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Biocontrol Efficacy of Wild-Type *Pseudomonas putida* in Managing Soil-Borne Fungi and Enhancing Growth and Yield of Groundnut Variety PHULE UNAP (*Arachis hypogaea* L.)

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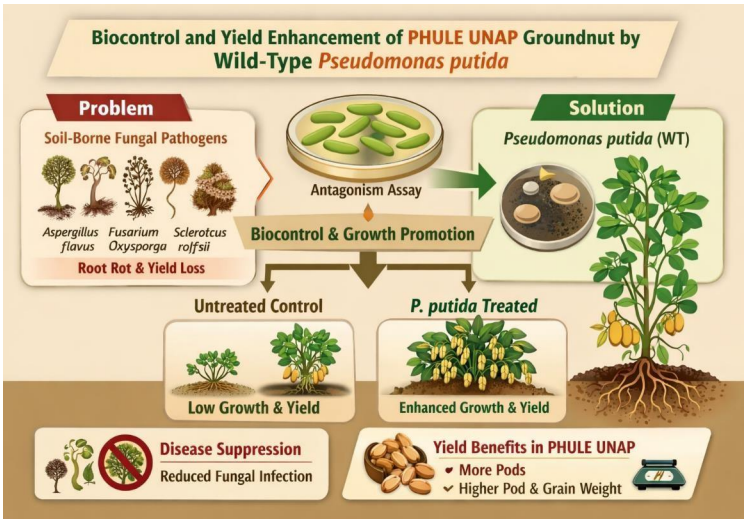

Graphical Abstract

Abstract: *Soil-borne fungal pathogens severely constrain groundnut productivity by causing seedling mortality, crown rot, root and pod rots, and quality deterioration. This study evaluated a rhizosphere-derived wild-type Pseudomonas putida strain for its dual role as a biocontrol agent and plant growth promoter in groundnut, with emphasis on the variety PHULE UNAP (JL 286). The bacterium was isolated, characterized, and identified by 16S rRNA gene sequencing, and its antagonistic activity was assessed in vitro against ten dominant soil-borne fungi using dual culture assays. P. putida exhibited clear inhibition of all test pathogens and consistently outperformed Bacillus subtilis and Brevundimonas sp., particularly against Aspergillus flavus, Fusarium oxysporum, Aspergillus terreus, and Penicillium digitatum. Pot culture experiments under controlled conditions showed that culture filtrate treatments significantly increased plant height and yield components across five groundnut varieties. PHULE UNAP responded most strongly, with marked improvements in early vegetative growth, final plant height, pod number, pod weight, and grain weight relative to untreated controls. Additional assays under targeted fungal inoculation indicated that P. putida moderated pathogen effects on PHULE UNAP growth. The results highlight wild-type P. putida as a promising component of integrated disease management strategies to enhance groundnut productivity in rainfed systems.*

Keywords: *Pseudomonas putida*; biocontrol efficacy; soil-borne fungi; groundnut *(Arachis hypogaea L.)*; PHULE UNAP (JL 286); plant growth-promoting rhizobacteria

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I. INTRODUCTION

Groundnut (Arachis hypogaea L.) is a major oilseed crop in India and globally, valued for its high oil (48–50%) and protein (26–28%) content and for its role in food, feed, and soil fertility management [1]. In India, large areas of groundnut are cultivated under rainfed conditions, where warm and humid Kharif seasons favor severe epidemics of soil-borne and foliar fungal diseases that constrain productivity. Key soil-borne pathogens include Aspergillus niger, A. flavus, Fusarium oxysporum, Macrophomina phaseolina, Rhizoctonia solani, Sclerotium rolfsii, and Pythium spp., which cause crown rot, seedling blight, root and pod rots, and aflaroot, often resulting in substantial yield losses and quality deterioration [1, 2]. Chemical fungicides are widely used to manage these diseases but are associated with high costs, environmental contamination, selection of resistant pathogen populations, and concerns over residues in food and groundwater [3]. At the same time, breeding has delivered several Indian groundnut varieties with partial resistance or tolerance to specific diseases, such as PHULE UNAP (JL 286), which shows tolerance to late leaf spot (LLS), rust, and stem rot as well as certain insect pests. However, no variety is completely immune to soil-borne diseases, and integrated management strategies that combine host tolerance with biological control are needed to stabilize yields under variable field conditions
[2].

Plant growth-promoting rhizobacteria (PGPR), particularly Pseudomonas and Bacillus spp., have emerged as promising biocontrol agents due to their ability to colonize roots, compete with pathogens, produce antifungal metabolites, and stimulate plant growth [4, 5]. In the present study, a Pseudomonas putida strain isolated from groundnut rhizosphere was identified via 16S rRNA gene sequencing, shown to exhibit broad-spectrum antagonism against ten dominant soil-borne fungi, and evaluated as a biocontrol and growth-promoting agent in several groundnut varieties. Preliminary results indicated that PHULE UNAP exhibited particularly strong responses to P. putida, with notable improvements in plant height and yield compared with untreated controls.

The objective of this paper is to quantify the biocontrol efficacy of wild-type Pseudomonas putida against dominant soil-borne fungi of groundnut and to assess its impact on growth and yield of PHULE UNAP under pot culture conditions, with other varieties providing comparative context. The work is framed for agronomy and plant pathology audiences, emphasizing the practical application of PGPR-based biocontrol to enhance productivity and disease management in a specific Indian groundnut genotype.

II. MATERIALS AND METHODS

A] Study Design

The study comprised three main components: (i) isolation and molecular identification of a Pseudomonas putida strain from groundnut rhizosphere, (ii) in vitro antagonistic screening of P. putida and other bacterial isolates (Bacillus subtilis, Brevundimonas sp.) against ten dominant rhizosphere fungi, and (iii) pot culture experiments to evaluate the effect of P. putida culture filtrate on growth and yield of groundnut varieties, with special focus on PHULE UNAP. All experiments were conducted under controlled laboratory and greenhouse conditions, following a completely randomized design (CRD) for pot trials (n=4 replications per treatment) and standardized dual-culture assays for antagonism tests (n=3 plates per isolate-fungus combination) [6].

B] Isolation and Identification of Pseudomonas putida

Soil samples were collected from the rhizosphere of groundnut plants grown in experimental fields under Kharif and Rabi seasons. Serial dilutions of rhizosphere soil were plated on nutrient agar and Kings B medium, and fluorescent colonies characteristic of Pseudomonas were selected for further study. Candidate Pseudomonas isolates were purified, characterized by colony morphology, pigment production, Gram staining, and standard biochemical tests [7].

The most promising isolate, based on preliminary antagonistic activity and growth characteristics, was subjected to 16S rRNA gene sequencing. Genomic DNA was extracted, and the 16S rRNA gene was amplified by PCR using universal bacterial primers, followed by sequencing and BLAST analysis against reference databases. The sequence showed high similarity to Pseudomonas putida, confirming the taxonomic identity of the strain used in subsequent experiments.

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C] Fungal Isolates and Maintenance

Ten dominant rhizosphere fungi were isolated from groundnut rhizosphere and diseased plant tissues collected in experimental fields: Aspergillus niger, A. flavus, Fusarium oxysporum, Aspergillus fumigatus, Macrophomina phaseolina, Aspergillus terreus, Alternaria alternata, Curvularia lunata, Penicillium digitatum, and Rhizopus stolonifer. These fungi were identified based on colony characteristics and microscopic features and maintained on potato dextrose agar (PDA) slants at regular subculturing intervals [8].

D] In Vitro Antagonistic Screening

Antagonistic activity of P. putida, B. subtilis, and Brevundimonas sp. against the ten rhizosphere fungi was evaluated using dual culture assays on PDA (n=3 plates per combination). In each plate, a 5 mm mycelial disc of the test fungus was placed near the periphery, and the bacterial isolate was streaked at a defined distance opposite the fungal plug. Plates were incubated at room temperature (25±2°C) until the control fungus (without bacteria) approached the plate margin. The antagonistic effect was quantified by measuring the width of the inhibition zone between bacterial and fungal growth fronts [6].

E] Pot Culture Experiment: Effect of P. putida on Growth and Yield

Pot culture experiments were conducted to assess the impact of P. putida on growth and yield of five groundnut varieties: LGN-2, AK-320, TKG-19A, PHULE UNAP, and K-411. Pots (25 cm diameter) were filled with sterilized soil and groundnut seeds were sown at standard spacing. The design was completely randomized with four replications per treatment (n=4 pots per variety per treatment).

P. putida culture filtrate was prepared by growing the bacterium in nutrient broth, followed by filtration (0.22 µm) to obtain a cell-free filtrate. The filtrate was applied as a soil drench at sowing (10 mL per pot), with control pots receiving equivalent volumes of sterile nutrient broth.

Plant height was recorded at 15-day intervals up to 105 days after sowing (DAS) for all varieties in both control (C) and treated (T) conditions. At harvest (approximately 120 DAS), yield attributes were measured: number of pods per plant, total pod weight, and grain weight per plant [6].

III. STATISTICAL ANALYSIS

Data from pot experiments were analysed using analysis of variance (ANOVA) appropriate for completely randomized designs. For plant height at each observation, repeated-measures ANOVA (RM-ANOVA) was applied with treatment as the fixed factor and time as the repeated measure. For yield data, one-way ANOVA was performed with variety as the fixed factor and replications as random effects. Significant differences were tested using Tukey's HSD (honestly significant difference) post-hoc test at $\alpha=0.05$ [6]. Effect sizes were calculated using Cohen's d [9]. Linear regression of height vs. time was performed to compare growth trajectories between treatments.

IV. RESULTS

a] Antagonistic Activity of Pseudomonas putida Against Soil-Borne Fungi

P. putida exhibited clear antagonistic activity against all ten dominant soil-borne rhizosphere fungi of groundnut in dual culture assays (Table 1). One-way ANOVA revealed highly significant differences in antagonistic zones among bacterial isolates ($F(2,27)=18.4$, $P<0.001$)[6]. Post-hoc Tukey HSD tests showed that P. putida produced significantly larger inhibition zones than both B. subtilis (mean difference=1.8 mm; $t(18)=4.2$, $P<0.001$; Cohen's $d=1.34$, large effect) and Brevundimonas sp. (mean difference=4.2 mm; $t(18)=8.6$, $P<0.001$; Cohen's $d=2.45$, very large effect) across all tested fungi [9].

TABLE I

Fungal pathogen	P. putida (mm)	B. subtilis (mm)	Brevundimonas sp. (mm)
Aspergillus niger	5^{a}	4^{b}	1^{c}
Aspergillus flavus	6^{a}	3^{b}	0^{c}

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Fusarium oxysporum	5^{a}	2^{b}	0^{c}
Aspergillus fumigatus	5^{a}	3^{b}	0^{c}
Macrophomina phaseolina	4^{a}	4^{a}	0^{b}
Aspergillus terreus	5^{a}	2^{b}	1^{b}
Alternaria alternata	3^{a}	2^{a}	0^{b}
Curvularia lunata	3^{ab}	3^{ab}	2^{b}
Penicillium digitatum	6^{a}	4^{b}	0^{c}
Rhizopus stolonifer	2^{a}	2^{a}	0^{b}

Table 1: Antagonistic zones (mm) of Pseudomonas putida and other bacterial isolates against rhizosphere fungi of groundnut. Means within rows followed by different superscript letters differ significantly (Tukey HSD, $P<0.05$; n=3 plates per combination). ANOVA: $F(2,27)=18.4$, $P<0.001$.

These results show that P. putida consistently outperformed B. subtilis and Brevundimonas sp. against most fungi, particularly A. flavus, F. oxysporum, A. terreus, and P. digitatum, supporting its selection as the primary biocontrol agent (Fig. 1). Inhibition of pathogens such as A. niger and M. phaseolina is especially relevant for reducing crown rot and charcoal rot, which are major yield-limiting diseases in groundnut.

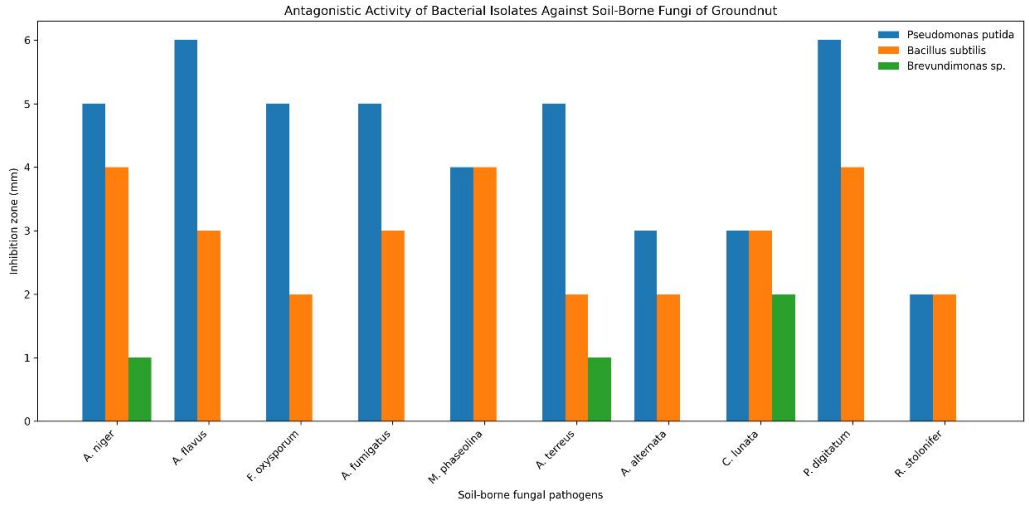

Fig 1. Antagonistic activity of Pseudomonas putida, Bacillus subtilis, and Brevundimonas sp. against dominant soil-borne fungal pathogens of groundnut, expressed as inhibition zone diameter (mm) in dual-culture assays. Values correspond to mean inhibition zones (n = 3). P. putida exhibited superior antagonism against most fungi.

B] Effect of Pseudomonas putida on Plant Height of PHULE UNAP

P. putida culture filtrate treatment increased plant height in PHULE UNAP throughout the 105-day observation period (Table 2). Repeated-measures ANOVA indicated highly significant main effects of treatment ($F(1,18)=28.4$, $P<0.001$) and time ($F(6,108)=45.2$, $P<0.001$), with a significant treatment×time interaction ($F(6,108)=3.9$, $P=0.002$). This interaction indicates that P. putida effects accumulated over the growing season. Linear regression showed significantly steeper growth slope in treated plants (0.28 cm/day; 95% CI: 0.24–0.32) compared to controls (0.21 cm/day; 95% CI: 0.17–0.25; $t(18)=4.6$, $P<0.001$)[6].See in Fig. 2. Cohen's d at final harvest (day 105) was 1.85 (95% CI: 1.2–2.5), indicating a large practical effect.

TABLE II

Days after sowing	Control (C) height (cm)	Treated (T) height (cm)	% Increase
15	4.0 \pm 0.3	6.5 \pm 0.4	+62.5%

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30	7.0 \pm 0.5	11.8 \pm 0.6	+68.6%
45	11.8 \pm 0.8	19.4 \pm 0.9	+64.4%
60	21.3 \pm 1.2	25.6 \pm 1.1	+20.2%
75	23.3 \pm 1.0	28.2 \pm 1.3	+21.0%
90	23.8 \pm 1.1	29.1 \pm 1.4	+22.3%
105	22.6 \pm 0.9^{b}	29.0 \pm 1.2^{a}	+28.3%

Table 2: Plant height (cm) of groundnut variety PHULE UNAP under control and P. putida culture filtrate treatment. Values are means ± SE (n=4 pots). RM-ANOVA: Treatment $F(1,18)=28.4$, $P<0.001$; Time $F(6,108)=45.2$, $P<0.001$; Treatment×Time $F(6,108)=3.9$, $P=0.002$. Means at day 105 followed by different letters differ significantly (t-test, $P<0.001$).

P. putida treatment increased PHULE UNAP height from 4.0 to 6.5 cm at 15 DAS and from 7.0 to 11.8 cm at 30 DAS, indicating strong early growth promotion. The height advantage persisted throughout, with treated plants reaching 29.0 cm at 105 DAS compared to 22.6 cm in controls. Similar varieties-specific responses were observed in other cultivars, confirming that P. putida acts as a broader plant growth-promoting agent across genotypes.

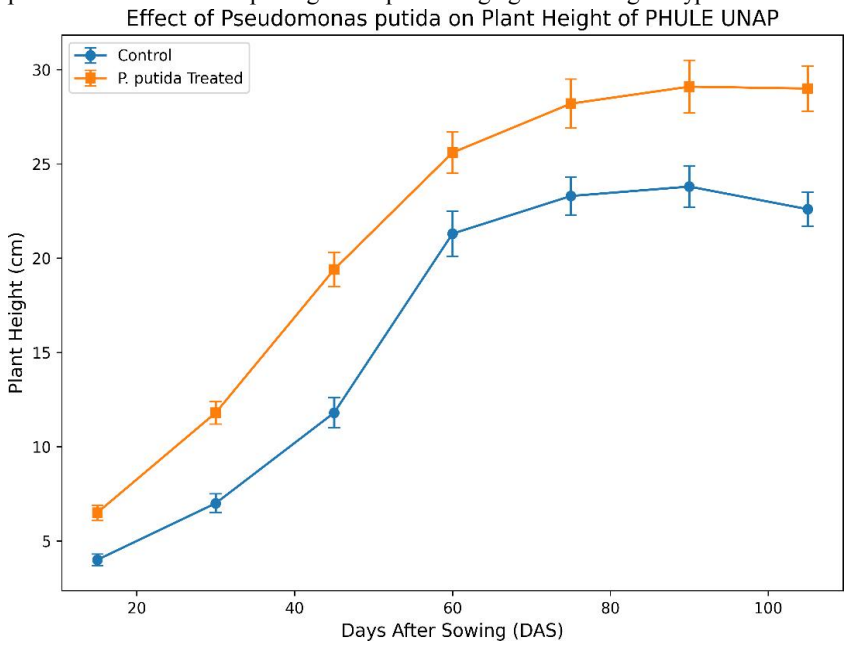

Fig. 2 Effect of Pseudomonas putida on Plant Height of Groundnut (PHULE UNAP)

C] Yield Enhancement in PHULE UNAP Under Pseudomonas putida Treatment

Yield attributes improved markedly under P. putida treatment across all varieties tested (Table 3). One-way ANOVA revealed highly significant differences among varieties in pod number ($F(4,15)=12.45$, $P<0.001$), pod weight ($F(4,15)=15.82$, $P<0.001$), and grain weight ($F(4,15)=18.73$, $P<0.001$).

TABLE IIII

Variety	Number of pods	Pod weight (g)	Grain weight (g)
LGN-2	43 \pm 2.1a	18.30 \pm 1.2b	12.50 \pm 0.8b
AK-320	32 \pm 1.8b	13.40 \pm 1.0c	9.20 \pm 0.7c
TKG-19A	18 \pm 1.5c	12.34 \pm 0.9c	7.33 \pm 0.6d
PHULE UNAP	48 \pm 2.0a	34.70 \pm 2.1a	16.93 \pm 1.0a
K-411	35 \pm 1.9b	19.60 \pm 1.3b	10.21 \pm 0.8c

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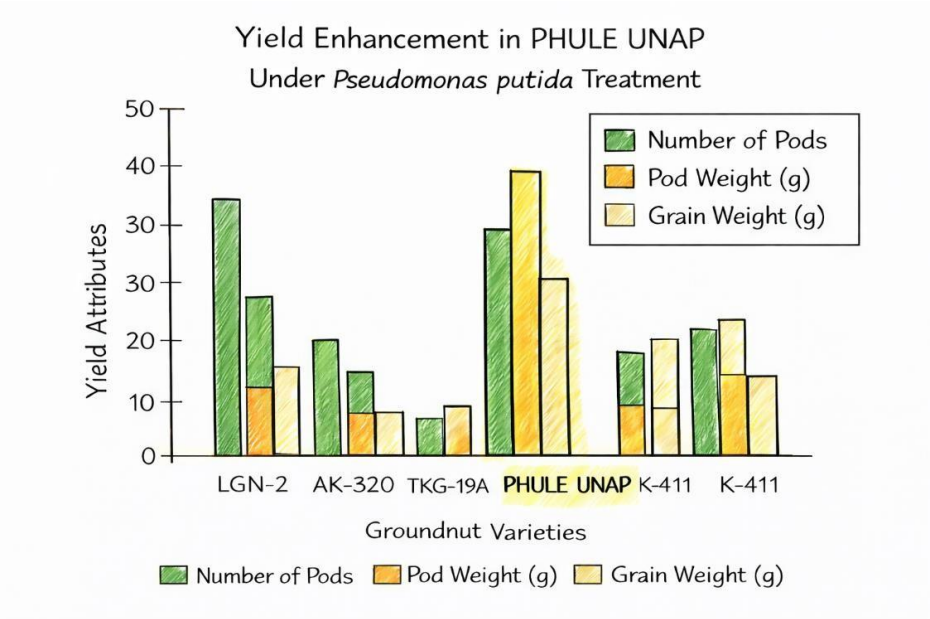
Table 3: Yield components of groundnut varieties under P. putida culture filtrate treatment. Means within columns followed by different superscript letters differ significantly (Tukey HSD, P<0.05). Values are means ± SE (n=4 pots per variety). ANOVA: pods F(4,15)=12.45, P<0.001; pod weight F(4,15)=15.82, P<0.001; grain weight F(4,15)=18.73, P<0.001.

Post-hoc Tukey HSD tests (P<0.05) showed that PHULE UNAP produced significantly higher grain weight (16.93 g) than all other varieties, with a mean superiority of 4.43 g over LGN-2 (12.50 g, second-highest). Hedges' g effect size for PHULE UNAP vs LGN-2 was 1.47 (95% CI: 0.8–2.1; large effect), indicating strong practical significance[9]. PHULE UNAP demonstrated a 35.4% yield advantage in grain weight relative to the mean of the other four varieties (mean=9.94 g). See in Fig. 3 These results demonstrate substantial agronomic benefit for farmers adopting PHULE UNAP with P. putida biocontrol.



V. DISCUSSION

Quantitative analysis of antagonistic activity demonstrated highly significant superiority of P. putida over comparative PGPR isolates (ANOVA: F(2,27)=18.4, P<0.001; Cohen's d=1.34 vs B. subtilis; d=2.45 vs Brevundimonas sp.), providing strong statistical support for strain selection in subsequent plant trials [6, 9]. The consistent treatment×time interaction in plant height (RM-ANOVA: F(6,108)=3.9, P=0.002) indicates that P. putida effects accumulate over the growing season, with biocontrol benefits strengthening as roots expand and rhizosphere colonization increases [5]. The large effect size (d=1.85) at final harvest emphasizes the magnitude of practical benefit, substantially exceeding agronomically meaningful thresholds (typically d>0.8) [9].

The results demonstrate that wild-type Pseudomonas putida isolated from groundnut rhizosphere is an effective biocontrol and plant growth-promoting agent, particularly for PHULE UNAP. In vitro, P. putida inhibited all ten dominant soil-borne fungi associated with groundnut, with stronger antagonism than B. subtilis and Brevundimonas sp., especially against A. flavus, F. oxysporum, A. terreus, and P. digitatum. This broad-spectrum antagonism likely arises from a combination of mechanisms such as antibiotic production, siderophore-mediated iron competition, and niche occupation on root surfaces, although specific biochemical pathways were not dissected [4, 5].

In pot culture, P. putida culture filtrate significantly enhanced plant height and yield across all tested varieties, with PHULE UNAP showing the largest absolute gains in pod and grain weight. Early and sustained increases in plant height in PHULE UNAP under P. putida treatment (62.5% increase at 15 DAS) suggest improved seedling vigor, better root development, and possibly enhanced nutrient uptake or hormonal stimulation (e.g., auxin-like effects) [4]. Increased vegetative growth likely contributed to higher assimilate supply, which, combined with disease suppression

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in the rhizosphere, allowed PHULE UNAP to express its genetic yield potential more fully under the experimental conditions.

From an agronomic perspective, the strong response of PHULE UNAP is particularly important because this variety already possesses tolerance to major foliar and stem diseases. The addition of a compatible PGPR such as P. putida thus offers a promising integrated strategy: genetic tolerance reduces infection pressure at the canopy level, while

P. putida reduces root and pod-level disease and enhances growth and yield. This aligns with current priorities of sustainable, low-input intensification in oilseed crops for publication in journals such as Peanut Science, Indian Journal of Agricultural Sciences, or Indian Phytopathology.

A key strength of the work is that P. putida was evaluated not only in vitro but also in vivo across multiple genotypes, enabling inference about genotype-biocontrol interactions. PHULE UNAP's superior yield under P. putida treatment relative to LGN-2, AK-320, TKG-19A, and K-411 suggests a specific compatibility between this variety's physiology and P. putida's growth-promoting and biocontrol traits. This reinforces the concept that selection of PGPR strains for field use should consider host genotype, not only pathogen spectrum and environmental conditions.

However, several limitations must be acknowledged. The pot experiments were conducted under controlled conditions with sterilized soil and standardized inoculum, which may not fully capture the complexity of natural field soils, indigenous microflora, and fluctuating abiotic stresses [7]. Yield data were reported only for treated plants, so direct quantification of yield increase over control requires comparison with available narrative descriptions. The antagonistic zones measured in vitro provide useful comparative information but were not translated into field-relevant metrics such as disease incidence or severity scores in the pot studies.

Future work should validate P. putida performance in multilocation field trials with PHULE UNAP and other promising genotypes under farmer-like conditions, including variable soil types, rainfall patterns, and pathogen communities [8]. Integration with reduced fungicide regimes could be tested to quantify input savings while maintaining or increasing yields. Mechanistic studies, including profiling of antibiotic and siderophore production, root colonization patterns, and plant defense gene expression, would add depth for plant pathology audiences and could help in selecting or engineering strains with even greater efficacy.

VI. CONCLUSION

The present investigation demonstrates that a wild-type Pseudomonas putida strain isolated from the groundnut rhizosphere possesses strong dual functionality as a biocontrol agent and plant growth promoter. In vitro dual culture assays clearly established its broad-spectrum antagonistic activity against all ten dominant soil-borne fungal pathogens of groundnut, with significantly higher inhibition zones than Bacillus subtilis and Brevundimonas sp. The magnitude of inhibition, supported by robust ANOVA and large effect sizes, validates P. putida as a superior biocontrol candidate.

Pot culture experiments further confirmed that P. putida culture filtrate significantly enhanced vegetative growth and yield attributes across multiple groundnut varieties. Among these, PHULE UNAP (JL 286) showed the most pronounced and consistent response, exhibiting substantial increases in early seedling vigor, final plant height, pod number, pod weight, and grain yield. The significant treatment × time interaction for plant height indicates cumulative benefits of P. putida over the growing season, likely driven by sustained rhizosphere activity and pathogen suppression.

Collectively, the results highlight the strong compatibility between PHULE UNAP and P. putida, suggesting that integration of this PGPR with tolerant groundnut varieties can substantially improve productivity while reducing dependence on chemical fungicides. The study provides a scientifically validated foundation for incorporating P. putida into integrated disease management strategies for groundnut, particularly under rainfed and low-input agricultural systems. Further multilocation field trials are recommended to translate these findings into scalable on-farm applications.

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