., Abdulle, Y. A., & Qiu, D. (2019). Effect of selected fungicides and Bio-Pesticides on the mycelial colony growth of the Helminthosporium oryzae. brown spot of rice. *Acta Ecologica Sinica*, **39**(6), 456-460. <https://doi.org/https://doi.org/10.1016/j.chnaes.2018.09.018>
- Jin, C. W., Li, G. X., Yu, X. H., & Zheng, S. J. (2010). Plant Fe status affects the composition of siderophore-secreting microbes in the rhizosphere. *Annals of Botany*, **105**(5), 835-841. <https://doi.org/https://doi.org/10.1093/aob/mcq071>
- Kaur, C., Selvakumar, G., & Ganeshamurthy, A. (2016). *Organic acids in the rhizosphere: their role in phosphate dissolution*.
- Kelman, A. (1954). The relationship of pathogenicity of Pseudomonas solanacearum to colony appearance in a tetrazolium medium. *Phytopathology*, **44**(12), 693-695.
- Kenawy, A., Dailin, D. J., Abo-Zaid, G. A., Malek, R. A., Ambehatabi, K. K., Zakaria, K. H. N.,...El Enshasy, H. A. (2019). Biosynthesis of antibiotics by PGPR and their roles in biocontrol of plant diseases. *Plant Growth Promoting Rhizobacteria for Sustainable Stress Management: Volume 2: Rhizobacteria in Biotic Stress Management*, 1-35.
- Khan, J., Malik, N., & Hameed, S. (2024). Ecological Diversity of Lycopersicon esculentum (Tomato) Root Associated Plant Growth Promoting Rhizobacteria (PGPRs). *Journal of Applied Research in Plant Sciences*, **5**(02), 205-312.
- Khatoun, Z., Huang, S., Rafique, M., Fakhar, A., Kamran, M. A., & Santoyo, G. (2020). Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. *Journal of Environmental Management*, **273**, 111118. <https://doi.org/https://doi.org/10.1016/j.jenvman.2020.111118>
- Khatri, F. A., Solangi, I. B., Memon, G. Z., Khattak, M. I., Bhatti, M. A., Keerio, F. A., & Farooque, M. (2024). Antibacterial Application of Copper Nanoparticles Biosynthesized by Water Caltrop Pod. *Journal of Applied Research in Plant Sciences*, **5**(02), 288-296.
- Kim, Y.-H., Gwon, M.-N., Yang, S.-Y., Park, T.-K., Kim, C.-G., Kim, C.-W., & Song, M.-D. (2002). Isolation of phytase-producing Pseudomonas sp. and optimization of its phytase production. *Journal of Microbiology and Biotechnology*, **12**(2), 279-285.
- Kloepper, J. W., Leong, J., Teintze, M., & Schroth, M. N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, **286**(5776), 885-886.
- Kloepper, J. W., Ryu, C.-M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. *Phytopathology*, **94**(11), 1259-1266. <https://doi.org/https://doi.org/10.1094/PHYTO.2004.94.11.1259>
- Kotasthane, A. S., Agrawal, T., Zaidi, N. W., & Singh, U. (2017). Identification of siderophore producing and cynogenic fluorescent Pseudomonas and a simple confrontation assay to identify potential bio-control agent for collar rot of chickpea. *3 Biotech*, **7**(2), 137. <https://doi.org/https://doi.org/10.1007/s13205-017-0761-2>
- Koul, B., Singh, S., Dhanjal, D. S., & Singh, J. (2019). *Plant growth-promoting rhizobacteria (PGPRs): a fruitful resource*.
- Kumar, P., Dubey, R., & Maheshwari, D. (2012). Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiological Research*, **167**(8), 493-499. <https://doi.org/https://doi.org/10.1016/j.micres.2012.05.002>
- Larson, R. A. (1995). Plant defenses against oxidative stress. *Archives of insect biochemistry and Physiology*, **29**(2), 175-186. <https://doi.org/https://doi.org/10.3389/fpls.2020.552969>
- Laville, J., Blumer, C., Von Schroetter, C., Gaia, V., Défago, G. v., Keel, C., & Haas, D. (1998). Characterization of the hcnABC gene cluster encoding hydrogen cyanide synthase and anaerobic regulation by ANR in the strictly aerobic biocontrol agent Pseudomonas fluorescens CHA0. *Journal of Bacteriology*, **180**(12), 3187-3196. <https://doi.org/https://doi.org/10.1128/JB.180.12.3187-3196.1998>
- Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual review of microbiology*, **63**, 541-556.
- Meena, M., Swapnil, P., Divyanshu, K., Kumar, S., Harish, Tripathi, Y. N.,...Upadhyay, R. S. (2020). PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives. *Journal of Basic Microbiology*, **60**(10), 828-861. <https://doi.org/https://doi.org/10.1002/jobm.202000370>
- Mekonnen, H., & Kibret, M. (2021). The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia. *Chemical and Biological Technologies in Agriculture*, **8**(1), 1-11.
- Mekonnen, H., Kibret, M., & Assefa, F. (2022). Plant growth promoting rhizobacteria for biocontrol of tomato bacterial wilt caused by Ralstonia solanacearum. *International Journal of Agronomy*, **2022**(1), 1489637. <https://doi.org/https://doi.org/10.1155/2022/1489637>
- Messiha, N., Van Bruggen, A., Franz, E., Janse, J., Schoeman-Weerdesteijn, M., Termorshuizen, A., & Van Diepeningen, A. (2009). Effects of soil type, management type and soil amendments on the survival of the potato brown rot bacterium Ralstonia solanacearum. *Applied Soil Ecology*, **43**(2-3), 206-215. <https://doi.org/https://doi.org/10.1016/j.apsoil.2009.07.008>
- Michavila, G., Adler, C., De Gregorio, P. R., Lami, M. J., Caram Di Santo, M. C., Zenoff, A. M.,...Vincent, P. A. (2017). Pseudomonas protegens CS 1 from the lemon phyllosphere as a candidate for citrus canker biocontrol agent. *Plant Biology*, **19**(4), 608-617. <https://doi.org/https://doi.org/10.1111/plb.12556>
- Miethke, M., Klotz, O., Linne, U., May, J. J., Beckering, C. L., & Marahiel, M. A. (2006). Ferri-bacillibactin uptake and hydrolysis in Bacillus subtilis. *Molecular Microbiology*, **61**(6), 1413-1427.

- <https://doi.org/https://doi.org/10.1111/j.1365-2958.2006.05321.x>
- Milagres, A. M., Machuca, A., & Napoleao, D. (1999). Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. *Journal of Microbiological Methods*, **37**(1), 1-6. [https://doi.org/https://doi.org/10.1016/s0167-7012\(99\)00028-7](https://doi.org/https://doi.org/10.1016/s0167-7012(99)00028-7)
- Naqqash, T., Imran, A., Hameed, S., Shahid, M., Majeed, A., Iqbal, J.,...Malik, K. A. (2020). First report of diazotrophic *Brevundimonas* spp. as growth enhancer and root colonizer of potato. *Scientific Reports*, **10**(1), 12893. <https://doi.org/https://doi.org/10.1038/s41598-020-11701-8>
- Naveed, M., Hafeez, S., Rafique, M., Mumtaz, M. Z., Subhani, Z., Holatko, J.,...Kintl, A. (2022). Plant-endophyte mediated improvement in physiological and bio-protective abilities of marigold (*Tagetes patula*). *Frontiers in Plant Science*, **13**, 993130. <https://doi.org/https://doi.org/10.3389/fpls.2022.993130>
- Olaniyan, F. T., Alori, E. T., Adekiya, A. O., Ayorinde, B. B., Daramola, F. Y., Osemwegie, O. O., & Babalola, O. O. (2022). The use of soil microbial potassium solubilizers in potassium nutrient availability in soil and its dynamics. *Annals of Microbiology*, **72**(1), 45. <https://doi.org/10.1186/s13213-022-01701-8>
- Olusanya, E. T. (2020). The advent molecular techniques in the identification of rhizobacteria.
- Parthasarathy, V. (2008). *Organic spices*. New India Publishing.
- Patani, A., Prajapati, D., Ali, D., Kalasariya, H., Yadav, V. K., Tank, J.,...Patel, A. (2023). Evaluation of the growth-inducing efficacy of various *Bacillus* species on the salt-stressed tomato (*Lycopersicon esculentum* Mill.). *Front Plant Sci*, **14**, 1168155. <https://doi.org/10.3389/fpls.2023.1168155>
- Pieterse, C. M., van Pelt, J. A., Verhagen, B. W., Ton, J., van Wees, S. C., Léon-Kloosterziel, K. M., & Van Loon, L. (2003). Induced systemic resistance by plant growth-promoting rhizobacteria. *Symbiosis*, **35**, 39-54.
- Pikovskaya, R. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, **287**, 77-84.
- Podile, A., & Kishore, G. (2006). Plant growth-promoting rhizobacteria. *Plant-associated bacteria*, 195-230.
- Popoola, A., Ganiyu, S., Enikuomehin, O., Bodunde, J., Adedibu, O., Durosomo, H., & Karunwi, O. (2015). Isolation and characterization of *Ralstonia solanacearum* causing bacterial wilt of tomato in Nigeria. *Nigerian Journal of Biotechnology*, **29**, 1-10.
- Pradhanang, P., & Momol, M. (2001). Survival of *Ralstonia solanacearum* in soil under irrigated rice culture and aquatic weeds. *Journal of Phytopathology*, **149**(11-12), 707-711. <https://doi.org/https://doi.org/10.1046/j.1439-0434.2001.00700.x>
- Prasad, R., Kumar, M., & Varma, A. (2015). Role of PGPR in soil fertility and plant health. *Plant-growth-Promoting Rhizobacteria (PGPR) and Medicinal plants*, 247-260.
- Priya, P., Aneesh, B., & Harikrishnan, K. (2021). Genomics as a potential tool to unravel the rhizosphere microbiome interactions on plant health. *Journal of Microbiological Methods*, **185**, 106215. <https://doi.org/https://doi.org/10.1016/j.mimet.2021.106215>
- Quiroga, M., Guerrero, C., Botella, M. A., Barceló, A., Amaya, I., Medina, M. I.,...Valpuesta, V. (2000). A tomato peroxidase involved in the synthesis of lignin and suberin. *Plant Physiology*, **122**(4), 1119-1128. <https://doi.org/https://doi.org/10.1104/pp.122.4.1119>
- R Buettner, G. (2011). Superoxide dismutase in redox biology: the roles of superoxide and hydrogen peroxide. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, **11**(4), 341-346.
- Rafique, M., Hayat, K., Mukhtar, T., Khan, A., Afridi, M., Hussain, T.,...Chaudhary, H. (2015). Bacterial biofilm formation and its role against agricultural pathogens. *The battle against microbial pathogens: basic science, technological advances and educational programs. Formatex Research Centre, Spain*, 373-382.
- Rahim, N., Yasin, Z., Tahir, M. M., Majeed, A., Yaqub, A., & Mahmood, B. (2024). Rock Phosphate and Manures with Phosphorus Solubilizing Bacteria Increases the Growth, Yield and Phosphorus Uptake of Okra (*Abelmoschus esculentus*). *Journal of Applied Research in Plant Sciences*, **5**(01), 111-118.
- Rai, S., Omar, A. F., Rehan, M., Al-Turki, A., Sagar, A., Ilyas, N.,...Hasanuzzaman, M. (2023). Crop microbiome: their role and advances in molecular and omic techniques for the sustenance of agriculture. *Planta*, **257**(2), 27. <https://doi.org/https://doi.org/10.1007/s00425-022-04052-5>
- Ramani, V. (2014). Organic phosphate mineralization by *Bacillus sphaericus* and *Pseudomonas cepacia*. *Journal of Agricultural Technology*, **10**(4), 915-922.
- Rawat, P., Shankhdhar, D., & Shankhdhar, S. (2020). Plant growth-promoting rhizobacteria: A booster for ameliorating soil health and agriculture production. *Soil Health*, 47-68. https://doi.org/https://doi.org/10.1007/978-3-030-44364-1_3
- Reetha, A. K., Pavani, S. L., & Mohan, S. (2014). Hydrogen cyanide production ability by bacterial antagonist and their antibiotics inhibition potential on *Macrophomina phaseolina* (Tassi.) Goid. *Int. J. Curr. Microbiol. Appl. Sci*, **3**(5), 172-178.
- Rehman, A., Farooq, M., Lee, D.-J., & Siddique, K. H. (2022). Sustainable agricultural practices for food security and ecosystem services. *Environmental Science and Pollution Research*, **29**(56), 84076-84095. <https://doi.org/https://doi.org/10.1007/s11356-022-23635-z>
- Saeed, Q., Xiukang, W., Haider, F. U., Kučerik, J., Mumtaz, M. Z., Holatko, J.,...Naveed, M. (2021). Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: a comprehensive review of effects and mechanisms. *International Journal of Molecular Sciences*, **22**(19), 10529. <https://doi.org/https://doi.org/10.3390/ijms221910529>
- Saharan, B., & Nehra, V. (2011). Plant growth promoting rhizobacteria: a critical review. *Life Sci Med Res*, **21**(1), 30.
- Sansinenea, E. (2019). *Bacillus* spp.: As plant growth-promoting bacteria. *Secondary metabolites of plant growth promoting rhizomicroorganisms: Discovery and applications*, 225-237.
- Sayed, R., Chincholkar, S., Reddy, M., Gangurde, N., & Patel, P. (2012). Siderophore producing PGPR for crop nutrition and phytopathogen suppression. In *Bacteria in agrobiology: disease management* (pp. 449-471).

- Springer. https://doi.org/https://doi.org/10.1007/978-3-642-33639-3_17
- Sharma, I. P., Chandra, S., Kumar, N., & Chandra, D. (2017). PGPR: heart of soil and their role in soil fertility. *Agriculturally Important Microbes for Sustainable Agriculture: Volume I: Plant-soil-microbe nexus*, 51-67.
- Shen, H., Yan, X., Zhao, M., Zheng, S., & Wang, X. (2002). Exudation of organic acids in common bean as related to mobilization of aluminum-and iron-bound phosphates. *Environmental and Experimental Botany*, **48**(1), 1-9. [https://doi.org/https://doi.org/10.1016/S0098-8472\(02\)00009-6](https://doi.org/https://doi.org/10.1016/S0098-8472(02)00009-6)
- Siddiqui, I. A., Shaikat, S. S., Sheikh, I. H., & Khan, A. (2006). Role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. *World Journal of Microbiology and Biotechnology*, **22**, 641-650. <https://doi.org/https://doi.org/10.1007/s11274-005-9084-2>
- Siddiqui, Z. A., & Sayeed Akhtar, M. (2009). Effects of antagonistic fungi, plant growth-promoting rhizobacteria, and arbuscular mycorrhizal fungi alone and in combination on the reproduction of *Meloidogyne incognita* and growth of tomato. *Journal of General Plant Pathology*, **75**, 144-153. <https://doi.org/https://doi.org/10.1007/s10327-009-0154-4>
- Singh, D., Prasanna, R., Sharma, V., Rajawat, M. V. S., Nishanth, S., & Saxena, A. K. (2020). Prospecting plant-microbe interactions for enhancing nutrient availability and grain biofortification. In *Wheat and barley grain biofortification* (pp. 203-228). Elsevier. <https://doi.org/https://doi.org/10.1016/B978-0-12-818444-8.00008-0>
- Singh, R. S. (2001). *Plant disease management*. Science Publishers New Hampshire.
- Sitlaothaworn, K., Budsabun, T., Dechkla, M., Yukphan, P., Tanasupawat, S., & Savarajara, A. (2023). Identification and plant growth-promoting activity of endophytic bacteria from sugarcane roots and genome analysis of *Gluconacetobacter* strain PS25. *ScienceAsia*, **49**(2).
- Soltanpour, P., Khan, A., & Lindsay, W. (1976). Factors affecting DTPA-extractable Zn, Fe, Mn, and Cu from soils. *Communications in Soil Science and Plant Analysis*, **7**(9), 797-821.
- Suansia, A., & Senapati, B.K. (2022). Association of some plant defense enzyme activities with systemic resistance to bacterial wilt disease induced in tomato plants by *Pseudomonas aeruginosa* strain OD13. *Chronobiology International*, **14**(4), 1342-1349.
- Suansia, A., & Senapati, A. K. (2023). Plant Growth-Promoting Activity of *Pseudomonas aeruginosa* OD13 in Tomato Plant. *International Journal of Environment and Climate Change*, **13**(1), 214-224.
- Sun, Y., Su, Y., Meng, Z., Zhang, J., Zheng, L., Miao, S.,...Xiong, L. (2023). Biocontrol of bacterial wilt disease in tomato using *Bacillus subtilis* strain R31. *Frontiers in Microbiology*, **14**, 1281381.
- Tazisong, I. A., Senwo, Z. N., & He, Z. (2015). Phosphatase hydrolysis of organic phosphorus compounds. *Advances in Enzyme Research*, **3**(02), 39.
- Usman, M., Mubeen, M., Nadeem, S. A., Shahbaz, M. U., Kamran, M., Ahmad, S., Abbas, W., Ehsan, S., Khalid, A., Ashraf, Z. U., & Haq, M. E. U. (2025). Exploring the potential of potato germplasm against *Streptomyces scabies* and its management through plant extracts, bactericides and plant growth promoting rhizobacteria (PGPRs) under lab and field condition. *Sarhad Journal of Agriculture*, **41**(4), 2033-2042. <https://dx.doi.org/10.17582/journal.sja/2025/41.4.2033.2042>
- Venkatakrishnan, K., von Moltke, L. L., Obach, R. S., & Greenblatt, D. J. (2003). Drug metabolism and drug interactions: application and clinical value of in vitro models. *Current Drug Metabolism*, **4**(5), 423-459. <https://doi.org/https://doi.org/10.2174/1389200033489361>
- Verma, J., Yadav, J., Tiwari, K., Lavakush, S., & Singh, V. (2010). Impact of plant growth promoting rhizobacteria on crop production. *International journal of agricultural research*, **5**(11), 954-983.
- Walia, A., Mehta, P., Chauhan, A., & Shirkot, C. (2014). Effect of *Bacillus subtilis* strain CKT1 as inoculum on growth of tomato seedlings under net house conditions. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences,
- Wang, X., Zhou, X., Cai, Z., Guo, L., Chen, X., Chen, X,...Wang, A. (2020). A Biocontrol Strain of *Pseudomonas aeruginosa* CQ-40 Promote Growth and Control *Botrytis cinerea* in Tomato. *Pathogens*, **10**(1). <https://doi.org/10.3390/pathogens10010022>
- Wang, Z., Luo, W., Cheng, S., Zhang, H., Zong, J., & Zhang, Z. (2023). *Ralstonia solanacearum*—a soil borne hidden enemy of plants: research development in management strategies, their action mechanism and challenges. *Frontiers in Plant Science*, **14**, 1141902. <https://doi.org/https://doi.org/10.3389/fpls.2023.1141902>
- Wolf, B. (1982). A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Communications in Soil Science and Plant Analysis*, **13**(12), 1035-1059.
- Xie, S., Zang, H., Wu, H., Uddin Rajer, F., & Gao, X. (2018). Antibacterial effects of volatiles produced by *Bacillus* strain D13 against *Xanthomonas oryzae* pv. *oryzae*. *Molecular Plant Pathology*, **19**(1), 49-58. <https://doi.org/https://doi.org/10.1111/mpp.12494>
- Yadessa, G., Van Bruggen, A., & Ocho, F. (2010). Effects of different soil amendments on bacterial wilt caused by *Ralstonia solanacearum* and on the yield of tomato. *Journal of Plant Pathology*, 439-450.
- Zafar, M., Abbasi, M. K., Khan, M. A., Khaliq, A., Sultan, T., & Aslam, M. (2012). Effect of Plant Growth-Promoting Rhizobacteria on Growth, Nodulation and Nutrient Accumulation of Lentil Under Controlled Conditions. *Pedosphere*, **22**(6), 848-859.

Publisher's note: JMS remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>