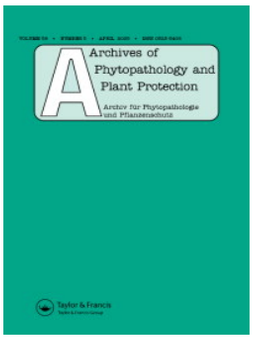

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RESEARCH ARTICLE

Biocontrol and growth promotion of groundnut by *Pseudomonas putida* GN1 against soilborne pathogens

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ABSTRACT

Groundnut root rot (*Macrophomina phaseolina*) and stem rot (*Sclerotium rolfsii*) are significant threats to yield in both rainfed and irrigated systems. This study aimed to isolate and evaluate plant growth-promoting rhizobacteria (PGPR) from groundnut soils in Tamil Nadu for their potential as biocontrol agents and growth enhancers. Fourteen PGPR strains were tested, with *Pseudomonas putida* GN1 (ON307464), *Burkholderia cepacia* KKM1 (OM908755) and *Pseudomonas* sp. K1 (ON408243) showing notable pathogen suppression. *P. putida* GN1 was the most effective, inhibiting *M. phaseolina* by 53.93% and *S. rolfsii* by 46.06%, while also boosting seed germination and seedling vigour. Additionally, *P. putida* GN1 improved drought tolerance by enhancing root growth characteristics. These results highlight *P. putida* GN1 as a promising biocontrol and plant growth-promoting agent for groundnut, particularly in drought-prone areas.

ARTICLE HISTORY
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KEYWORDS
Groundnut; root rot; stem rot; rhizoscanner; siderophore; *Pseudomonas putida* GN1

Introduction

India ranks first globally in groundnut area and production, contributing 40% of the area under cultivation and 33% of the total world production. Among oilseed crops, groundnut holds a prominent position in India and Tamil Nadu boasts the highest productivity at 1604 kg/ha, followed by Gujarat at 1190 kg/ha. Approximately 85% of India's groundnut is sown

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during the kharif season under rainfed conditions, with some cultivation during the rabi season in Tamil Nadu, Andhra Pradesh and Karnataka.

Groundnut crops encounter considerable challenges due to fungal and bacterial diseases, particularly soil-borne pathogens, which lead to substantial yield losses. These fungi are both seed and soil-borne, and their pathogenic propagules are pervasive in soils, rendering long-term chemical management ineffective. Biological control presents an environmentally safe and economically viable alternative. The utilisation of indigenous beneficial microbes with multifaceted traits offers a promising method for managing these diseases.

Ramesh and Korikanthimath (2010) demonstrated that seed treatment with talc-based formulations of bacterial antagonists can effectively combat *Macrophomina phaseolina* and promote groundnut growth under residual moisture conditions. Isolation, characterisation and field evaluation of plant growth-promoting rhizobacteria (PGPRs) with diverse beneficial traits are crucial (Sulthana et al. 2018). The root cap shows a crucial role in protecting the growing tip of crop plants, secreting mucilage to facilitate soil penetration and possibly aiding communication with soil microbiota. PGPRs, by colonising internal tissues, remain protected from harsh environments and require fewer nutrients by Pandey et al. (2019). These beneficial microbes improve crop growth under drought conditions through nitrogen fixation, phosphorus availability, siderophore accumulation and the synthesis of organic acids and plant growths inducing compounds, including ACC deaminase, glucanase and chitinase (Indiragandhi et al. 2008; Niu et al. 2017; Singh et al. 2019). Direct effects of Plant (Plant Growth-Promoting Bacteria (PGPB) on plant growth include the production and/or synthesis of phytohormones such as auxins, gibberellins, ethylene, cytokinins, and abscisic acid (Basu et al. 2021) potassium, zinc and phosphorus solubilisation and the creation of iron chelating compounds (siderophore) (Mir et al. 2021). Influence phytohormone signalling through volatile organic compounds (Wang et al. 2015). Given this background, this study aims to investigate whether rhizosphere bacterial strains exhibit antagonistic activity, PGPR activity and drought tolerance mechanisms.

Materials and methods

Isolation of PGPR from groundnut rhizosphere

Bacterial strains were meticulously isolated from rhizosphere soil samples gathered across various groundnut-growing districts in Tamil Nadu. The process began by carefully uprooting plants with intact root systems and adhering soil. Excess soil was removed gently, leaving a sufficient amount of rhizosphere soil attached to the roots. Ten grams of this soil was then

mixed with 100 mL of sterilised water in a 250 mL Erlenmeyer flask, followed by vigorous shaking to ensure thorough suspension. The resultant mixture was subjected to serial dilution to achieve appropriate concentrations. Specifically, 1 mL of the aliquot from the 10^{-5} and 10^{-6} dilutions was aseptically transferred onto sterilised Petri dishes containing nutrient agar medium. The plates were then gently swirled to ensure even distribution of the sample and incubated at 28 ± 2 °C for 24 h. Post-incubation, the bacterial broth was carefully observed under UV light at 366 nm. Colonies exhibiting morphologically typical of *Bacillus* sp. and *Pseudomonas* sp. were selected for further isolation. These colonies were subsequently purified by streaking onto Nutrient Agar and King's B (KB) medium to ensure the acquisition of pure cultures.

In vitro screening of PGPR against root rot and stem rot pathogens

In this study, fourteen isolates of PGPR cultures were meticulously screened for their antagonistic properties against *M. phaseolina* and *Sclerotium rolfsii*. Each isolate was lined in a straight, approximately 4 cm in length, on plates containing Potato Dextrose Agar medium, with the streak positioned 1 cm from the edge of the plate. To test the antagonistic interaction, a 9 mm mycelial disc of *M. phaseolina* and *S. rolfsii* was placed at the opposite end of the plate, furthest from the bacterial streak (Vidhyasekaran et al. 1997). The plates were incubated under controlled conditions at 27 ± 2 °C for four days, during which the interaction between the PGPR isolates and the fungal pathogens was closely monitored. Post-incubation, the mycelial growth of the pathogens and the resulting zones of inhibition were meticulously measured to assess the efficacy of each PGPR isolate in suppressing the fungal pathogens.

Extraction of crude antibiotics produced by antagonistic bacteria

Bacterial strains demonstrating significant antagonistic activity were further analyzed for the production of crude antibiotics. These strains were cultured in Pigment Production Medium (PPM) broth at room temperature for 5 days. Following incubation, the bacterial cultures were subjected to centrifugation at 5000 rpm to separate the bacterial cells. The supernatant was then acidified to pH 2.0 using concentrated HCl, facilitating the extraction process. An equal measurement of benzene was added to the acidified, and the combination was vigorously agitated to ensure thorough mixing. The benzene layer, containing the crude antibiotics, was then evaporated using a water bath to eliminate the solvent. The remaining residues were dissolved in 0.1 N NaOH and stored for further analysis of their antimicrobial properties.

Extraction of 2,4-Diacetylphloroglucinol

The mining of 2,4-Diacetylphloroglucinol (2,4-DAPG) from antagonistic bacterial cultures was carried out by culturing the bacteria in 100 mL of PPM broth for 4 days in room temperature in a shaker. After the incubation period, the cultures were centrifuged at 3500 rpm for 5 min to obtain a clear supernatant, as described by Rosales et al. (1995).

Effect of 2,4-DAPG on the growth of soil borne pathogens

The inhibitory effects of the extracted 2,4-DAPG on the mycelia growth of groundnut soil borne pathogens were evaluated using the poisoned food technique (Schmitz 1930). Two concentrations of 2,4-DAPG, 0.1% and 0.5%, were tested by incorporating the compound into the growth medium of the pathogens. The extent of mycelial growth inhibition was measured, providing insights into the potential application of 2,4-DAPG as a biocontrol agent against these soilborne pathogens, highlighting its role in reducing pathogen proliferation.

Siderophore production

The capacity of bacterial antagonists to produce siderophores was assessed using a plate growth assay method, as outlined by Louden et al. (2011). Bacterial isolates were streaked onto succinate medium added with Chrome Azurol S (CAS), Fe^{3+} and hexadecyltrimethyl ammonium bromide. The plates were nurtured at room temperature for 3 days. Siderophore formation was indicated by the bright yellowish fluorescent zone around the bacterial colonies against the dark blue background of the medium, signifying the chelation of iron by the siderophores, which is essential for pathogen suppression.

Quantification of siderophore formation

To measure siderophore formation, bacterial strains were grown in KB broth for 3 days, followed by centrifugation at 2000 rpm for 10 min to separate the cells from the supernatant. The supernatant was then adjusted to pH 2.0 and combined ethyl acetate extracts were air-dried and reconstituted in 5 mL of 50% ethanol. This solution was then mixed with Hathway's reagent, and the absorbance was measured at 700 nm as described by Reeves et al. (1983).

Hydrogen cyanide production

The production of hydrogen cyanide (HCN) by bacterial isolate was assessed using a modified protocol based on Ahmad et al. (2008). Bacterial culture was inoculated onto Tryptic Soy Agar plates, which

serve as a nutrient-rich medium supporting bacterial growth. To detect HCN production, 1.5 cm diameter filter paper discs soaked in a freshly prepared picric acid were carefully placed on the inside of the Petri dish lids. These dishes were then sealed with parafilm to prevent gas exchange and were protected for three days. The production of HCN was inferred from the color change of the picric acid-soaked filter paper discs, which transitioned from yellow to orange or red.

Indole acetic acid production

To quantify the ındole acetic acid (IAA), an essential plant hormone, bacterial strains were cultured in Tryptic Soy Broth added with 100 µg/mL of tryptophan, which serves as a forerunner for IAA synthesis. The cultures were grown on a rotary shaker for 30 h to ensure optimal bacterial growth and IAA production and then centrifuged for 10 min at 2000 rpm to get the supernatant, which contained the secreted IAA. From which 1 mL aliquot was mixed with 2 mL of Salkowski reagent, and the mixture was nursed at room temperature for 30 min leads to color development and the resulting solution was measured at 530 nm to determine IAA concentration (Mir et al. 2021).

Testing the PGPR activity of isolated bacterial strains

PGPR potential of rhizospher isolates was evaluated through the standard roll towel method, a widely accepted technique for assessing seed germination and seedling vigour. Bacterial cultures were initially grown in KB broth for 48 h to achieve optimal bacterial density. The cultures were centrifuged at 10,000 rpm for 5 min, and the bacterial pellet was resuspended in sterile distilled water for seed treatment. Groundnut seeds were surface-sterilised using a suitable disinfectant, dried with sterile blotting paper and then soaked in the bacterial suspension for 4 h to ensure thorough coating. The treated seeds were subsequently placed on wet blotters and incubated under controlled conditions. After 10 days, the germination percentage, root length, shoot length and vigour index of the seedlings were recorded to define the properties of the bacterial treatments on seedling development (Agrawal and Agrawal 2013).

Drought tolerant mechanism

To investigate the drought tolerance mechanism conferred by PGPR strains, a talc-based formulation of the bacteria was prepared and used for seed treatment. The treated seeds were sown in trays filled with sterilised soil to ensure a controlled environment free from external microbial influences. After 15 days of growth, the root characteristics of the

seedlings were analyzed using the BioVis PSM Root-Rhizoscanner software. This advanced imaging system provided detailed measurements of root architecture, including root length, surface area and branching patterns, allowing for a comprehensive assessment of root growth under drought stress conditions.

Molecular identification of PGPR

The bacterial isolates that exhibited notable antagonistic activity were further analyzed for their ability to produce siderophores, HCN, DAPG, phenazine and IAA, important characters associated with plant growth promotion and biocontrol. For molecular identification, genomic DNA was extracted from these isolates by the protocol demonstrated by Thiruvengadam et al. (2022). The 16S rRNA gene sequencing was performed and sequences were compared with those of known PGPR strains in the NCBI database, facilitating accurate identification of the bacterial isolates and confirmation of their phylogenetic relationships.

Statistical analysis

Data collected from the greenhouse experiments were exposed to rigorous statistical analysis using analysis of variance for Completely Randomized Design and Randomized Block Design. The critical differences among treatments were evaluated at a 5% significance level using the Least Significant Difference method, as implemented in the IRISAT statistical software package. This statistical approach ensured that the observed differences in plant growth parameters were statistically significant and could be reliably attributed to the effects of the bacterial treatments.

Results

Antagonistic activity of PGPR against soil-borne pathogens in vitro

Among the fourteen PGPR isolates tested for their antagonistic activity against the soil-borne pathogens *M. phaseolina* and *S. rolfsii* through dual culture assays, isolate *Pseudomonas putida* GN1 demonstrated the highest efficacy. This isolate not only suppressed the fungal growth of both pathogens but also showed a clear zone of inhibition, indicating its potent antagonistic capabilities. The effectiveness of *P. putida* GN1 could be accredited to its capability to produce a range of antimicrobial metabolites, including HCN, DAPG and siderophores, which are known to destroy the development of phytopathogens. These findings underscore

the importance of exploring and utilising native PGPR isolates like GN1 to enhance crop health and productivity under varying environmental conditions. This isolate exhibited a notable mycelial growth inhibition of 53.93% against *M. phaseolina* and 46.06% against *S. rolfsii*. Following GN1, isolates K1 and KKM1 demonstrated significant antagonistic activity with inhibition rates of 48.31% and 40.44%, respectively, in compared to the control (Figures 1 and 2).

Sclerotial production and germination

Isolate *P. putida* GN1 also significantly reduced the sclerotial production of *S. rolfsii*, yielding only 22.69 sclerotia per plate, whereas isolates K1 and KKM1 recorded 34.53 sclerotia per plate. In contrast, the control plates produced a substantial 248.01 sclerotia per plate (Figure 3). The germination of sclerotia was similarly suppressed by *P. putida* GN1, showing a germination rate of 34.65%, compared to 100% germination in the control. Isolate K1 showed a moderate inhibition with a germination rate of 42.57%.

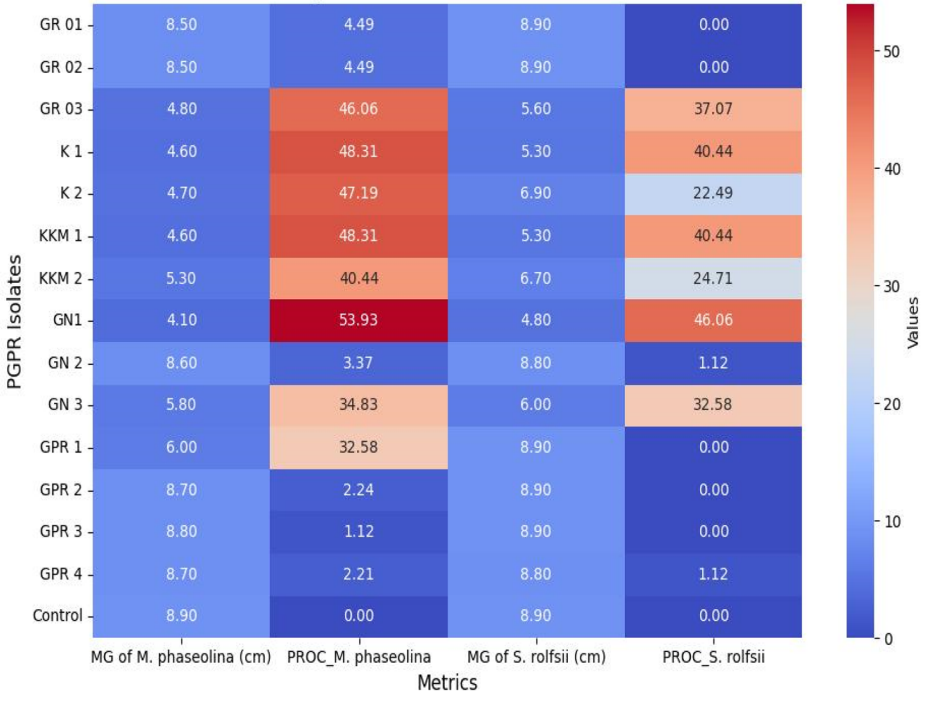

Figure 1. Effect of PGPR isolates on the growth of *macrophomina phaseolina* and *sclerotium rolfsii*. MG, mycelial growth; PROC, percent reduction over control.

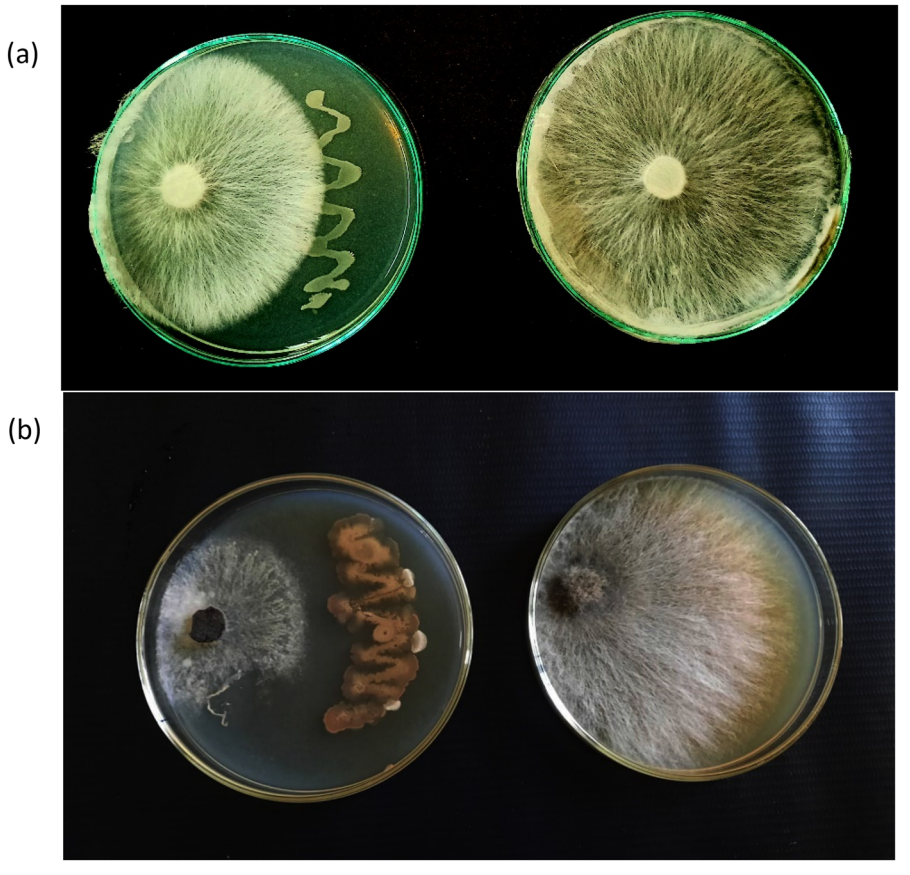

Figure 2. *In vitro* antagonistic activity of *Pseudomonas putida* GN1 against soilborne pathogens (a) *S. rolfsii* and (b) *M. Phaseolina.*

Siderophore production

Siderophore production was assessed by culturing the effective bacterial isolates, including *P. putida* GN1, *Pseudomonas* sp. K1 and *Burkholderia cepacia* KKM1, on CAS agar plates. All tested isolates, with the exception of *Bacillus subtilis*, produced yellow pigmentation in the blue medium, indicative of siderophore production. In contrast, *B. subtilis* produced a pink coloration, differentiating it from the siderophore-producing isolates (Table 1).

Quantification of siderophore production

P. putida GN1 produced the maximum amount of salicylate-type siderophore (17.62 µg/0.5 mL culture filtrate), followed by *Pseudomonas* sp. K1 (13.48 µg). *Klebsiella* produced the least (4.20 µg/0.5 mL) (Figure 4).

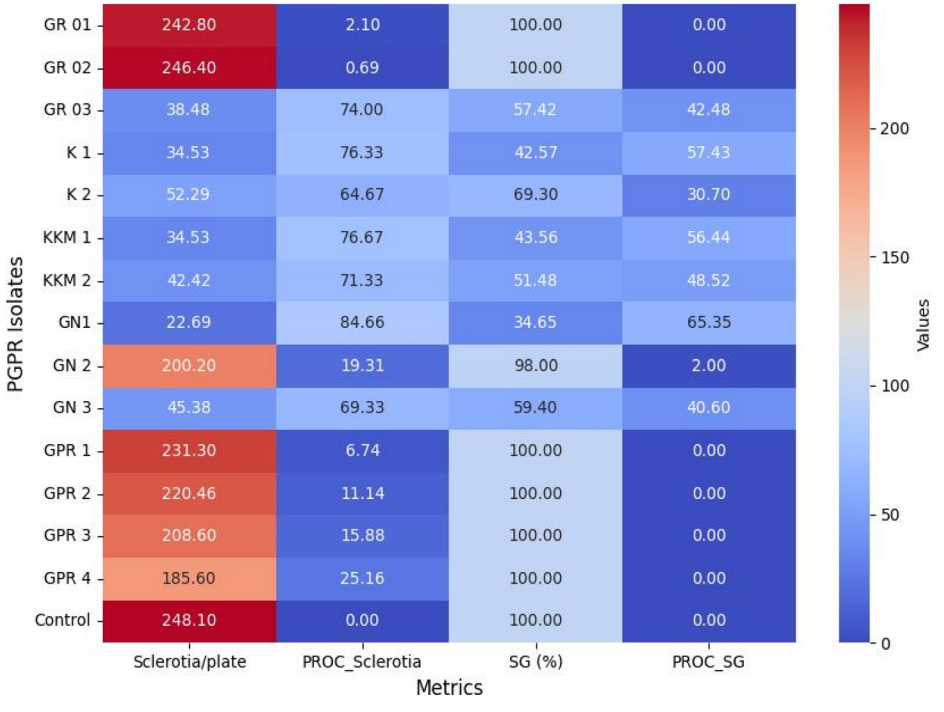

Figure 3. Effect of PGPR isolates on the number of sclerotia and sclerotial germination. MG, mycelial growth; PROC, percent reduction over control.

Table 1. Efficacy of bacterial antagonists in the production of siderophore and HCN.

S. No	PGPR strains	Siderophore production	HCN
1	*Pseudomonas putida* – GN1	+	+++
2	*Pseudomonas* spp. – K1	+	++
3	*Burholderia cepacia –KKM 1*	+	++
4	*Klebsiela*	+	+
5	PA 23	+	+++
6	*Bacillus subtilis* – TNAU	+	–

+ = Appearance of light red colour.
+++ = Appearance of deep red colour.
– = Appearance of pink colour.

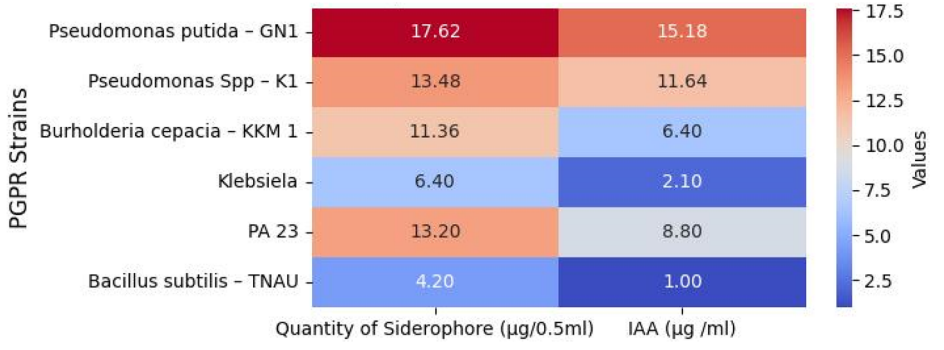

Figure 4. Efficacy of bacterial antagonists in the production of siderophore, HCN and IAA.

HCN production

P. putida GN1 and *Pseudomonas* sp. K1 produced strong HCN, while *B. subtilis* and *Klebsiella* tested negative for HCN production (Table 1).

IAA production

P. putida GN1 formed the supreme amount of IAA (15.18 µg/mL), followed by *Pseudomonas* sp. K1 (11.64 µg) and *B. cepacia* KKM1 (6.40 µg). *Klebsiella* produced the least IAA (1.0 µg) (Figure 4).

Effect of antibiotics from P. putida on mycelial growth of M. phaseolina and S. rolfsii

Crude antibiotics 2,4-DAPG from *P. putida* GN1 and *Pseudomonas* sp. K1 were tested at 0.1% and 0.5% concentrations. At 0.5% concentration, 2,4-DAPG from *P. putida* GN1 and *Pseudomonas* sp. K1 showed 68.71% and 60.89% inhibition of *S. rolfsii* mycelial growth, respectively. Against *M. phaseolina*, the same antibiotics showed 70.22% and 59.33% inhibition, respectively (Figure 5).

Plant growth promotion activity

P. putida GN1 considerably improved the vigour index of groundnut plantlets to 3945.67, compared to 3880.94 for TNAU- *B. subtilis* and 3063.6 for *Pseudomonas* sp. K1. GN1 also showed the highest germination rate (97.40%), root length (20.21 cm) and shoot length (20.30 cm), whereas non-treated seeds had a germination rate of 88.00%, shoot length of 13.67 cm, root length of 11.00 cm and vigour index of 2170.96 (Figure 6).

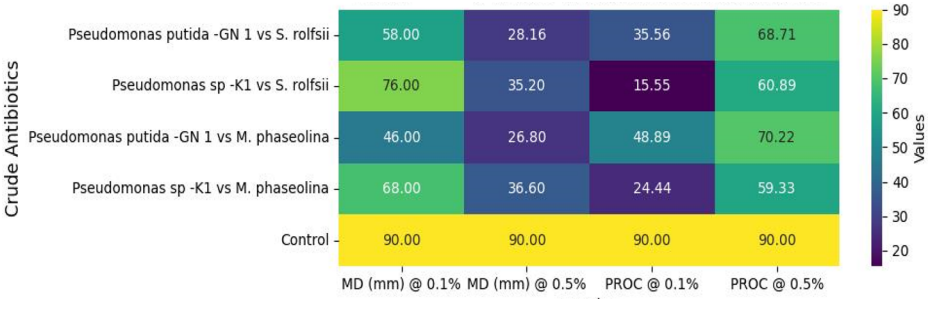

Figure 5. Effect of crude antibiotics of *P. putida* (GN 1) on *S. rolfsii and M. Phaseolina*. MD, mycelial diameter. PROC, percent reduction over control.

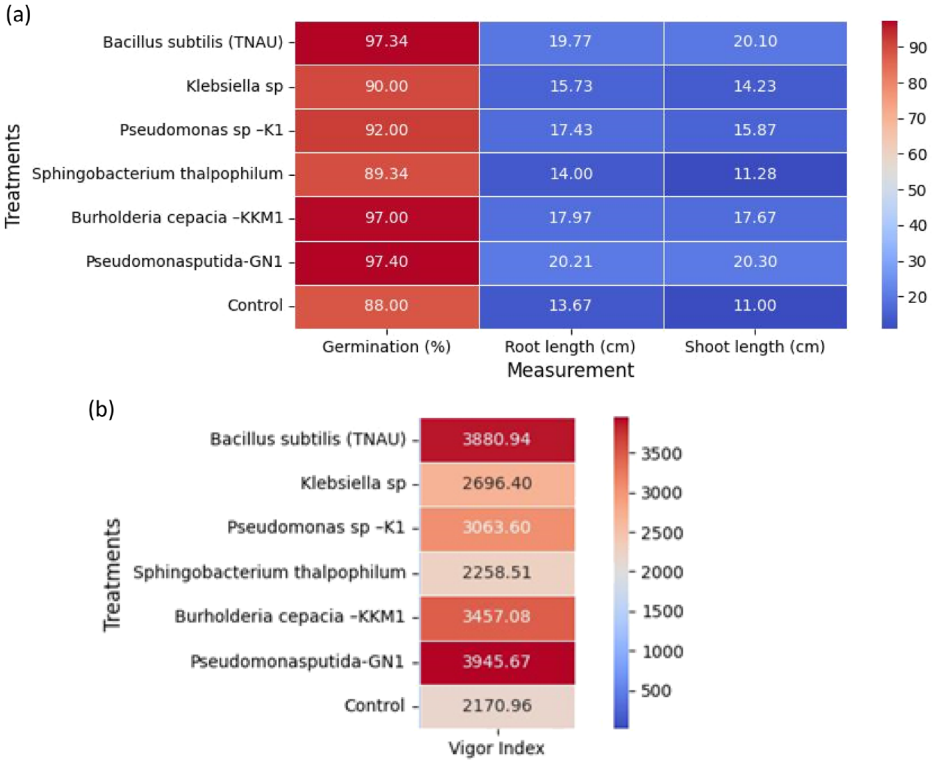

Figure 6. Effect of PGPR on plant growth promotion in groundnut on roll towel methods. (a) Growth attributes and (b) vigour index.

Drought tolerance studies

Application of *P. putida* GN1 (10 g/kg of seed) resulted in a total root length of 2906.13 mm, compared to 886.99 mm in the control. GN1-treated plants also had higher root tips (584), forks (501), root diameter (4.05 mm) and root volume (79470.02 mm^3), compared to other PGPR treatments and controls (Figures 7 and 8).

Molecular characterisation

Bacterial sequences were aligned using MEGA 7.0 software. Sequences were compared with gene bank data using BLAST. Accession numbers for 16S rDNA sequences are: GN1 (*P. putida* ON307464), KKM1 (*B. cepacia* OM908755) and K1 (*Pseudomonas* sp. ON408243). The phylogenetic relationships among the bacterial isolates were analyzed using the neighbor-joining method with MEGA 7.0 software, which facilitated the generation of a detailed phylogenetic tree, as illustrated in Figure 9. This tree was constructed from 16S rRNA gene sequences,

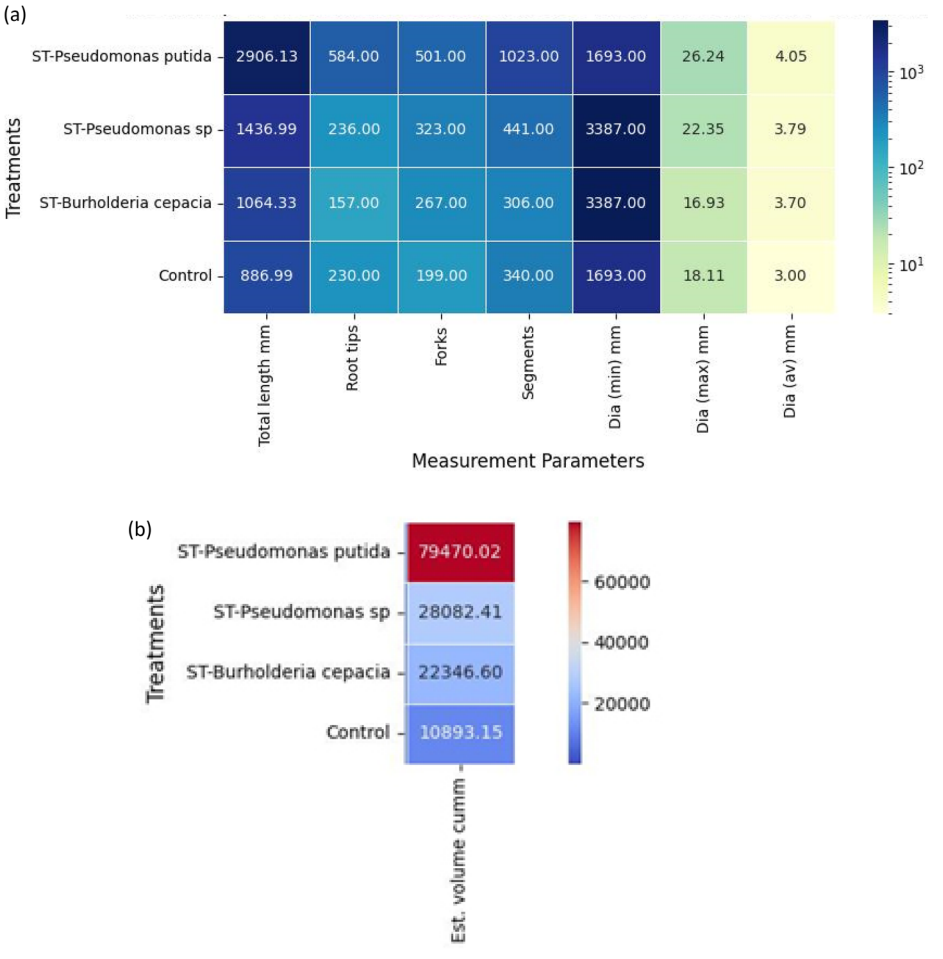

Figure 7. Effect of PGPR on root architectures using rhizoscaner. (a). Root anatomical characteristics and (b) root volume.

offering a comprehensive view of the evolutionary relationships and genetic similarities among the isolates. The use of the neighbor-joining method, known for its effectiveness in constructing phylogenetic trees with minimal computational complexity, allowed for an accurate representation of the genetic distance between the isolates. The resulting phylogenetic tree not only highlights the genetic diversity among the isolates but also underscores the potential evolutionary pathways that might have led to the development of specific traits beneficial for biocontrol and growth promotion in groundnut. This analysis provided a solid foundation for understanding the genetic basis of the antagonistic and growth-promoting activities exhibited by these bacterial strains, particularly *P. putida* GN1, in the context of soilborne pathogen management. By analyzing the branching patterns and genetic distances, this method facilitated the classification of the isolates and their

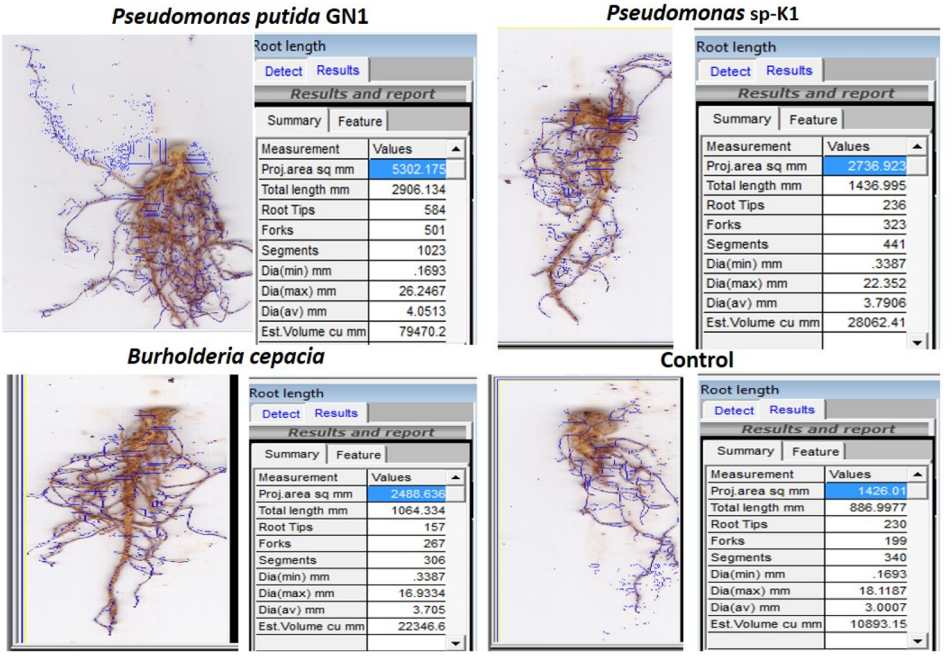

Figure 8. RhizoScanner analysis of PGPR-treated groundnut root architecture.

alignment with known bacterial species. This analysis is crucial for understanding the taxonomic positioning of *P. putida* GN1 and its related strains within the broader context of PGPR.

Discussion

P. putida GN1 against soil-borne pathogens of groundnut

The isolation, characterisation and practical assessment of PGPR activity with diverse beneficial possessions are essential for advancing biocontrol strategies and enhancing plant health (Pradhan et al. 2017). In our study, *P. putida* GN1 demonstrated effective biocontrol against *M. phaseolina* and *S. rolfsii*, showcasing its potential as a promising PGPR. This outcome is consistent with previous research indicating that certain bacterial and fungal species can suppress soilborne pathogens. For instance, *Streptomyces* species have been reported to constrain the mycelial growth of *S. rolfsii* effectively (Leona et al. 2020). Similarly, other PGPR, such as *Trichoderma* species and *Pseudomonas fluorescens* isolated from groundnut rhizosphere, exhibited significant antagonistic activity against *M. phaseolina* (Mahendra et al. 2022). *Ralstonia officinalis* and *P. fluorescens* showed a strong *in vitro* activity in relation growth limitation of *R. solanacearum* as well as limiting the development of bacterial wilt disease on potato plants under greenhouse conditions (Abd El-Wahed

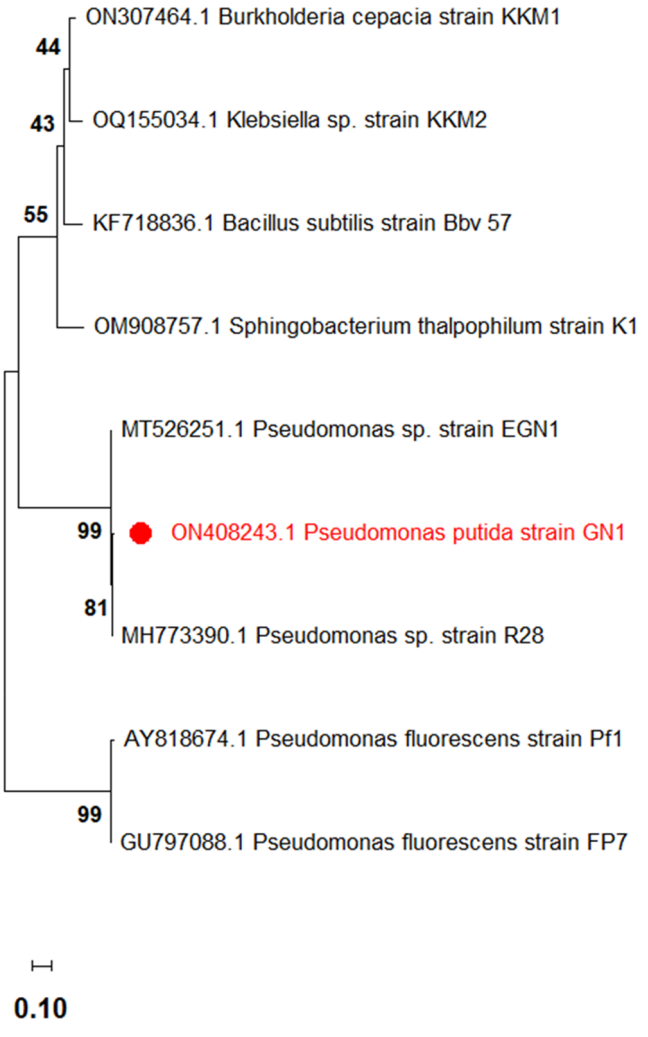

Figure 9. Molecular characterization of *Pseudomonas putida* GN1.

et al. 2023) *P. putida* T6SS. Many routes have been explored to develop biocontrol agents capable of manipulating the microbial composition of the rhizosphere and phyllosphere (Bernal et al. 2017). These findings reinforce the potential of PGPR in managing soilborne diseases and promoting plant growth, aligning with our results and highlighting the value of integrating such beneficial microorganisms into agricultural practices.

Production of secondary metabolites

PGPR are recognised for their capacity to synthesise a diverse array of secondary metabolites, such as siderophores, HCN, IAA and DAPG. These bioactive compounds are instrumental in both the suppression of soilborne pathogens and the enhance the plant growth. Siderophores, for

instance, chelate iron from the soil environment, limiting its availability to pathogens and thus inhibiting their growth. HCN, a volatile compound, disrupts the cellular respiration of pathogenic fungi, further enhancing the protective effect of PGPR. Indole-3-acetic acid, a well-known phytohormone, not only aids in root development and overall plant vigour but also indirectly contributes to the plant's defense mechanisms. Furthermore, 2,4-DAPG is a potent antimicrobial agent that has been extensively documented for its role in the biocontrol of many plant pathogens, including those causing root and stem rot in groundnut. The synergistic action of these secondary metabolites underscores the multifaceted approach by which PGPR like *P. putida* GN1 enhance plant health and yield, making them invaluable in sustainable agriculture and integrated pest management strategies (Pradhan et al. 2017). Siderophores, for instance, bind iron, thereby depriving pathogens of this essential nutrient and giving PGPR a competitive edge in the rhizosphere (Ghosh et al. 2020; Scavino and Pedraza 2013; Al-Sman et al. 2019). In our study, *P. putida* GN1 demonstrated the production of siderophores, HCN, IAA, DAPG and phenazines. These metabolites are likely key factors in its effective antagonism against *S. rolfsii* and *M. phaseolina*. Our findings align with previous research, which underscores the importance of these metabolites in pathogen suppression and improve the crop growth (Lu et al. 2021). Similarly, the production of IAA by *P. putida, Pseudomonas aeruginosa, Pseudomonas libanensis, Bacillus megaterium, Bacillus cereus* and *B. subtilis* isolated from the rhizosphere of chilli (Hyder et al. 2020). The capacity of *P. putida* GN1 to produce these beneficial compounds supports its possible as a valuable disease control agent in managing soil borne diseases and promoting the health of groundnut plants. Applying *P. putida* ASU15 at the same time of pathogen inoculation showed reduction in disease severity (69.9%), higher than application before pathogen inoculation (54.9%) (Abo-Elyousr et al. 2021).

Plant growth promotion activity

PGPR activity are significantly boost plant productivity and enhance control against both biotic and abiotic stresses (Kaushal and Wani 2016). In our study, *P. putida* GN1 notably improved the shoot-to-root ratio in groundnut seedlings. This observation aligns with existing research demonstrating that PGPRs can effectively enhance overall seedling vigour (Leona et al. 2020). Furthermore, the impact of rhizobacteria on growth and stress tolerance is also supported by studies on other bacterial species, such as *Sphingomonas* sp., which has been shown to improve crop growth and increase drought tolerance by modifying the rhizosphere bacterial community (Luo et al. 2019). These findings highlight the

beneficial role of rhizobacteria on promoting robust growth and stress resilience in groundnut plants, confirming their potential as effective tools for sustainable agriculture.

Alteration of root system architecture

P. putida GN1 demonstrated an important effect on the root system architecture of groundnut plants, notably enhancing root length, the number of root tips, root forks, root diameter and root volume. Extended root systems are crucial for improving water uptake, especially under drought conditions, which is essential for drought resilience (Vadez et al. 2008). Our results align with existing literature showing that modifications in root structure, including increased lateral roots and root hairs, contribute to more effective water and nutrient absorption (Wang et al. 2015). Specifically, groundnut plants treated with *P. putida* GN1 exhibited a higher root growth character. This expanded root system improves the plant's capability to access more nutrient from the soil, thereby supporting improved overall crop growth and drought tolerance (Bresson et al. 2013).

Root volume

The notable increase in root volume in groundnut plants treated with *P. putida* GN1 underscores its efficacy as a seed treatment for improving plant growth, especially under rainfed conditions. This enhancement aligns with similar studies that reported significant improvements in root and shoot lengths, as well as seedling vigour, with other beneficial microbes such as *B. subtilis* G-1 and various *Pseudomonas* strains (de Boer et al. 2003; Shifa et al. 2014;). These observations reinforce the potential of *P. putida* GN1 as a powerful agent for both biocontrol and plant growth promotion in agricultural settings. The increased root volume and improved growth metrics suggest that *P. putida* GN1 contributes effectively to soil health and plant productivity, offering valuable benefits for crop management and sustainability.

Conclusion

P. putida GN1 demonstrates considerable promise as a biocontrol agent against soilborne pathogens *M. phaseolina* and *S. rolfsii* affecting groundnut. This efficacy is largely attributed to its production of various antimicrobial compounds, including HCN, DAPG and siderophores. HCN acts as a potent antimicrobial agent that can directly inhibit pathogen growth, while DAPG contributes by disrupting the development of pathogenic fungi. Siderophores produced by *P. putida* GN1 chelate iron,

depriving pathogens of this essential nutrient and further suppressing their proliferation. Additionally, *P. putida* GN1 enhances plant growth and alters root architecture, making it a valuable tool for improving groundnut productivity, particularly under drought conditions. This study, the first to analyze the effect of *P. putida* GN1 on groundnut, demonstrates its suitability as a seed treatment for rainfed cultivation. The strain's ability to promote crop growth by secreting plant growth hormones and improving root characteristics such as length, tips, forks, hairs, diameter and volume further underscores its utility. When applied as a seed treatment, *P. putida* GN1 effectively colonises roots, providing protection against plant pathogens and enhancing overall plant health. This highlights its promise in sustainable agriculture and its potential to boost groundnut production under challenging environmental conditions.

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Authors' contribution

Conceptualisation, M. P.; Formal analysis, M.P., A.T., M. K. and V.R.; Methodology, P.I., I.J. Resources, G.K.; Software, V.R.; Supervision, M. K.; Writing – original draft, M.P. and M.K. Writing – review and editing, M.K., I.J. and M.P. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

All authors declared no conflict of interest.

Ethical approval

This study does not require ethical approval from the Institutional Review Board.

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