PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.7861

eISSN 2348-1900

RESEARCH ARTICLE

Efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* in managing groundnut leaf blight disease caused by *Alternaria alternata*

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Received: 21 February 2025; Accepted: 20 May 2025; Available online: Version 1.0: 05 June 2025

Cite this article: Chitra K, Dhanalakshmi K, Ejilane J, Balisasikumar C, Sivasankari DT, Dineshkumar P, Jayaseelan C, Karthikeyani VK & Ramasamy M. Efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* in managing groundnut leaf blight disease caused by *Alternaria alternata*. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.7861

Abstract

This study evaluated five *Pseudomonas fluorescens* (Pf) strains and ten *Bacillus subtilis* (Bs) strains for their antagonistic effects against *Alternaria alternata* under *in vitro* conditions. Among them, Pf1 and Bs1 demonstrated the highest inhibition of mycelial growth. Subsequent greenhouse and field trials focused on the most effective strains (Pf1, Pf2, Bs1 and Bs5). Under greenhouse conditions, treatments involving Seed Treatment (ST) and Foliar Spray (FS) significantly reduced disease intensity. Field trials confirmed that seed treatment with Pf1 at 10 g/kg of seed, followed by two foliar sprays at 30 and 40 days after sowing at 0.2 %, effectively reduced disease incidence and enhanced yield. The results suggest that biocontrol agents, particularly Pf1, can serve as an eco-friendly alternative to chemical fungicides for managing leaf blight in groundnut.

Keywords: *Bacillus subtilis*; biological control; groundnut leaf blight; plant growth promotion; *Pseudomonas fluorescens*

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop globally, but its productivity is significantly affected by various fungal diseases, including leaf blight caused by *Alternaria alternata* (Fr.) Keissler. The disease is widespread and can cause severe yield losses if left unmanaged. Traditionally, chemical fungicides have been used for disease control, but their extensive application has led to concerns such as pathogen resistance, environmental contamination and human health risks (1, 2). Consequently, sustainable and eco-friendly alternatives, such as biological control using antagonistic microorganisms, have gained attention (3).

Pseudomonas fluorescens and *Bacillus subtilis* are well-documented biocontrol agents known for their ability to suppress plant pathogens through mechanisms such as competition, production of antibiotics and induction of systemic resistance in plants (4-6). Fluorescent *Pseudomonas* strains produce secondary metabolites, including phenazines and lipopeptides, that inhibit fungal growth (7, 8). Similarly, *Bacillus subtilis* produces antifungal compounds such as iturin, surfactin and fengycin, which play a key role in biocontrol (9). The present study aims to evaluate the efficacy of these beneficial bacterial strains against *A. alternata* under *in vitro*, greenhouse and field conditions to develop a sustainable disease management strategy for groundnut leaf blight.

Material and Methods

Isolation of the pathogen

Groundnut leaves exhibiting typical leaf blight symptoms were collected and pathogen isolation was carried out using the tissue segment method on Potato Dextrose Agar (PDA) medium (10). The fungus was purified using the hyphal tip method and maintained on PDA slants at 4 °C.

Isolation of biocontrol agents

Rhizosphere soil from healthy groundnut plants was used to isolate *Pseudomonas* and *Bacillus* strains. Serial dilution plate methods were employed to isolate the strains on King's B (KB) medium for *Pseudomonas* and nutrient agar medium for *Bacillus* (11, 12). The Pf1 strain was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Preparation of bacterial inoculum

Pseudomonas and *Bacillus* strains were cultured in King's B and nutrient agar broths, respectively. Bacterial suspensions were prepared to a final concentration of 10^8 cfu/ml, mixed with talc powder and carboxymethyl cellulose (CMC) for seed treatment applications (13).

In vitro screening of antagonistic activity

Initially, five *P. fluorescens* strains (Pf1–Pf5) and ten *B. subtilis* strains (Bs1-Bs10) were screened against *A. alternata* using the dual culture technique (14). The zone of inhibition was measured and the most effective strains (Pf1, Pf2, Bs1 and Bs5) were selected for further greenhouse and field evaluations.

Screening under greenhouse and field conditions

Pot culture experiments evaluated the effectiveness of Pf1, Pf2, Bs1 and Bs5 as ST, FS and combined seed treatment plus foliar spray (ST+FS). Field trials were conducted at Coconut Research Station, Aliyar Nagar, using a randomized complete block design. Disease intensity and yield parameters were recorded at 90 Days After Sowing (DAS) (15, 16).

Results

Pathogenicity and symptomatology

Pathogenicity tests confirmed that *A. alternata* caused characteristic leaf blight symptoms, with chlorotic lesions progressing to necrotic patches, leading to severe defoliation (17).

In vitro screening results

Among the five *Pseudomonas* strains, Pf1 exhibited the highest inhibition of *A. alternata* (16.2 mm inhibition zone), followed by Pf2 (15.3 mm). Similarly, Bs1 recorded the highest inhibition among *Bacillus* strains (21 mm), followed by Bs5 (19.3 mm) (18).

Pot culture evaluation

Seed treatment plus foliar spray of Pf1 significantly reduced disease intensity (24.17 %), followed by Pf2 (26.21 %), Bs1 (27.13 %) and Bs5 (28.21 %). Growth parameters, including plant height and number of leaflets, were also improved (19).

Field trial results

Field trials demonstrated that seed treatment with Pf1 (10 g/kg) plus foliar spray at 30 and 45 DAS significantly reduced disease incidence (23.23 %) and increased yield (1342 kg/ha) compared to the control (59.23 % disease incidence and 940 kg/ha yield). Mancozeb (chemical control) recorded the lowest disease incidence (20.14 %) and highest yield (1417 kg/ha), but the biocontrol treatments showed promising results as an eco-friendly alternative (20).

Discussion

The efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* strains in managing groundnut leaf blight disease caused by *Alternaria alternata* was evaluated under *in vitro*, greenhouse and field conditions. The results demonstrated the significant potential of biocontrol agents in suppressing pathogen growth and reducing disease incidence, ultimately enhancing crop yield.

In vitro antagonistic activity

The *in vitro* screening of bacterial strains revealed that *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* Bs1 exhibited the highest inhibition of *A. alternata* mycelial growth (Tables 1 and 2). Pf1 recorded the largest inhibition zone (16.20 mm) and reduced mycelial growth by 51.18 %, while Bs1 exhibited a superior inhibition zone of 21 mm and a 53.33 % reduction in mycelial growth. Other strains, such as Pf2 and Bs5, also showed significant inhibitory effects but were slightly less effective than Pf1 and Bs1. These findings are consistent with previous reports indicating that Pseudomonas and Bacillus strains exert antagonistic effects against fungal pathogens through the production of antifungal compounds and competition for nutrients.

Table 1. Effect of Pseudomonas strains on radial mycelial growth of *A. alternata*

Strains	Mycelial growth (mm)	Percent reduction over control	Inhibition zone (mm)
Pf1	43.1 a	51.18	16.20 a
Pf2	48.1 b	45.52	15.30 a
Pf3	47.2 b	46.54	11.20 b
Pf4	52.1 c	40.96	7.10 c
Pf5	55.2 c	37.48	5.10 d
Control	88.3 d	0	0

Table 2. Effect of *Bacillus* strains on the mycelial growth of the pathogen

Treatments	Mycelial growth (mm)	Per cent reduction over control	Inhibition zone (mm)
Bs1	41.3 a	53.33 a	21.0 a
Bs5	43.3 ab	51.07 b	19.3 b
Bs9	45.3 bc	48.81 cd	17.3 b
Bs6	45.2 bc	48.92 cd	15.2 bc
Bs8	50.2 e	43.27 f	16.4 bc
Control	88.5 f	-	-

Efficacy of biocontrol agents under greenhouse conditions

Greenhouse studies demonstrated that the combined application of ST and FS significantly reduced disease intensity compared to individual treatments (Table 3). Pf1 (ST+FS) exhibited the greatest disease suppression, reducing the Percent Disease Index (PDI) to 24.17 %, representing a 57.79 % reduction over control. Pf2 (ST+FS), Bs1 (ST+FS) and Bs5 (ST+FS) also showed substantial disease suppression, with PDI reductions of 54.23 %, 52.62 % and 50.74 %, respectively. Mancozeb, a synthetic fungicide, recorded the lowest PDI (21.32 %), confirming its high efficacy. However, the biocontrol treatments provided a promising eco-friendly alternative.

Table 3. Effect of biocontrol agents against leaf blight pathogen under pot culture conditions

Treatments (%)	PDI	Percent reduction over control
Pf1 (ST+FS)	24.17 b (29.44)	57.79
Pf2 (ST+FS)	26.21 bc (30.79)	54.23
Bs1 (ST+FS)	27.13 c (31.39)	52.62
Bs5 (ST+FS)	28.21 c (32.08)	50.74
Mancozeb (FS)	21.32 a (27.49)	62.77
Control	57.27 j (49.19)	-

Field performance of biocontrol agents

Field trials confirmed the efficacy of biocontrol treatments in managing leaf blight disease under natural conditions (Table 4). The combined seed treatment and foliar spray of Pf1 resulted in the lowest disease incidence (23.23 %) and a corresponding yield increase (1342 kg/ha). Similarly, Pf2, Bs1 and Bs5 treatments significantly improved yield while reducing disease severity. Mancozeb-treated plants recorded the highest yield (1417 kg/ha) and the lowest PDI (20.14 %), but the marginal difference between biocontrol treatments and chemical fungicide suggests that biocontrol agents can serve as a viable alternative for sustainable groundnut production.

Table 4. Effect of biocontrol agents against *A. alternata* under field conditions

Treatments	PDI	Percent reduction over control	Yield (kg/ha)
Pf1 (ST+FS)	23.23 (28.82) b	60.78	1342 b
Pf2 (ST+FS)	28.11 (32.02) c	52.54	1319 c
Bs1 (ST+FS)	30.44 (33.49) d	48.60	1299 d
Mancozeb	20.14 (26.65) a	65.99	1417 a
Control	59.23 (50.33) g	-	940 f

Mechanisms of disease suppression and growth promotion

The efficacy of *P. fluorescens* and *B. subtilis* in disease management can be attributed to their diverse mechanisms, including the production of secondary metabolites, competition with pathogens and induction of systemic resistance in plants. Fluorescent Pseudomonas strains are known to produce phenazines, lipopeptides and siderophores that inhibit fungal growth. Likewise, *B. subtilis* synthesizes antifungal compounds such as iturin, surfactin and fengycin, which suppress fungal pathogens. These beneficial microbes also enhance plant vigor, as evident from improved growth parameters such as plant height and the number of leaflets in treated plants.

Implications for sustainable agriculture

The present study supports the adoption of biocontrol strategies as an eco-friendly alternative to chemical fungicides. The application of Pf1 and Bs1 as seed treatments followed by foliar sprays offers a sustainable and effective approach to groundnut disease management. Future research should focus on optimizing the formulation and field stability of these biocontrol agents to enhance their commercial viability.

Conclusion

The findings of this study highlight the potential of *P. fluorescens* Pf1 and *B. subtilis* Bs1 as biocontrol agents against groundnut leaf blight. The combined application of seed treatment and foliar spray effectively reduced disease incidence and improved yield. These biocontrol agents can be integrated into sustainable disease management programs, reducing reliance on chemical fungicides and minimizing environmental impact.

Acknowledgements

The Work was supported by TNAU, Coimbatore.

Authors' contributions

KC and KD conducted the research deign participated in data collection and drafted the manuscript; KC, JE and TSD performed the statistical data analysis; CB, PD, CJ and KKV involved in preparation and alignment of data. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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