

## Synergistic Effects of *Trichoderma harzianum* and Fluorescent Pseudomonads on Sheath Blight Management and Growth Promotion in Rice

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Rice (*Oryza sativa* L.) is among the most important staple crops worldwide and forms the dietary base for more than half of the global population. In India, it contributes significantly to food and nutritional security, economic stability, and the livelihood of millions of smallholder farmers. However, rice productivity is adversely affected by several diseases, of which sheath blight, caused by *Rhizoctonia solani* Kühn, is the second most destructive disease after rice blast (Senapati *et al.* 2022; Choudhary *et al.* 2020). The disease causes considerable yield losses ranging from 20% to 50% and is prevalent in regions characterized by high humidity and intensive rice cultivation. In susceptible rice cultivars, grain losses to the tune of 40% are reported annually with this disease (Goswami *et al.* 2017). The pathogen persists in soil as sclerotia and infects rice plants at the tillering stage, leading to lesions on leaf sheaths that can extend to flag leaves, ultimately reducing photosynthesis and grain filling (Singh *et al.* 2019). Chemical fungicides have long been used to control sheath blight, but their effectiveness is often short-lived, and their continuous use poses risks of environmental contamination, pathogen resistance, and disruption of beneficial soil microflora. This has necessitated the exploration of sustainable and eco-friendly approaches for disease management.

Biological control using beneficial microorganisms has emerged as a promising and environmentally safe alternative for managing soil-borne pathogens. Among the well-known microbial antagonists, *Trichoderma harzianum* and *Pseudomonas fluorescens* have received particular attention for their antagonistic potential against several plant pathogens and their capacity to promote plant growth (Kashyap *et al.* 2020; Srivastava *et al.* 2012). *Trichoderma* species are known to suppress pathogens through mechanisms such as mycoparasitism, production of hydrolytic enzymes (chitinases and glucanases), competition for nutrients and space, and secretion of antifungal metabolites (Rai *et al.* 2016; Kashyap *et al.* 2017). They also improve plant vigor by producing phytohormones like indole acetic acid, gibberellins, and cytokinins, enhancing root colonization and nutrient uptake. Similarly, *Pseudomonas fluorescens*, a well-characterized plant growth-promoting rhizobacterium (PGPR), exhibits biocontrol activity through the production of siderophores, antibiotics, and other metabolites that inhibit pathogen growth, while simultaneously inducing systemic resistance in plants (Srivastava *et al.* 2013; Solanki *et al.* 2014). The combined application of these microbial antagonists can therefore provide a dual benefit of growth promotion and disease



suppression, aligning well with sustainable agricultural practices.

The present study was conducted to evaluate the potential of different isolates of *T. harzianum* and *P. fluorescens* (Table 1) for growth promotion and sheath blight management in rice under controlled pot conditions. It was hypothesized that application of these bioagents through seed bio-priming or seedling root dipping would improve rice growth and significantly reduce sheath blight severity through antagonism and plant defense activation. The experiment was performed on rice variety Pusa Basmati-1 in a completely randomized design with ten treatments and three replications. Six isolates of *T. harzianum* (IRRI-1, SV-3, SV-19, SV-21, SV-26, and SV-27) and two isolates of *P. fluorescens* (PF-2 and PF-4) were tested for antagonistic using *in-vitro* dual culture assay. The bioagents were also applied by two methods: seed bio-priming, where rice seeds were coated with microbial formulations and incubated for 12 hours before sowing, and seedling root dipping, where 25-day-old seedlings were dipped in microbial suspensions before transplanting. For the seed bio-priming treatments, ten different treatments were employed. In treatment T1, seeds were bioprimed with

*fluorescent Pseudomonas* isolate Pf-2 at the rate of 1 g per 100 g of seed. In T2, *fluorescent Pseudomonas* isolate Pf-4 was used at the same rate. Treatments T3 to T7 involved seed bio-priming with different isolates of *Trichoderma harzianum* at 1 g per 100 g of seed- specifically, isolates IRRI-1 (T3), SV-3 (T4), SV-19 (T5), SV-21 (T6), and SV-26 (T7). In T8, seeds were bioprimed with *T. harzianum* isolate SV-27 at 1 g per 100 g of seed. Treatment T9 consisted of chemical seed treatment with Carbendazim at 2 g per kg of seed, while T10 served as the untreated control (seed without any treatment). Similarly, for root dipping treatments, ten different treatments were carried out. In T1, seedlings were subjected to root dipping with *Pseudomonas fluorescens* isolate Pf-2 at 10 g per litre of water, while T2 involved root dipping with *Pseudomonas fluorescens* isolate Pf-4 at the same rate. Treatments T3 to T8 involved root dipping with various isolates of *Trichoderma harzianum* at 10 g per litre of water, namely IRRI-1 (T3), SV-3 (T4), SV-19 (T5), SV-21 (T6), SV-26 (T7), and SV-27 (T8). Treatment T9 included root dipping with Carbendazim at 2 g per litre of water, and T10 served as the untreated control (root dipping without any treatment). An untreated control and a chemical check (Carbendazim) were included for comparison.

**Table 1:** Effect of different bio agents of *P. fluorescens* and *T. harzianum* on the *in-vitro* growth of *Rhizoctonia solani*

Treatments details	Fungal colony (cm) in <i>in vitro</i> dual culture technique			
	After 3 days	Percent inhibition over control	After 7 days	Percent inhibition over control
<i>Trichoderma harzianum</i> (IRRI-1)	1.50	68.75	2.12	77.44
<i>Trichoderma harzianum</i> (SV-3)	1.60	66.66	2.50	73.40
<i>Trichoderma harzianum</i> (SV-19)	2.89	60.62	3.00	68.05
<i>Trichoderma harzianum</i> (SV-21)	1.68	65.00	2.50	73.40
<i>Trichoderma harzianum</i> (SV-26)	1.90	60.41	2.45	73.40
<i>Trichoderma harzianum</i> (SV-27)	1.89	60.62	2.40	74.46
<i>Pseudomonas fluorescens</i> (PF-2)	1.62	66.45	3.10	67.02
<i>Pseudomonas fluorescens</i> (PF-4)	1.71	64.37	2.90	69.14
Control	4.80		9.40	
CD(P=0.05)	0.217		0.476	

CD: Critical difference

The pathogen *R. solani* was isolated from diseased rice plants showing typical sheath blight symptoms and purified on potato dextrose agar. Pathogenicity was confirmed by inoculating healthy plants and fulfilling Koch's postulates. Disease severity was quantified as

relative lesion height (RLH), calculated by dividing lesion length by total plant height and multiplying by 100. Growth parameters, including plant height, root and shoot length, fresh and dry biomass, and chlorophyll content (measured using a SPAD meter), were recorded at harvest.



Statistical analyses were performed using ANOVA under a completely randomized design at a 5% significance level. The results of the study indicated that all the test *T. harzianum* isolates tested for antagonistic activity against *R. solani* were found antagonistic against *R. solani* due to considerable mycelial growth inhibition of the latter. Among the *T. harzianum* isolates, IRRI-1 followed by SV-3 was found most aggressive (Table 1). Similarly, two *P. fluorescens* isolates (PF-2 and PF-4) also inhibited the growth of *R. solani*. The antagonistic potential of *T. harzianum* against the majority of the fungal pathogen is well documented. The *R. solani* being grown in a dual culture may be deprived of the nutrients and space for growth. This may be another reason for the inhibition of the growth of *R. solani* because of the tough competition posed by *T. harzianum* in dual culture. The production of volatile and non-volatile secondary metabolites by *T. harzianum* is another reason for the inhibitory effect against *R. solani*. These volatile and non-volatile secondary metabolites by their toxicity may result in the inhibition of fungal growth. Meena *et al.* (2003) tested three *Trichoderma* species for the production of volatiles and found that *T. harzianum* was effective in suppressing the growth by 80% and sclerotia formation by 33.5 % of *R. solani* f. sp. *sasakii* *in vitro*. It has been reported that *T. harzianum* and *T. viride*, when tested in dual culture against *R. solani* *in vitro*, started inhibiting the growth of *R. solani* after seven

days and covered the entire petri dish within 10 days of inoculation. Thus, the present study's finding regarding growth inhibition by *T. harzianum* conforms with the report of earlier workers mentioned above. *P. fluorescens* is a bacterial bioagent that acts by producing siderophores (Fe chelating compounds). Since chelated Iron (Fe) cannot be utilized by the fungal organism, they become Fe-deficient, and their growth, as well as virulence and aggressiveness, are inhibited. This activity is evident in the present finding also as two isolates of *P. fluorescens* have been inhibitory against the radial/mycelial growth of *R. solani*.

The results of the present study revealed that all microbial treatments significantly improved rice growth compared to the untreated control. Among all isolates, *T. harzianum* IRRI-1 was the most effective, producing the tallest plants (99.66 cm), longest roots (21.56 cm), and highest shoot length (77.53 cm) under the seed bio-priming method (Table 2). Chlorophyll content also increased significantly (SPAD value 39.7) under this treatment, indicating enhanced photosynthetic efficiency and plant health. *T. harzianum* SV-3 and *P. fluorescens* PF-2 were the next most effective treatments, showing considerable improvement in plant height and biomass accumulation. Seedling root dipping treatments also resulted in improved growth compared to the control, although the response was slightly lower than that of seed bio-priming.

**Table 2:** Effect of seed bioprimering and seedling root dip with selected isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* on growth parameters of rice

Treatments	Seed Bioprimering				Seedling Root Dip			
	Plant height (cm)	Root length (cm)	Shoot length (cm)	Chlorophyll content (SPAD)	Plant height (cm)	Root length (cm)	Shoot length (cm)	Chlorophyll content (SPAD)
T1	99.66	21.56	77.53	39.7	95.20	22.56	75.63	43.7
T2	93.76	21.13	72.20	37.5	92.73	22.26	70.46	36.37
T3	93.30	18.50	74.80	36.1	92.80	19.63	73.16	34.7
T4	91.30	18.06	73.23	38.7	90.80	18.03	72.76	37.1
T5	92.63	17.56	75.06	35.2	91.60	17.63	73.96	34.6
T6	92.33	16.80	75.53	37.8	91.86	16.73	75.13	42.5
T7	92.06	16.73	75.33	37.4	91.30	15.66	72.63	27.7
T8	90.50	17.56	72.93	37.0	90.43	17.43	73.00	27.0
T9	82.63	16.13	66.50	37.2	81.80	15.02	66.80	37.5
T10	73.70	12.36	61.33	23.1	71.36	11.53	59.83	22.5
CD ( $p \leq 0.05$ )	3.51	0.65	1.42	1.13	3.15	0.76	1.14	1.58

CD: Critical difference



Fresh and dry biomass measurements further confirmed the growth-promoting potential of these bioagents. *T. harzianum* IRR1-1-treated plants recorded the highest root and shoot fresh weights (16.66 g and 36.91 g, respectively) and dry weights (8.23 g and 26.60 g, respectively) (Table 3). These enhancements can be attributed to improved nutrient uptake, root system development, and enhanced metabolic activity resulting from microbial interactions in the rhizosphere. Similar findings were reported by Harman (2004) and Bisen *et al.* (2016), who demonstrated that *Trichoderma* species enhance nutrient acquisition and stimulate root growth through hormonal and enzymatic regulation. Likewise, *Pseudomonas fluorescens* strains are known to produce auxins and siderophores that promote

root elongation and improve plant nutrient status (Reddy *et al.* 2008).

All microbial treatments significantly reduced sheath blight severity compared to the untreated control, which recorded an RLH of 9.36%. *T. harzianum* IRR1-1 reduced disease severity to 3.41%, corresponding to a 63.56% reduction. *T. harzianum* SV-3 and *P. fluorescens* PF-4 also exhibited strong antagonistic effects, reducing disease severity by 57.58% and 56.41%, respectively (Table 3). Under the root dipping method, *T. harzianum* IRR1-1 and *P. fluorescens* PF-4 achieved the highest reductions of 64.11% and 51.79%, respectively. These results highlight the superior antagonistic efficacy of *T. harzianum* IRR1-1 in suppressing *R. solani* infection.

**Table 3:** Effect of seed biopriming and seedling root dip with *Trichoderma harzianum* and *Pseudomonas fluorescens* on fresh and dry weight of rice root and shoot

Treatments	Seed Biopriming				Seedling Root dip			
	Root fresh weight (g)	Root dry weight (g)	Shoot Fresh Weight (g)	Shoot dry weight	Root fresh weight	Root dry weight	Shoot Fresh Weight	Shoot dry weight
T1	16.66	8.23	36.91	26.60	16.66	6.26	30.32	21.67
T2	15.42	6.28	26.44	18.76	15.42	5.55	26.58	17.66
T3	13.59	3.48	25.82	18.71	13.59	3.88	26.17	18.61
T4	14.73	4.15	25.51	18.80	14.73	4.17	24.91	17.39
T5	14.82	3.23	35.91	26.44	14.82	2.92	29.32	20.80
T6	15.18	3.96	26.36	19.06	15.18	2.97	24.54	18.05
T7	14.89	4.04	27.24	19.65	14.89	2.55	26.33	18.88
T8	15.83	4.07	26.80	18.70	15.83	4.08	26.14	18.36
T9	12.82	2.99	25.59	17.12	12.82	2.43	25.18	16.13
T10	10.69	2.32	17.18	10.59	10.69	1.53	16.92	8.93
CD(P=0.05)	2.01	1.56	3.22	3.57	2.01	1.73	3.69	2.65

CD: Critical difference

The disease suppression observed in this study can be attributed to several direct and indirect mechanisms. *Trichoderma* species are known to parasitize pathogenic hyphae by coiling around them and secreting cell wall-degrading enzymes such as chitinase and glucanase (De França *et al.* 2015). They also produce antibiotics and volatile compounds that inhibit pathogen growth. Additionally, *Trichoderma* and *Pseudomonas* species can

trigger induced systemic resistance (ISR) in plants by stimulating defense-related enzymes and phenolic compounds, enhancing the plant's ability to resist subsequent infections (Vinale *et al.* 2008; Alfano *et al.* 2007). The activation of these biochemical defenses explains the reduction in sheath blight severity and improved overall plant health observed in the treated plants.



A comparison between the two application methods revealed that seed bio-priming was more effective in enhancing plant growth and chlorophyll content, while seedling root dipping provided slightly better disease suppression (Table 4). This difference could be due to the early establishment of beneficial microbes in the rhizosphere during germination in the case of bio-priming, whereas root dipping ensured direct colonization of root tissues at the transplanting stage, where pathogen attack usually begins. Both methods, therefore, have distinct advantages and could be integrated for maximum benefit. The study demonstrates the potential of *Trichoderma*

*harzianum* and *Pseudomonas fluorescens* as eco-friendly bioagents for the dual purpose of growth enhancement and sheath blight management in rice. The isolates exhibited strong antagonistic activity against *R. solani*, improved physiological and morphological growth parameters, and reduced disease severity significantly. Among all treatments, *T. harzianum* IRRI-1 consistently produced the best results across both methods. The dual application strategy of seed bio-priming for growth promotion and seedling root dipping for disease suppression can be recommended for sustainable rice cultivation.

**Table 4:** Effect of seed biopriming and seedling root dip with *Trichoderma harzianum* and *Pseudomonas fluorescens* on sheath blight disease in rice

Treatment	Seed Biopriming			Seedling Root Dip		
	Lesion length (cm)	RLH (%)	Reduction (%)	Lesion length (cm)	RLH (%)	Disease Reduction (%)
T1	3.40	3.41	63.56	3.53	3.70	64.11
T2	3.73	3.97	57.58	4.76	5.13	50.24
T3	3.83	4.10	56.19	4.46	4.80	53.44
T4	4.23	5.46	41.66	5.30	5.83	43.45
T5	5.06	5.46	41.66	5.80	6.63	35.69
T6	5.63	6.09	34.93	5.50	5.98	41.99
T7	4.73	5.13	45.19	5.56	6.05	41.31
T8	3.96	4.08	56.41	4.50	4.97	51.79
T9	5.36	6.48	30.76	5.00	6.11	40.73
T10	6.90	9.36	–	7.36	10.31	–
CD ( $p \leq 0.05$ )	1.40			1.62		

CD: Critical difference; RLH: Relative lesion height

In conclusion, integrating microbial bioagents such as *T. harzianum* and *P. fluorescens* into rice disease management practices offers an effective, economical, and environmentally safe approach for controlling sheath blight while enhancing crop growth and productivity. The combined use of these microorganisms not only reduces dependence on chemical fungicides but also improves soil health and resilience of rice plants. The findings highlight the potential of biological control as a core component of integrated disease management strategies and contribute to the long-term sustainability of rice production systems. Further field evaluations and formulation standardization are recommended to validate these findings and facilitate large-scale adoption by farmers.

### Authors' Contributions

All authors contributed equally for preparing the final version of the manuscript.

### Conflict of Interest

Authors declare no conflict of interest.

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