

EVALUATION OF NATIVE ISOLATES OF *Trichoderma* AGAINST FUNGAL PATHOGENS OF RICE, WITH EMPHASIS ON *Fusarium proliferatum*

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ABSTRACT

Rice (*Oryza sativa* L.) is a major staple food crop of Nepal; however, its productivity is constrained by fungal diseases, including blast (*Pyricularia oryzae*), sheath blight (*Rhizoctonia solani*), foot rot/seedling blight (*Fusarium proliferatum*). The present study aimed to evaluate the biocontrol potential of native *Trichoderma* isolates against major fungal pathogens of rice and to assess their effects on rice seedling vigor and suppression of *F. proliferatum*-induced seedling blight. Forty *Trichoderma* isolates were screened for antagonistic activity against *F. proliferatum*, *P. oryzae*, and *R. solani* and dual culture assays, followed by seed biopriming and screenhouse evaluation. Among the tested isolates, TL6-36B, TL2-50A, and TL4-81A completely inhibited the growth of all three pathogens in the dual culture assay. TL1-2A also showed strong antagonistic activity and significantly improved seed germination and seedling growth. Based on their *in vitro* performance and growth-promoting effects, ten promising isolates were further evaluated in pot experiments under screenhouse conditions using rice cv. Khumal-4. The selected isolates significantly reduced the incidence and severity of seedling blight caused by *F. proliferatum*. In the seed treatment experiment, TL1-2A reduced disease severity by up to 74.55%, whereas TL4-81A reduced it by 68.41% compared with the pathogen-inoculated control. In soil treatment by drenching, TL4-81A and TL1-2A reduced disease severity by 68.95% and 67.89%, respectively. These isolates also improved seed germination and seedling vigor. Overall, native *Trichoderma* isolates, particularly TL1-2A and TL4-81A, showed promising biocontrol activity against *F. proliferatum* and may have potential for integrated management of rice seedling blight in Nepal.

Keywords: Biocontrol, *Trichoderma*, plant growth promotion, rice pathogens

INTRODUCTION

Rice (*Oryza sativa* L.) is a major staple food crop of Nepal, cultivated on nearly 1.43 million hectares of land and producing about 5.72 million metric tonnes of paddy annually (MoALD, 2023/24). Despite its importance, rice production in Nepal is constrained by several abiotic and biotic stresses that reduce both yield and grain quality. Among biotic factors, fungal diseases have remained a major constraint (Manandhar 1987). Several economically important fungal pathogens affect rice crops in Nepal, including *Bipolaris oryzae* (brown spot), *Fusarium proliferatum* (foot rot/bakanae), *Pyricularia oryzae* (blast), *Rhizoctonia solani* (sheath blight), *Sarocladium oryzae* (sheath rot),

Sclerotium oryzae (stem rot), and *Ustilaginoidea virens* (false smut), among others (Manandhar, 2017a, b).

Among these diseases, bakanae or foot rot, a destructive seed-borne disease, has become an important problem in fine and aromatic rice. Although the disease was considered to be of minor importance before the 1980s, it became more prominent following the release of the susceptible cultivar Khumal-4 in 1987 and has since caused significant yield losses of up to 40% in affected areas (Desjardins et al., 2000; Paudel et al., 2024). The disease is primarily seed-borne, and seed infection levels of up to 83% have been reported in Nepal (Manandhar, 2017b). The disease causes seedling blight in nurseries with or without typical characteristic elongation symptoms. Both local and improved rice varieties are susceptible to bakanae (Desjardins et al., 2000). The popular medium-fine cultivars Khumal-4 and Taichung-176, which are widely grown in the mid-hills including Nuwakot and Kathmandu Valley, are highly susceptible to bakanae/seedling blight (Manandhar, 2017a).

Management of rice diseases in Nepal relies mainly on chemical fungicides, supplemented by resistant varieties and cultural practices. However, increasing concerns about environmental safety and human health have encouraged greater attention to alternative disease-management approaches. Biological control offers an environmentally safe and sustainable option for managing rice diseases (Eziashi et al., 2007). Several bacterial and fungal antagonists have been investigated, among which *Trichoderma* species are widely used because of their strong antagonistic activity, plant growth-promoting effects, and ability to induce host resistance (Harman, 2006; Jogaiah et al., 2017; Li et al., 2018).

In Nepal, the use of commercial strains of *Trichoderma* spp. and *Pseudomonas fluorescens* for disease management is increasing (Manandhar, 2017b; Manandhar and Timila, 2019), but their efficacy remains a concern. The effectiveness of *Trichoderma* varies depending on the strain, pathogen virulence, and environmental conditions (Rokaya et al., 2023; Yadav et al., 2024, 2026). Therefore, identification and evaluation of native *Trichoderma* isolates with high biocontrol potential are important. This study aimed to evaluate indigenous *Trichoderma* isolates for their antagonistic activity against major fungal pathogens of rice, including *Fusarium proliferatum*, *Pyricularia oryzae*, and *Rhizoctonia solani*, and to assess their effectiveness in enhancing rice seedling vigor and suppressing seedling blight caused by *F. proliferatum*.

MATERIALS AND METHODS

Trichoderma isolates

Forty native *Trichoderma* isolates used in this study were maintained on potato dextrose agar (PDA) slants at Nepal Plant Disease and Agro Associates (NPDA), Balaju, Kathmandu. The isolates were originally obtained from soil collected from different locations of Likhu Rural Municipality, Nuwakot district. For revival, cultures were transferred onto freshly prepared PDA plates and incubated at 25°C for three days. Actively growing hyphal tips were then sub-cultured onto new PDA plates and incubated for seven days. The *Trichoderma* isolates were confirmed based on their cultural and morphological characteristics following Barnett and Hunter (1972).

Sporulated cultures were flooded with sterile water and gently scraped with a sterile loop to harvest spores. The suspension was filtered through muslin cloth and adjusted to a concentration of 1×10^9 spores/ml using a hemocytometer. This suspension was used for seed biopriming and pot experiments.

Rice pathogens

Three economically important rice pathogens - *Fusarium proliferatum* (seedling blight/foot rot/bakanae), *Pyricularia oryzae*, and *Rhizoctonia solani* were used in dual culture assays. The pathogens were originally isolated from rice seeds and plants and maintained on PDA slants at NPDA, Balaju, Kathmandu.

For *F. proliferatum*, a rice seed sample of the cv. Khumal-4 was obtained from the National Agronomy Research Centre of Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur. The rice seeds were plated on three-layered water-soaked blotter paper in Petri plates and incubated under a 12-hour alternate cycle of light and darkness at 25 ± 2 °C for seven days. Whitish mycelial growth on the rice seeds was examined under a stereomicroscope and identified using a compound microscope. The mycelial growth was then transferred to PDA and incubated at 25 ± 2 °C for culture.

For *P. oryzae*, a rice seed sample of the cv. Taichung-176 was collected from Dhikure, Nuwakot. The rice seeds were plated on three-layered water-soaked blotter paper and incubated as described above for *F. proliferatum*. Fungal growth on the incubated seeds was examined under a stereomicroscope, identified using a compound microscope, and transferred to PDA plates for culture.

For *R. solani*, infected leaf sheaths were collected from a rice field at Dhikure, Nuwakot. The samples were washed with tap water, cut into approximately 1 cm² pieces, and surface-sterilized with 1% NaOCl for one minute. The sterilized sheath pieces were air-dried under a laminar airflow cabinet, placed on water agar plates, and incubated at 25 ± 2 °C for three days. The fungal growth was examined under a compound microscope, transferred aseptically to PDA plates, and incubated at 25 ± 2 °C for culture.

Dual-culture assay

Dual-culture assays were conducted to assess the ability of the *Trichoderma* isolates to restrict the growth of the major rice pathogens. For *F. proliferatum* and *P. oryzae*, 6-mm mycelial discs from seven-day-old cultures were placed on dual culture plates three days before inoculation with *Trichoderma* to allow establishment of the relatively slower-growing pathogens (Nyakeko et al., 2026; Dieumerici et al., 2026). For *R. solani*, the pathogen and *Trichoderma* discs were inoculated on the same day.

Discs of the pathogen and *Trichoderma* isolates were placed 0.5 cm from the edge at opposite ends of 9 cm Petri plates and incubated at 25 ± 2 °C. The experiment was arranged in a completely randomized design with five replications, with one Petri plate serving as one replication. Plates inoculated only with the pathogen served as controls. Radial growth of the pathogens was recorded daily until the pathogen in the control plates reached the edge of the Petri plate. The percent inhibition of pathogen growth was calculated using the formula by Vincent (1947).

$$P_i (\%) = [(C - T)/C] \times 100$$

Where, C is the radial growth of the pathogen in control plates, and T is the radial growth of the pathogen in the presence of *Trichoderma* isolates.

Seed biopriming with *Trichoderma*

Seeds of rice cv. Taichung-176 were soaked overnight in respective *Trichoderma* suspensions of all 40 isolates. Seeds soaked in sterile water served as control. The bioprimed seeds were air-dried and placed on water-soaked blotter papers (60 × 45 cm) with four replications per treatment. Each replication consisted of 100 seeds arranged in a 12 × 8 pattern, with the remaining 4 seeds placed randomly. The blotters were rolled, placed in polythene bags, and incubated at 25 ± 2 °C for 10 days.

The number of germinated seeds, plumule length, and radicle length were recorded. Seedling vigor index was calculated as follows:

$$\text{Seedling vigor index (\%)} = (\text{Plumule length} + \text{Radical length}) \times \text{Germination percentage}$$

Evaluation of *Trichoderma* efficacy against *F. proliferatum*

Based on the results of dual culture assay and seed biopriming experiment, ten promising *Trichoderma* isolates were selected for pot experiments under screenhouse conditions. Two application methods, seed treatment and soil drenching, were evaluated.

For seed treatment, surface-sterilized seeds of rice cv. Khumal-4 were soaked in sterile water for 24 h, followed by soaking in *F. proliferatum* suspension (1×10^6 conidia/ml) for 24 h and then in the respective *Trichoderma* isolate suspension (1×10^9 conidia/ml) for an additional 24 h (Pal et al., 2019). The pathogen-treated control consisted of seeds soaked only in *F. proliferatum* suspension, whereas the untreated control consisted of seeds soaked in sterile water.

For soil drenching, seeds were soaked in sterile water for 24 h and subsequently soaked in an *F. proliferatum* suspension (1×10^6 conidia/ml) for 24 h. The pots were drenched with the respective *Trichoderma* suspension at 50 ml per pot 24 h before sowing. Seeds were sown at the rate of 50 seeds per pot in approximately 400 g of potting mixture consisting of soil, sand, and vermicompost in a ratio of 3:1:1). The potting mixture was autoclaved before use to minimize the effects of native microorganisms and maintain controlled experimental condition.

The experiment was arranged in a completely randomized design with five replications. Screenhouse temperatures ranged from 30-35 °C during the day and 23-28 °C at night. Seedlings were manually irrigated daily. Disease incidence, disease severity, and seedling growth parameters were recorded 20 days after sowing.

Disease severity was assessed using a modified 1–5 scoring scale based on the method described by Zainudin et al. (2008). The scoring criteria were as follows: 1 = healthy and symptomless seedlings; 2 = stunted seedlings with yellow leaves; 3 = thin seedlings with narrow pale-yellow leaves; 4 = seedlings with abnormal elongated internodes and chlorotic or brownish leaves; and 5 = seedlings showing visible fungal growth on infected tissues or dead seedlings.

Disease incidence and severity were assessed using the following formulas:

$$\text{Disease incidence \%} = \frac{\text{Total number of infected plants in pot}}{\text{Total number of plants in pot}} \times 100$$

$$\text{Disease severity \%} = \frac{\text{Sum of all numerical rating}}{\text{Number of seedlings observed} \times \text{Highest score (5)}} \times 100$$

Data analysis

The data were subjected to analysis of variance (ANOVA), and treatment means were compared using Duncan's multiple range test at the 5% probability level. Statistical analyses were performed using GENSTAT, 15th edition.

RESULTS

Effect of selected *Trichoderma* isolates on pathogen growth in dual culture

Dual culture assays were conducted to evaluate the ability of 40 *Trichoderma* isolates to suppress the growth of three major pathogens, *R. solani*, *P. oryzae*, and *F. proliferatum*. After seven days of incubation, significant differences were observed among *Trichoderma* isolates in their ability to inhibit pathogen growth (Table 1, Fig. 1).

Against *R. solani*, inhibition percentage varied significantly among the tested isolates, ranging from 17.76% to 100%. The highest inhibition was recorded with TL1-2A and TL6-36B (100%), followed by TL2-50A (98.90%), TL2-56A (98.33%), TL6-24B (98.90%), TL4-81A (98.83%), and TL6-29A (95.67%). Several other isolates, including TL6-27A, TL6-26B, TL1-5B, TL1-4A, and TL5-21A, also showed strong inhibition (>80%) against *R. solani*.

For *P. oryzae*, inhibition values ranged from 15.42% to 100%. Complete inhibition (100%) was recorded with TL1-2A, TL6-36B, TL2-48A, TL2-50A, TL2-56B, TL1-73A, TL6-24B, and TL4-81A. TL5-21A also showed high inhibition (91.77%). Other isolates showed moderate to low inhibition, ranging from approximately 20%-60%.

For *F. proliferatum*, the inhibitory effect of the *Trichoderma* isolates against *F. proliferatum* varied considerably, ranging from 7.37% to 100%. Complete inhibition (100%) was recorded with TL6-36B, TL2-48A, TL2-50A, TL2-56A, TL2-56B, TL1-73A, and TL4-81A. Other isolates, including TL1-5B, TL1-8A, TL6-32B, and TL1-62B, and TL1-72A, exhibited moderate inhibition, ranging from approximately 33% to 43%.

Table 1. Dual culture assay of forty *Trichoderma* isolates against *Rhizoctonia solani*, *Pyricularia oryzae*, and *Fusarium proliferatum* after 7 days of incubation at 25 ± 2 °C

<i>Trichoderma</i> isolates	Inhibition percent			<i>Trichoderma</i> isolates	Inhibition percent		
	<i>R. solani</i>	<i>P. oryzae</i>	<i>F. proliferatum</i>		<i>R. solani</i>	<i>P. oryzae</i>	<i>F. proliferatum</i>
TL1-2A	100 ^a	100 ^a	77 ^b	TL6-32C	60.49 ^{f-k}	40.53 ^{e-h}	10 ^{kl}
TL1-3A	64.47 ^{d-k}	32.4 ^{g-j}	21.58 ^{f-l}	TL6-34A	49.13 ^{h-m}	30.47 ^{h-j}	16.84 ^{h-l}
TL1-4A	85 ^{a-c}	29.9 ^{h-j}	31.04 ^{e-h}	TL6-34B	77.6 ^{a-g}	50.13 ^{c-f}	20.79 ^{f-l}
TL1-5A	71.48 ^{c-h}	34.77 ^{g-j}	22.11 ^{f-k}	TL6-36A	54.61 ^{g-k}	38.4 ^{f-h}	25.26 ^{d-j}
TL1-5B	86.67 ^{a-c}	32.29 ^{g-j}	42.89 ^e	TL6-36B	100 ^a	100 ^a	100 ^a
TL1-5C	71.9 ^{c-h}	28.87 ^{h-j}	23.68 ^{e-k}	TL2-42B	61.97 ^{e-k}	34.07 ^{g-j}	14.47 ^{i-l}
TL1-8A	81.1 ^{a-f}	29.93 ^{h-j}	41.84 ^e	TL2-48A	44.69 ^{j-m}	100 ^a	100 ^a
TL5-15A	17.76 ⁿ	15.42 ^k	7.37 ^l	TL2-49A	54.77 ^{g-l}	36.19 ^{g-i}	23.15 ^{c-k}
TL5-18A	35.13 ^{l-n}	24.43 ^{i-k}	18.15 ^{h-l}	TL2-50A	98.9 ^a	100 ^a	100 ^a
TL5-18B	29.68 ^{mn}	23.5 ^{jk}	13.95 ^{i-l}	TL2-54A	68.27 ^{c-j}	52.1 ^{b-e}	21.84 ^{f-l}
TL5-18C	30.28 ^{mn}	22.73 ^{jk}	19.65 ^{g-l}	TL2-54B	69.83 ^{c-i}	41.47 ^{e-h}	11.62 ^{j-l}
TL5-21A	80 ^{a-f}	91.77 ^a	20.52 ^{f-l}	TL2-56A	98.33 ^a	49.8 ^{c-f}	100 ^a
TL6-24B	98.9 ^a	100 ^a	23.68 ^{e-k}	TL2-56B	47.4 ^{i-m}	100 ^a	100 ^a
TL6-26B	88 ^{a-d}	39.59 ^{f-h}	25.26 ^{d-j}	TL2-56C	60.23 ^{f-k}	56.73 ^{bc}	12.36 ^{j-l}
TL6-27A	89.33 ^{a-c}	29.53 ^{h-j}	10.53 ^{kl}	TL1-62B	80.43 ^{a-e}	36.08 ^{g-i}	33.68 ^{c-g}
TL6-29A	95.67 ^{ab}	31.1 ^{g-j}	27.63 ^{d-i}	TL1-73B	85.67 ^{a-d}	32.8 ^{g-j}	11.84 ^{j-l}
TL6-30A	42.7 ^{k-m}	53.07 ^{b-d}	16.57 ^{h-l}	TL1-72A	67.23 ^{c-j}	43.14 ^{d-g}	34.47 ^{c-f}
TL6-31A	53.92 ^{g-l}	34.1 ^{g-j}	20.26 ^{f-l}	TL1-73A	58.87 ^{f-k}	100 ^a	100 ^a
TL6-32A	48.9 ^{h-m}	39.2 ^{f-h}	37.1 ^{c-e}	TL4-81C	65.86 ^{c-k}	62.35 ^b	21.58 ^{f-l}
TL6-32B	73.5 ^{b-g}	38.83 ^{f-h}	38.15 ^{cd}	TL4-81A	98.83 ^a	100 ^a	100 ^a
SEM $\pm$	6.92	5.14	7.3	SEM $\pm$	6.92	5.14	7.3
CV %	17.4	12.4	19.5	CV	17.4	12.4	19.5
p-value	<.001**	<.001**	<.001**	p-value	<.001**	<.001**	<.001**

Treatment means followed by the same letters within columns are not significantly different based on DMRT value at <1% (**) and <5% (*) significance level. CV = Coefficient of variance; SEM = Standard error of the means

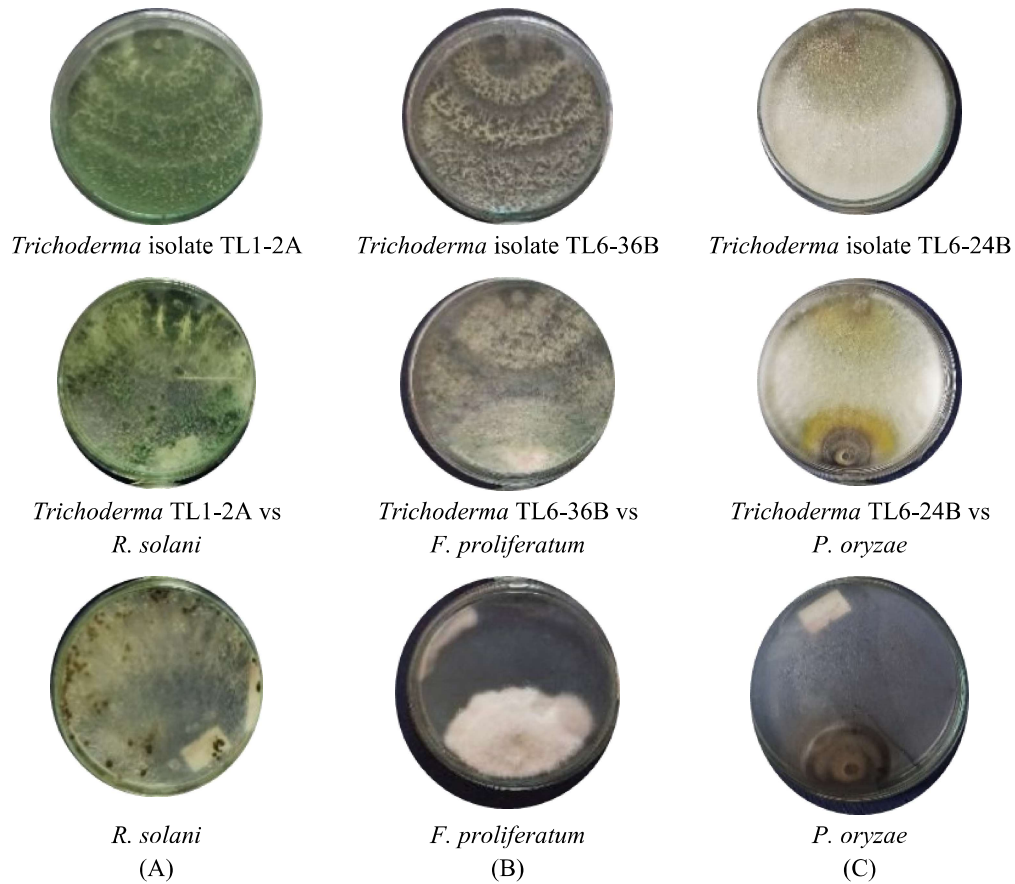


Fig. 1. Dual culture of *Trichoderma* isolates with pathogens: (A) TL1-2A against *Rhizoctonia solani*, (B) TL6-36B against *Fusarium proliferatum*, and (C) TL6-24B against *Pyricularia oryzae* at 7 days after inoculation.

Among the tested isolates, TL6-36B, TL2-50A, and TL4-81A showed broad-spectrum antagonistic activity, completely inhibiting the growth of all three pathogens. TL2-48A, TL2-56B, and TL1-73A completely inhibited both *P. oryzae* and *F. proliferatum* but showed TL1-2A and TL6-24B showed strong inhibition against *R. solani* and *P. oryzae*, but their inhibition of *F. proliferatum* was lower.

In addition to mycelial growth inhibition, the effect of *Trichoderma* isolates on sclerotia formation by *R. solani* was also evaluated. The highest number of sclerotia was recorded in the *R. solani* control plate (108 sclerotia), followed by dual cultures with TL5-15A, TL5-18B, TL5-18C, and TL1-5B (Data not shown). In contrast, the lowest number of sclerotia recorded in the dual culture with TL2-50A, with seven sclerotia (Fig. 2). Regression analysis showed a moderate negative correlation between inhibition percent and sclerotial count ($r = -0.67$, $p < 0.001$; $R^2 = 0.45$), indicating that greater inhibition of mycelial growth was generally associated with lower sclerotia production. These

results suggest that some *Trichoderma* isolates can suppress both mycelial growth and sclerotial formation of *R. solani* (Fig. 2 and 3).

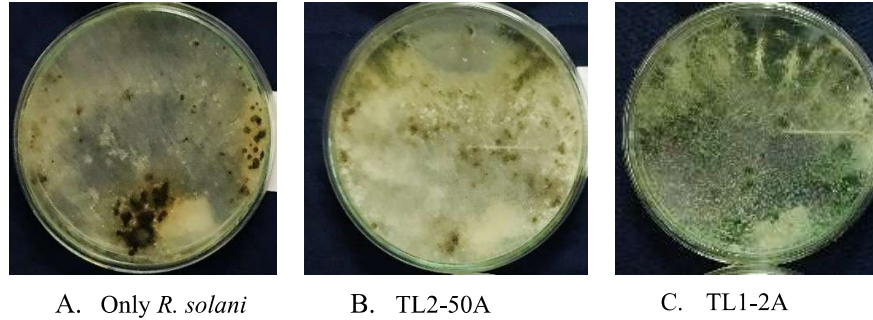


Fig. 2. Sclerotia production in dual culture plates (A) Control plate with no *Trichoderma* inoculation, (B) plate with TL2-50A isolate and (C) plate with TL1-2A isolate

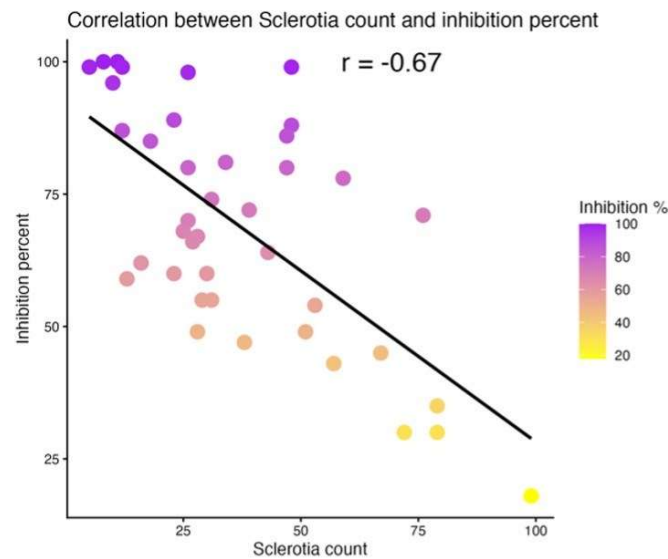


Fig. 3. Correlation between inhibition percent of *Rhizoctonia solani* and sclerotia production in dual culture with *Trichoderma* isolates.

Seed bioprimer assay

All 40 *Trichoderma* isolates were evaluated for their effects on seed germination and seedling vigor of rice cv. Taichung 176. Significant differences were observed among the isolates for radicle length, plumule length, germination percentage, and seedling vigor index ($p < 0.001$; Table 2). TL1-2A recorded the highest radicle length (10.3 cm), plumule length (8.23 cm), germination (99%), and seedling vigor index (1835). TL2-48A, TL2-50A, and TL6-24B also performed well for several

growth parameters. The untreated control recorded the lowest germination (70.33%), lowest radicle length (5.73 cm), plumule length (4.03 cm), and seedling vigor index (687).

Table 2. Effect of seed biopriming with selected *Trichoderma* isolates on rice seed germination and seedling vigor (cv. Taichung) at 10 days after incubation at 25 ± 2 °C

<i>Trichoderma</i> isolates	Radicle length (cm)	Plumule length (cm)	Germination percent	Seedling vigor
TL1-2A	10.3 ^a	8.233 ^a	99 ^a	1835 ^a
TL1-3A	8.1 ^{m-q}	6.167 ^{de}	84.67 ^{m-o}	1208 ^{jk}
TL1-4A	6.233 ^w	4.567 ^{o-s}	76.93 ^p	831 ^q
TL1-5A	7.373 ^{tu}	5.867 ^{e-j}	88.23 ^{ij}	1168 ^{kl}
TL1-5B	6.667 ^v	4.5 ^{q-s}	97 ^{a-d}	1084 ^{mn}
TL1-5C	8.5 ^{h-m}	5.767 ^{f-j}	85 ^{l-o}	1211 ^{jk}
TL1-8A	8.5 ^{h-m}	6.833 ^c	88 ^{i-k}	1349 ^{ef}
TL5-15A	7.5 ^{s-u}	4.9 ^{n-p}	88 ^{i-k}	1092 ^{mn}
TL5-18A	7.567 ^{t-u}	4.867 ^{n-q}	86 ⁱ⁻ⁿ	1069 ^{mn}
TL5-18B	9.633 ^{bc}	6.867 ^c	88 ^{i-k}	1452 ^c
TL5-18C	8.2 ^{k-o}	6.1 ^{d-g}	91 ^h	1301 ^{f-h}
TL5-21A	7.867 ^{o-s}	6.333 ^d	85.33 ^{k-o}	1211 ^{jk}
TL6-24B	8.8 ^{f-i}	7.267 ^b	94.67 ^{d-f}	1521 ^b
TL6-26B	8.933 ^{c-g}	6.367 ^d	84.67 ^{m-o}	1295 ^{f-h}
TL6-27A	9.533 ^{bc}	6.833 ^c	87 ^{j-m}	1424 ^{cd}
TL6-29A	7.747 ^{q-t}	6.12 ^{d-f}	83 ^o	1151 ^l
TL6-30A	8.667 ^{g-j}	5.717 ^{g-k}	86.67 ^{j-n}	1247 ^{h-j}
TL6-31A	6.85 ^v	5.067 ^{mn}	84 ^{no}	1001 ^o
TL6-32A	7.8 ^{p-s}	5.6 ^{i-l}	95 ^{d-f}	1273 ^{g-i}
TL6-32B	9.46 ^{b-d}	6.8 ^c	90.33 ^{hi}	1469 ^c
TL6-32C	8.187 ^{l-p}	4.423 ^{rs}	86 ⁱ⁻ⁿ	1084 ^{mn}
TL6-34A	6.9 ^v	4.543 ^{p-qrs}	91.67 ^{gh}	1049 ^{no}
TL6-34B	7.71 ^{q-t}	6.1 ^{d-h}	95.33 ^{c-e}	1317 ^{fg}
TL6-36A	8.633 ^{g-j}	5.367 ^{k-m}	94 ^{c-g}	1316 ^{fg}
TL6-36B	8.467 ^{i-m}	5.557 ^{j-l}	91 ^h	1276 ^{g-i}
TL2-42B	8.72 ^{g-i}	5.903 ^{e-j}	94.67 ^{d-f}	1384 ^{de}
TL2-48A	9.673 ^b	6.857 ^c	92.33 ^{f-h}	1526 ^b
TL2-49A	9.267 ^{c-e}	5.303 ^{lm}	95 ^{d-f}	1384 ^{de}

contd...

<i>Trichoderma</i> isolates	Radicle length (cm)	Plumule length (cm)	Germination percent	Seedling vigor
TL2-50A	9.617 ^{bc}	5.91 ^{e-j}	98 ^{a-c}	1522 ^b
TL2-54A	8.4 ^{i-m}	5.057 ^{mn}	96.33 ^{a-e}	1296 ^{f-h}
TL2-54B	7.633 ^{r-ai}	5.12 ^{mn}	74 ^q	944 ^p
TL2-56A	8.273 ^{j-n}	4.813 ^{n-q}	88 ^{i-k}	1152 ^l
TL2-56B	7.3 ^u	5.523 ^{j-l}	87.67 ^{i-l}	1124 ^{lm}
TL2-56C	8.55 ^{g-l}	6.023 ^{d-i}	83 ^o	1210 ^{jk}
TL1-62B	8.793 ^{f-i}	5.653 ^{i-l}	88 ^{i-k}	1271 ^{g-i}
TL1-73B	7.557 ^{r-ai}	4.86 ^{n-q}	98.33 ^{ab}	1221 ^{i-k}
TL1-72A	8.6 ^{g-k}	4.777 ^{n-r}	97 ^{a-d}	1298 ^{f-h}
TL1-73A	7.957 ^{n-r}	4.82 ^{n-q}	96 ^{b-e}	1227 ^{ij}
TL4-81C	8.907 ^{c-h}	4.933 ^{no}	82.67 ^o	1144 ^l
TL4-81A	9.133 ^{d-f}	4.333 st	97 ^{a-d}	1306 ^{fg}
Control	5.733 ^x	4.033 ^t	70.33 ^r	687 ^r
SEM±	0.21	0.20	1.48	30.28
CV %	2.6	3.6	1.7	2.5
p-value	<.001**	<.001**	<.001**	<.001**

Treatment means followed by the same letters within columns are not significantly different based on DMRT value at <1% (**) and <5% (*) significance level. CV = Coefficient of variance; SEM = Standard error of the mean

Based on their antagonistic activity in the dual-culture assay and growth-promoting effects in the seed biopriming assay, ten promising isolates - TL1-2A, TL1-5B, TL6-24B, TL6-29A, TL6-36B, TL2-48A, TL2-50A, TL2-56B, TL1-73B, and TL4-81A - were selected for further evaluation against rice seedling blight caused by *F. proliferatum*.

Growth-promoting effects of *Trichoderma* on *F. proliferatum*-inoculated rice seedlings under screenhouse conditions

The effects of *Trichoderma* isolates on rice seedling growth under screenhouse conditions are presented in Table 3. Significant differences were among treatments were found for germination percentage, root length, and seedling vigor index in both seed treatment ($p < 0.05$) and soil drenching ($p < 0.001$) experiments. However, shoot length was not significantly affected by either application methods.

In the seed treatment experiment, *Trichoderma* isolates generally improved seed germination compared with the *Fusarium proliferatum*-inoculated positive control. The highest germination percentage was recorded with TL1-2A (86%), followed by TL4-81A (81%), whereas the positive control recorded the lowest germination (59.33%). Seedling vigor was also significantly improved by several *Trichoderma* treatments. TL4-81A recorded the highest vigor index (1785), followed by TL1-

2A (1738), compared with the positive control (1160). The negative control recorded a vigor index of 1701, which was statistically comparable with several effective *Trichoderma* treatments (Table 3).

In the soil drenching experiment, TL4-81A showed the strongest growth-promoting effect, with the highest germination percentage (87.33%) and seedling vigor index (1888). These values were significantly higher than those of the *F. proliferatum*-inoculated positive control, which recorded 61% germination and a vigor index of 1206. TL1-2A also performed well, recording 82.33% germination and a vigor index of 1662. Other isolates showed moderate improvements in seedling growth compared with the positive control.

Overall, the application of native *Trichoderma* isolates improved rice seedling establishment under screenhouse conditions. TL1-2A and TL4-81A showed the most consistent growth-promoting effects in both seed treatment and soil drenching methods.

Table 3. Plant growth-promoting effects of *Trichoderma* isolates on rice seedlings (cv. Khumal-4) inoculated with *Fusarium proliferatum* at 20 days after sowing under screenhouse conditions

Treatment	Seed treatment (biopriming)				Soil treatment (drenching)			
	Root length (cm)	Shoot length (cm)	Germination percent	Seedling vigour	Root length (cm)	Shoot length (cm)	Germination percent	Seedling vigour
TL1-2A	8.9 ^{ab} ± 0.13	11.32	86 ^a ± 0.47	1738 ^{ab} ± 7.24	9.83 ^{ab} ± 0.29	10.2	82.33 ^{ab} ± 0.61	1662 ^{a-c} ± 59.32
TL1-5B	7.55 ^b ± 0.30	12.35	77.33 ^{ab} ± 1.50	1550 ^{a-d} ± 53.86	6.50 ^{cd} ± 0.16	13.66	73.17 ^{b-d} ± 0.62	1473 ^{a-c} ± 26.48
TL6-24B	5.41 ^{cd} ± 0.08	12.45	78.33 ^{ab} ± 1.59	1385 ^{de} ± 64.14	6.44 ^{cd} ± 0.24	13.26	75.5 ^{b-d} ± 1.04	1484 ^{a-c} ± 56.20
TL6-29A	6.17 ^c ± 0.20	13.66	79.33 ^{ab} ± 0.75	1574 ^{a-d} ± 44.63	6.60 ^{cd} ± 0.23	13.74	64.67 ^{de} ± 2.91	1323 ^{bc} ± 68.15
TL6-36B	5.48 ^{cd} ± 0.09	13.78	77.33 ^{ab} ± 0.75	1489 ^{b-d} ± 22.93	6.31 ^{cd} ± 0.35	12.5	78.33 ^{a-c} ± 0.80	1466 ^{a-c} ± 26.94
TL2-48A	5.40 ^{cd} ± 0.18	14.03	72 ^b ± 0.78	1395 ^{de} ± 27.60	6.42 ^{cd} ± 0.25	12.23	77 ^{a-c} ± 1.75	1445 ^{a-c} ± 68.56
TL2-50A	5.74 ^{cd} ± 0.22	14.42	73.33 ^b ± 1.18	1483 ^{a-d} ± 34.98	7.12 ^{cd} ± 0.26	11.14	70.17 ^{c-e} ± 1.73	1302 ^{bc} ± 73.27
TL2-56A	5.98 ^c ± 0.18	13.65	73.67 ^b ± 1.32	1430 ^{c-e} ± 22.16	6.44 ^{cd} ± 0.36	11.23	75.67 ^{b-d} ± 1.38	1336 ^{bc} ± 74.06
TL1-73A	5.74 ^{cd} ± 0.22	15.25	78.33 ^{ab} ± 1.45	1642 ^{a-d} ± 30.50	6.08 ^{cd} ± 0.42	10.91	74.33 ^{b-d} ± 0.90	1259 ^{bc} ± 45.08
TL4-81A	8.06 ^b ± 0.21	13.83	81 ^{ab} ± 1.09	1785 ^a ± 50.10	8.23 ^{bc} ± 0.32	13.42	87.33 ^a ± 0.98	1888 ^a ± 28.66
Control†	4.37 ^d ± 0.12	15.28	59.33 ^c ± 1.06	1160 ^e ± 16.87	5.02 ^d ± 0.27	14.92	61 ^e ± 1.41	1206 ^c ± 24.29
Control††	10.02 ^a ± 0.10	11.99	77.33 ^{ab} ± 0.95	1701 ^{a-c} ± 23.73	11.38 ^a ± 0.53	11.99	72.33 ^{b-d} ± 0.64	1702 ^{ab} ± 51.38
SEM±	0.455	0.843	3.9	90.4	1.123	1.65	4.82	196.9
CV %	16.9	15.3	8.9	14.5	27	23	11.2	23.3
p-value	<.001**	NS	<.05*	<.001**	<.001**	NS	<.05*	<.001**

Treatment means followed by the same letters within columns are not significantly different based on DMRT value at a <1% (**) and <5% (*) significance level. CV = Coefficient of variance; SEM, Standard error of the means

† = Positive control = Seed treated with *F. proliferatum* but without *Trichoderma*

†† = Water-treated seed, without *F. proliferatum* and *Trichoderma*

Effect of *Trichoderma* on seedling blight disease incidence and severity

Typical seedling blight symptoms, including pinkish-white fungal growth around the collar region, appeared within 15 days after sowing. Seedlings showing these symptoms subsequently wilted or died (blighted) within the following 5 days (Fig. 4).



Fig. 4. Symptoms of seedling blight in rice (cv. Khumal-4): (A) normal (L) and stunted seedlings (R), (B) mycelial growth on germinating seed, (C) Diseased seedlings (L) and healthy ones (R).

Application of *Trichoderma* treatments significantly reduced seedling blight incidence and severity ($p < .001$) compared with the *F. proliferatum*-inoculated positive control in both seed treatment and soil drenching experiments. In the seed treatment experiment, all *Trichoderma* isolates reduced disease incidence compared with the positive control, which recorded 80.33% disease incidence. The lowest disease incidence was recorded in with TL4-81A (41.67%), followed by TL1-2A and TL1-73A (42.00% each). Disease severity was also significantly reduced by the *Trichoderma* treatments. TL1-2A produced the lowest disease severity (18.05%), followed by TL4-81A (22.40%) and TL1-73A (28.67%), compared with the positive control (70.93%). A similar trend was observed under soil drenching. TL1-2A and TL4-81A recorded the lowest disease incidence, at 41.00% and 41.33%, respectively, compared with 84.33% in the positive control (Table 4).

Table 4. Effect of *Trichoderma* isolates on disease incidence and severity of rice seedlings (cv. Khumal-4) inoculated with *Fusarium proliferatum* at 20 days after sowing under screenhouse conditions

Treatment†	Seed treatment (biopriming)				Soil treatment (drenching)			
	Disease incidence	Percent decrease	Disease severity	Percent decrease	Disease incidence	Percent decrease	Disease severity	Percent decrease
TL1-2A	42.00 ^b ± 0.94	47.71	18.05 ^b ± 0.57	74.55	41.00 ^b ± 0.54	51.38	24.27 ^b ± 0.83	67.89
TL1-5B	62.33 ^{fg} ± 0.38	22.40	51.6 ^c ± 1.28	27.25	53.33 ^{cd} ± 0.62	32.01	44.4 ^{cd} ± 1.07	41.26
TL6-24B	52.00 ^{dc} ± 0.96	35.26	49.87 ^c ± 1.75	29.69	50.67 ^c ± 0.72	39.91	43.47 ^{cd} ± 0.89	42.50

contd...

Treatment†	Seed treatment (biopriming)				Soil treatment (drenching)			
	Disease incidence	Percent decrease	Disease severity	Percent decrease	Disease incidence	Percent decrease	Disease severity	Percent decrease
TL6-29A	67.00 ^g ± 0.75	16.59	62.93 ^f ± 1.52	12.16	60.00 ^d ± 1.57	28.85	49.33 ^{cd} ± 2.29	34.74
TL6-36B	56.33 ^{ef} ± 1.62	29.87	55.07 ^e ± 0.29	22.36	56.33 ^{cd} ± 1.62	33.20	52.53 ^{de} ± 2.36	30.51
TL2-48A	49.33 ^c ± 1.24	38.59	34.53 ^d ± 1.20	51.31	52.67 ^{cd} ± 0.65	37.54	51.87 ^{de} ± 2.03	31.38
TL2-50A	48.00 ^{bc} ± 0.21	40.24	30.53 ^d ± 0.46	56.95	54.33 ^{cd} ± 0.92	35.57	63.07 ^e ± 1.48	14.06
TL2-56A	58.33 ^{ef} ± 1.48	27.38	52.8 ^e ± 1.78	25.56	55.00 ^{cd} ± 0.93	34.78	52.67 ^{de} ± 2.63	30.33
TL1-73A	42.00 ^b ± 0.66	47.71	28.67 ^{cd} ± 0.71	59.58	49.33 ^c ± 1.70	41.50	39.33 ^c ± 1.83	47.97
TL4-81A	41.67 ^b ± 0.71	48.12	22.4 ^{bc} ± 1.15	68.41	41.33 ^b ± 1.70	50.99	23.47 ^b ± 0.34	68.95
Control†	80.33 ^b ± 0.58		70.93 ^g ± 1.28		84.33 ^e ± 0.61		75.6 ^f ± 1.80	
Control††	5.33 ^a ± 0.58	93.36	9.33 ^a ± 0.39	86.84	6.33 ^a ± 0.57	92.49	11.87 ^a ± 0.54	84.29
SEM±	2.30		2.71		2.74		5.41	
CV %	11.20		16.4		13.30		21.10	
p-value	<.001**		<.001**		<.001**		<.001**	

Treatment means followed by the same letters within columns are not significantly different based on DMRT value at <1% (**) and <5% (*) significance level.

CV, Coefficient of variance; SEM, Standard error of the means

† = Pathogen control = Seed treated with *F. proliferatum* but without *Trichoderma*

†† = Untreated control = Water-treated seed, without *F. proliferatum* and *Trichoderma*

DISCUSSION

Trichoderma species are widely recognized as important biological control agents because of their antagonistic activity against a range of plant pathogens. They colonize diverse plant-associated habitats and have been investigated for the management of several rice diseases (Biswas & Datta, 2013; Doni et al., 2022). In the present study, all forty *Trichoderma* isolates showed some degree of antagonistic activity against *R. solani*, *P. oryzae*, and *F. proliferatum*, although the level of inhibition varied considerable among isolates and pathogens. These variations indicate that antagonistic activity is isolate-dependent and may also depend on the pathogen involved.

Three isolates, TL6-36B, TL2-50A, and TL4-81A, showed broad spectrum antagonistic activity as they completely inhibited the growth of all three tested pathogens in the dual culture assay. Other isolates showed varied effectiveness, indicating that the antagonistic capacity of individual *Trichoderma* isolates is not equally effective against different pathogens. The suppression of pathogen growth in dual culture may involve direct interaction between *Trichoderma* and the pathogens, including competition for space and nutrients, mycoparasitism, hyphal lysis, and production of hydrolytic enzymes and inhibitory metabolites (Moy et al., 2002; Timila et al., 2015). The differences observed among isolates in the present study may therefore reflect variation in their ability to colonize the substrate, compete with pathogens, produce extracellular enzymes, and release antifungal metabolites (Howell, 2007; Waghunde et al., 2016; Naeimi et al., 2017).

The *Trichoderma* isolates also differed in their effects on sclerotial production by *R. solani*. The untreated control produced the highest number of sclerotia, which suggests that isolates capable of strongly suppressing mycelial growth may also reduce the formation of survival structures. Despite the negative relationship between pathogen-growth inhibition and sclerotial production ($r = -0.67$; $R^2 = 0.45$), the moderate R^2 value indicates that inhibition of mycelial growth explained only part of the variation in sclerotial production. Other isolate-specific or environmental factors may also have contributed to the observed differences. Seed biopriming with all forty *Trichoderma* isolates significantly improved radicle length, plumule length, germination percentage, and overall seedling vigor of rice cv. Taichung 176 compared with the untreated control. These findings are consistent with previous reports of the plant growth-promoting effects of *Trichoderma* (Saxena et al., 2015). In the present study, TL1-2A produced the greatest overall improvement in terms of radicle, plumule length, germination percentage, and seedling vigor index. Several other isolates, including TL2-50A, TL2-48A, TL6-24B, and TL5-18B, also performed well for one or more parameters.

The results also showed that strong antagonistic activity did not always correspond to strong growth promotion. For example, TL4-81A showed strong antagonistic activity against all three test pathogens and performed well in the greenhouse experiment, but its seed biopriming treatment produced a lower vigor index than TL1-2A. This suggests that plant suppression and plant growth promotion may vary independently among *Trichoderma* isolates. Similar isolate-dependent differences have been reported previously (Li et al., 2015). The improvement in root and shoot growth observed with seed biopriming may be associated with several plant growth-promoting activities of *Trichoderma*. These include stimulation of root development through phytohormone-related mechanisms, improved nutrient availability, and enhanced nutrient uptake (Contreras-Cornejo et al., 2009; Harman, 2006; Rakshit et al., 2014). However, these mechanisms were not directly examined in the present study.

Under greenhouse conditions, all ten *Trichoderma* isolates significantly reduced the incidence and severity of seedling blight caused by *F. proliferatum*. The strongest disease suppression was obtained with TL1-2A and TL4-81A. These two isolates also produced highest germination and seedling vigor, indicating that their performance was not limited to disease suppression. Both seed treatment and soil drenching reduced disease incidence and severity compared with the pathogen-inoculated control. TL1-2A and TL4-81A were effective under both application methods. Although direct seed treatment may facilitate early association of *Trichoderma* with the germinating seed and developing root system, the present results do not demonstrate that seed treatment was consistently superior to soil drenching for every isolate. Therefore, the relative effectiveness of the two application methods should be interpreted on an isolate-specific basis. *Trichoderma* isolates TL4-81A and TL1-2A therefore showed the most consistent performance across the dual culture, seed biopriming, and greenhouse experiments. In contrast, some isolates that showed strong inhibition *in vitro* did not provide the same level of disease suppression under greenhouse conditions. This difference highlights the importance of evaluating potential biocontrol isolates under conditions that more closely represent the plant environment because strong *in vitro* antagonism does not necessarily translate into equivalent disease-control efficacy *in vivo*.

The disease-suppressive effect observed in the present study is consistent with earlier reports of the potential of *Trichoderma* species to suppress *Fusarium*-associated diseases (Calistru et al., 1997).

The superior performance of TL1-2A and TL4-81A under both laboratory and screenhouse conditions suggest that these native isolates are promising candidates for further evaluation. Field experiments, formulation studies, and molecular identification of the selected isolates would be necessary before recommending them for practical disease management.

CONCLUSIONS

Native *Trichoderma* isolates demonstrated significant potential as biocontrol and growth-promoting agents in rice. Dual culture assay revealed variable but significant inhibition of major rice pathogens, including *F. proliferatum*, *P. oryzae*, and *R. solani*, by the tested isolates, with TL6-36B emerging as the most potent antagonist against all three. In seed biopriming, most of the isolates enhanced seed germination and seedling vigor, with TL1-2A exhibiting the most pronounced growth-promoting activity. The biocontrol effects extended to screenhouse conditions, where *Trichoderma* treatments reduced seedling blight caused by *F. proliferatum*, with isolates TL1-2A, TL4-81A, and TL6-36B proving most effective across both seed and soil applications. Collectively, these findings show the strong potential of indigenous *Trichoderma* isolates as sustainable biocontrol agents for managing bakanae/seedling blight disease in rice.

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