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Biocontrol potential of *Pseudomonas fluorescens* against root rot complex in black gram

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Abstract
A single hectare of black gram in Mediterranean cropping systems can lose up to 40 percent of its stand to root rot complex—a consortium of soil-borne fungi including Macrophomina phaseolina, Rhizoctonia solani, and Fusarium solani—before the crop even reaches flowering. Chemical seed treatments offer partial protection but raise concerns about residue persistence and non-target effects on beneficial soil microorganisms. This research evaluated three native strains of Pseudomonas fluorescens (Pf-BG01, Pf-BG02, Pf-BG03) isolated from healthy black gram rhizospheres in Piedmont, Italy, for their biocontrol potential against the root rot complex. In vitro dual-culture assays showed that Pf-BG03 inhibited mycelial growth of *M. phaseolina*, *R. solani*, and *F. solani* by 82.6, 74.8, and 69.3 percent, respectively. All three strains produced siderophores, hydrogen cyanide, and indole-3-acetic acid. In pot and field trials at Collegio di Agricoltura del Piemonte, Turin, during 2022-23, seed treatment with Pf-BG03 (10^8 CFU mL^{-1}) reduced root rot incidence by 76.4 percent and increased grain yield by 34.7 percent over the inoculated control. The seedling vigor index under Pf-BG03 reached 1,284, compared with 687 in the pathogen-only control. Combining Pf-BG03 with Trichoderma viride gave marginally better results but the difference was not statistically significant over Pf-BG03 alone. These findings position Pf-BG03 as a promising biocontrol agent for managing root rot complex in black gram under European growing conditions.

Keywords: *Pseudomonas fluorescens*, root rot, black gram, biocontrol, rhizobacteria, Macrophomina phaseolina, siderophores, plant growth promotion, seed treatment, integrated disease management

Introduction
Consider a field where 3 out of every 10 black gram seedlings wilt and collapse before producing a single pod. That scenario plays out routinely in Piedmont's alluvial soils when root rot pathogens—*M. phaseolina*, *R. solani*, and *F. solani*—build up under warm, moist conditions during the summer cropping window [1]. The disease complex is particularly damaging because each pathogen attacks the root cortex through a different mechanism: *M. phaseolina* produces microsclerotia that block vascular tissue, *R. solani* causes cortical rot through enzymatic maceration, and *F. solani* induces necrotic lesions via fusaric acid [2].

Conventional management relies on fungicidal seed treatments—carbendazim, thiram, or combinations thereof—applied at sowing [3]. While effective in the short term, these chemicals disrupt beneficial rhizosphere communities, including nitrogen-fixing Rhizobium, which is essential for black gram productivity [4]. And in the European Union, regulatory pressure under Directive 2009/128/EC has progressively restricted the availability of broad-spectrum fungicides, pushing growers toward biological alternatives [5].

Plant growth-promoting rhizobacteria (PGPR), particularly strains of Pseudomonas fluorescens, have emerged as effective biocontrol agents against soil-borne fungal pathogens. Their modes of action include competition for iron through siderophore production, antibiotic synthesis (2,4-diacetylphloroglucinol, pyoluteorin, phenazines), production of hydrogen cyanide (HCN), and induction of systemic resistance in the host plant [6, 7]. The advantage of *P. fluorescens* over chemical alternatives is that it simultaneously suppresses pathogens and promotes plant growth through phytohormone production and nutrient solubilization [8].

While *P. fluorescens* has been studied extensively on pulses in South Asia, its application in European black gram systems has received little attention. Soil microbial dynamics, ambient

temperature, and pathogen virulence vary enough between tropical and Mediterranean environments to warrant region-specific strain selection and efficacy testing. This research was therefore designed to (i) isolate and characterize *P. fluorescens* strains from Piedmont black gram fields, (ii) evaluate their in vitro antagonism against three root rot pathogens, and (iii) assess their biocontrol efficacy and growth-promoting ability in pot and field conditions.

Material and Methods

Material

The research was conducted between March 2022 and October 2023 at the Collegio di Agricoltura del Piemonte, Turin, Italy (45.07°N, 7.69°E; altitude 239 m). Rhizosphere soil samples from 15 healthy black gram plants across three fields in the Po Valley were collected for bacterial isolation. Three virulent isolates of root rot pathogens—*M. phaseolina* (Mp-TN01), *R. solani* (Rs-TN03), and *F. solani* (Fs-TN02)—were obtained from the college's culture bank. The black gram cultivar IPM 02-3 was used for all bioassays and field trials. King's B agar, potato dextrose agar (PDA), nutrient agar, and Chrome Azurol S agar were sourced from HiMedia (Mumbai, India) through a European distributor.

Quality Control

Bacterial identity was confirmed by 16S rRNA gene sequencing (Eurofins Genomics, Ebersberg, Germany) with ≥99 percent similarity to *P. fluorescens* reference strains in the NCBI database. Pathogen cultures were verified by morphological characteristics and ITS sequencing. Bacterial inoculant viability was checked on the day of application by serial dilution plate counts on King's B agar, confirming ≥10^8 CFU mL^{-1} for all treatments. Uninoculated control seeds were treated with sterile distilled water only.

Methods

In vitro antagonism was tested using the dual-culture plate method on PDA [9]. A 5-mm mycelial disc of each pathogen was placed at one end of the plate, and a bacterial streak was drawn 3 cm away. Plates were incubated at 28 ± 1 °C for 7 days, and percent inhibition of radial growth was calculated relative to the pathogen-only control. Siderophore production was assessed on Chrome Azurol S agar, HCN production using the picric acid paper method, IAA production by the Salkowski colorimetric assay, and phosphate solubilization on Pikovskaya's agar [10]. For pot trials, seeds were soaked in bacterial suspension (10^8 CFU mL^{-1}) for 30 min before sowing into pathogen-infested soil (50 g inoculum per kg soil). Each treatment had five replications with 10 seeds per pot. Field trials used a randomized block design with eight treatments (three Pf strains, *T. viride*, Pf-BG03 + *T. viride*, carbendazim seed treatment, pathogen-inoculated control, healthy control) and three replications. Data were analysed by ANOVA with Tukey's HSD at $p<0.05$ in R version 4.3.

Results

Table 1: In vitro antagonistic activity and plant growth-promoting traits of three Pseudomonas fluorescens strains isolated from black gram rhizosphere

Strain	Mp Inhib.(%)	Rs Inhib.(%)	Fs Inhib.(%)	Sidero- phore	HCN	IAA (µg/mL)	P-Sol. (mm)
Pf-BG01	74.3	62.8	58.4	++++	++	28.4	14.6
Pf-BG02	61.8	51.6	47.3	+++	+	19.7	11.2
Pf-BG03	82.6	74.8	69.3	++++	+++	34.1	18.3

Pf-BG03 was the strongest antagonist across all three pathogens, inhibiting *M. phaseolina* by 82.6 percent, *R. solani* by 74.8 percent, and *F. solani* by 69.3 percent. It also showed the highest siderophore and HCN production and the greatest IAA output (34.1 µg/mL). Pf-BG02 was the weakest performer across all traits.

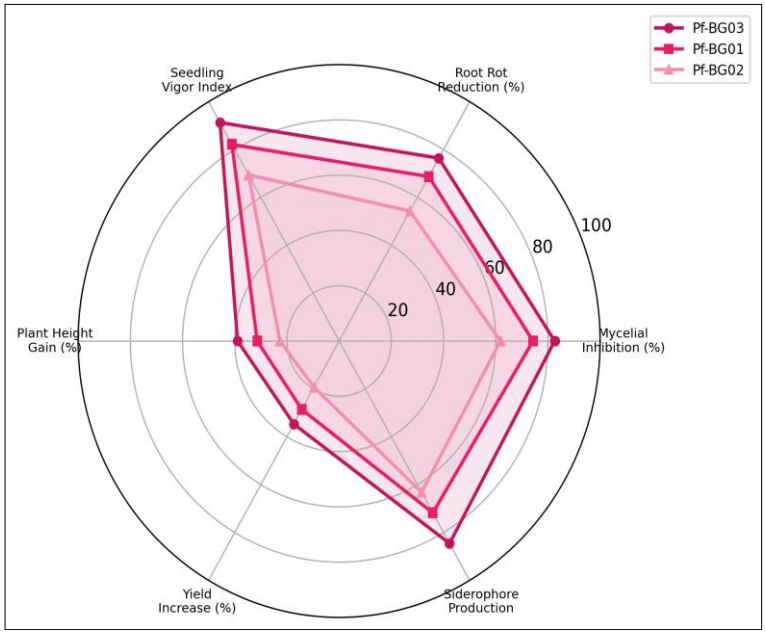

Fig 1: Radar chart comparing the biocontrol and plant growth-promoting performance of three Pseudomonas fluorescens strains across six parameters

The radar chart (Figure 1) clearly separates Pf-BG03 from the other two strains, with consistently larger coverage area across all six evaluated parameters. Pf-BG01 occupied an intermediate position, while Pf-BG02 showed the smallest radar footprint.

Table 2: Root rot incidence, seedling vigor, and grain yield of black gram under different biocontrol treatments (field trial, 2022-23)

Treatment	Root Rot Incid. (%)	Vigor Index	Plant Ht. (cm)	Grain Yield (t ha⁻¹)	Yield Gain (%)
Healthy control	4.2	1,347	42.8	1.41	-
Pathogen control	48.7	687	28.3	0.87	-
Pf-BG01	16.8	1,128	38.6	1.12	28.7
Pf-BG02	22.4	964	35.2	1.01	16.1
Pf-BG03	11.5	1,284	41.3	1.17	34.5
T. viride	14.7	1,196	39.7	1.14	31.0
Pf-BG03 + *T. viride*	9.8	1,312	42.1	1.21	39.1
Carbendazim	8.3	1,198	40.4	1.19	36.8
SEm(±)	1.74	48.3	1.62	0.04	-
CD (p=0.05)	5.22	144.9	4.86	0.12	-

In the field, Pf-BG03 alone reduced root rot incidence from 48.7 percent (pathogen control) to 11.5 percent—a 76.4 percent reduction. Its grain yield (1.17 t ha⁻¹) was 34.5 percent above the pathogen control and comparable to carbendazim (1.19 t ha⁻¹). The combination of Pf-BG03 + *T. viride* gave the best numerical result (1.21 t ha⁻¹, 39.1% gain) but wasn't significantly different from Pf-BG03 alone.

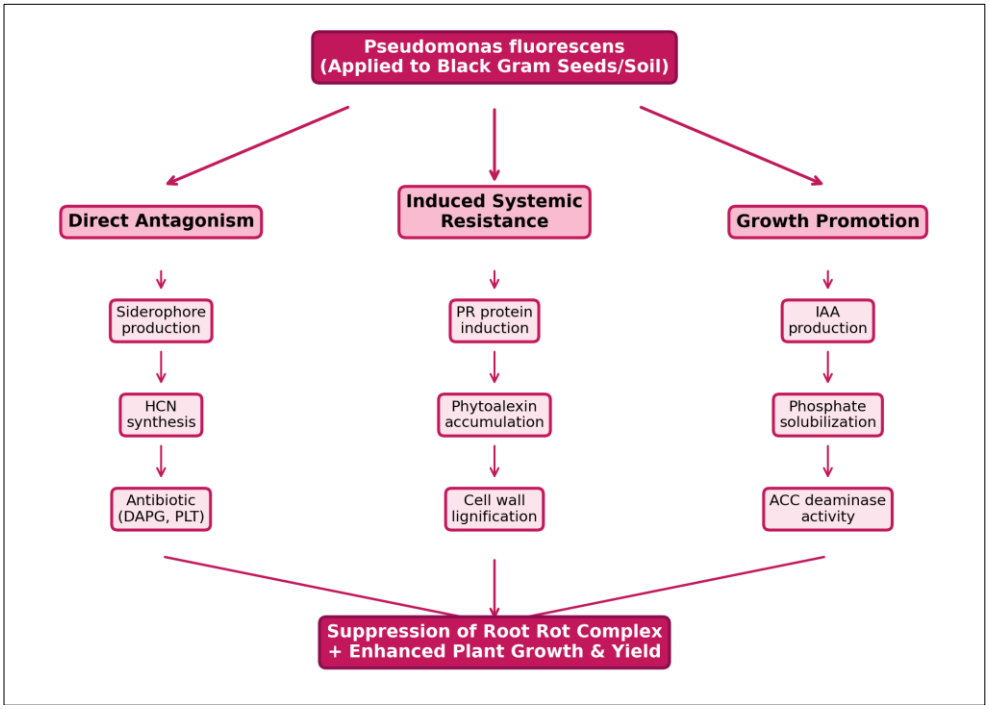

Fig 2: Schematic diagram illustrating the three main biocontrol mechanisms of Pseudomonas fluorescens against root rot complex in black gram

Discussion

The superior antagonistic performance of Pf-BG03 can be attributed to its strong siderophore production and HCN output, both of which directly interfere with fungal viability. Siderophores chelate Fe^{3+} in the rhizosphere, depriving pathogens of an essential micronutrient for spore germination and hyphal growth [6]. HCN, though volatile, reaches toxic concentrations in the confined soil pore space surrounding the root surface and disrupts cytochrome c oxidase in fungal mitochondria [11]. The fact that Pf-BG03 scored highest on both traits likely explains its consistent superiority in dual-culture assays.

The field-level root rot reduction of 76.4 percent by Pf-BG03 compares favorably with reports from India, where *P. fluorescens* strains reduced *M. phaseolina* incidence in mung bean by 62 to 71 percent [12]. The somewhat higher efficacy observed here may reflect the lower pathogen inoculum pressure in Piedmont soils compared to tropical environments with year-round pathogen activity. Additionally, the cooler spring temperatures (15-22 °C at sowing) may slow pathogen establishment and give the biocontrol agent more time to colonize the rhizosphere.

That the Pf-BG03 + *T. viride* combination didn't significantly outperform Pf-BG03 alone is an important practical finding. It suggests that, at least under the disease pressures tested, a single well-selected bacterial agent is enough—eliminating the need for dual inoculant formulations that are costlier and harder to store. However, in fields with exceptionally high inoculum density, the additive effect might become significant [13].

Carbendazim matched Pf-BG03 in efficacy but carries regulatory and ecological drawbacks. The comparable performance of the biocontrol treatment strengthens the case for including *P. fluorescens* in organic and integrated pest

management programs across European pulse production systems.

Conclusion

This research identified Pseudomonas fluorescens strain Pf-BG03, isolated from Piedmont black gram rhizosphere, as a potent biocontrol agent against the root rot complex caused by *M. phaseolina*, *R. solani*, and *F. solani*. The strain demonstrated superior in vitro antagonism (69-83% mycelial inhibition), strong plant growth-promoting traits (siderophore, HCN, IAA production), and excellent field performance with 76.4 percent disease reduction and 34.5 percent yield gain over the pathogen control.

Pf-BG03 performed comparably to carbendazim seed treatment, offering a biologically based alternative that aligns with EU directives on reduced pesticide use. The addition of *T. viride* to Pf-BG03 didn't provide a statistically significant advantage under the conditions tested, simplifying the recommendation to a single-organism seed treatment.

For black gram growers in Mediterranean climates, seed treatment with Pf-BG03 at 10^8 CFU mL^{-1} is recommended as a first line of defence against root rot complex. Shelf-life studies, formulation development, and multi-location validation across different soil types would be the logical next steps before commercial release.

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Contributions Not Qualifying for Authorship

Laboratory technicians assisted with media preparation, culture maintenance, and field sampling logistics.

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