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ORIGINAL ARTICLE

Potato stem canker and black scurf controlled by a *Trichoderma-Curtobacterium* consortium enhances yield

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ABSTRACT

Potato stem canker and black scurf, caused by *Rhizoctonia solani*, are the most devastating diseases affecting productivity, yield and tuber quality. This study evaluated the potential of an opportunistic fungus (*Trichoderma* spp.) and an endophytic bacterium to control these diseases. In dual-culture assay, both microbes exhibited strong antagonistic activity against *R. solani*. Molecular study identified the isolates as *Trichoderma yunnanense* strain PLPTr and *Curtobacterium citreum* strain PLPL. These antagonists were applied individually and in a microbial consortium for seed tuber treatment. The results showed that the consortium was more effective than either microbe applied alone. The experimental plots treated with the consortium displayed the lowest disease incidence, with 7.92% stem canker and 13.93% black scurf, as well as the lowest percent disease index, with 2.78% for stem canker and 9.03% for black scurf. Consortium-treated plants also exhibited a 44.7% increase in yield compared with pathogen-inoculated controls. Thus, the combined application of *T. yunnanense* and *C. citreum* as a seed tuber treatment represents a promising, eco-friendly strategy for controlling potato stem canker and black scurf while remarkably boosting potato yield.

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Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most important food crops, and a staple for millions of people. In 2022, global potato production covered approximately 17.8 million hectares, with an average yield of about 21 t ha^{-1} (FAO, 2023). Despite its importance, potato productivity remains severely decreased due to plant diseases. Among the diseases, stem canker and black scurf caused by *Rhizoctonia solani* (telomorph: *Thanatephorus cucumeris*) reduce the tuber yield significantly across the globe (Brewer and Larkin, 2005). Stem canker affects plants during early growth stages, impairing sprout emergence and damaging stolons and roots, which ultimately reduces tuber number and size. In contrast, black scurf compromises tuber quality through the formation of sclerotia and malformed tubers, resulting in substantial post-harvest losses (Das *et al*., 2014). The persistence of *R. solani* in agricultural systems is largely attributed to its broad host range and its capacity to survive in soil, plant debris, and seed tubers as resting structures, making effective management particularly challenging (El Bakali and Martin, 2006).

Several disease management strategies including cultural practices, resistant cultivars, and chemical or biological control are being used worldwide including Bangladesh (Tariq *et al*., 2010). In intensive potato production systems, use of fungicides remains the predominant approach for controlling soil-borne diseases (Panth *et al*., 2020). The excessive and indiscriminate use of fungicides has also raised numerous concerns due to the accumulation of toxic residues in agroecosystems, risks to human and environmental health, and disruption of beneficial soil microbiota. Consequently, there is increasing global emphasis on the development of sustainable, environmentally benign disease management strategies, including the use of biofungicides (Fenta and Mekonnen, 2024).

Use of antagonistic microbes has emerged as a promising alternative to chemical disease management. In Bangladesh and other potato-growing regions, numerous studies have evaluated fungal and bacterial biocontrol agents such as *Trichoderma* spp., *Pseudomonas fluorescens*, and *Bacillus* spp. against major potato pathogens, including *R. solani*, *Phytophthora infestans*, and *Fusarium* spp. These studies demonstrate that reduced disease incidence and severity, enhanced plant growth and yield, underscoring the potential of BCAs as sustainable disease management tools (Rakibuzzaman *et al*., 2021).

Among fungal BCAs, *Trichoderma* species are particularly notable due to their multifarious functions including disease control, plant growth promotion, and improvement of soil health. A good number of *Trichoderma* species show remarkable antagonistic activity against soil-borne pathogens like *R. solani*, *F. oxysporum*, and *Sclerotium rolfsii* through various mechanisms such as mycoparasitism, antibiosis, and induction of

host defense responses (Anees *et al*., 2010; Kumari *et al*., 2024; Karmakar *et al*., 2021). Endophytic bacteria also increase plant health by enhancing nutrient acquisition, modulating phytohormone levels, and suppressing pathogens. Members of the genus *Curtobacterium*, in particular, have been shown to enhance host defense and reduce disease severity in several crops (Tjamos *et al*., 2004; Lacava *et al*., 2007).

Still most microbial-based strategies rely on single-strain applications (Minchev *et al*., 2021). However, such approaches may be suboptimal under open field conditions due to variability of environment and limited functional diversity. In contrast, consortia consisting compatible microbes offer a more resilient and effective strategy for disease suppression and promotion of plant growth (Sudharani *et al*., 2014). For example, application of the consortium consisted of *P. aeruginosa* and *T. harzianum* significantly reduced Fusarium wilt and enhanced growth of banana (Wong *et al*., 2021), while co-inoculation of *B. subtilis* and *T. virens* enhanced potato resistance to *R. solani* (Das *et al*., 2014). Similarly, a consortium composed of *B. sonorensis* and *Funneliformis mosseae* incredibly stimulated the growth of tomato and capsicum (Desai *et al*., 2020). Nevertheless, the potential of fungal–bacterial consortia for managing potato stem canker and black scurf remains underexplored. Therefore, the present study aimed to evaluate the efficacy of a fungal–bacterial consortium for suppressing *R. solani*-induced diseases and enhancing potato growth and yield under field conditions.

Materials and Methods

Pathogenic fungus isolation, pathogenicity assessment and inoculum preparation

Potato tubers infested with stem canker and black scurf disease were collected from the Plant Pathology Research Field of Gazipur Agricultural University (GAU), Bangladesh. Infected specimens were subjected to isolation of *Rhizoctonia solani* following the plant tissue culture method. Briefly, tubers were cut into small pieces containing symptomatic tissues. The surface-sterilized tissue segments were then placed on Potato Dextrose Agar (PDA) medium and incubated for 3 days. At this stage, characteristic morphological features (off-white to tan-colored mycelium, right-angled (90°) branching, and frequent constrictions at the base of hyphae) were observed under a microscope. Hyphal tips from the initial growth were transferred to fresh culture plates and sub-cultured several times to obtain pure cultures. Finally, pure cultures were preserved at 5 °C on PDA slants (Wang *et al*., 2015).

Pathogenicity tests were performed in accordance with Koch's postulates. Inocula of all isolates were prepared using autoclaved wheat grains. The sterilized wheat was inoculated with mycelial discs from 7-day-old cultures and incubated for 30 days to ensure adequate fungal growth (Tasnim *et al*., 2024). The isolates were evaluated for virulence by

assessing pre- and post-emergence mortality of potato seedlings in pot culture experiments with three replications. Disease development was monitored and documented to determine the pathogen's role in inducing pre- and post-emergence seedling death. Finally, the most virulent isolate was selected and preserved for further use.

BCAs (Bio Control Agent) collection, culture conditions and potent antagonist selection trichoderma

Soil samples were collected from different locations at GAU, Bangladesh. A gradient dilution approach was employed to isolate opportunistic fungi *Trichoderma* from the collected samples. The isolates were initially cultured as single colonies on the PDA medium. Following the hyphal tip culture technique, young hyphal tips were excised using a cork borer and transferred to fresh, sterilized PDA plates to obtain pure cultures. Subculturing was repeated until contamination-free *Trichoderma* cultures were obtained. For subsequent studies, the pure strains were maintained at 4 °C (Long *et al*., 2023).

Assessment of antagonistic interaction between trichoderma and the pathogen

An *in vitro* screening was conducted with three replications to evaluate the antagonistic activity of the isolated *Trichoderma* strains against a virulent isolate of *R. solani* using the dual culture technique. A 5-mm mycelial disc of *Trichoderma* and *R. solani* was placed approximately 1 cm from the edge of the PDA plates on opposite sides. For the control treatment, *R. solani* was cultured alone on PDA plates (Rajendiran *et al*., 2010). The percentage reduction in the mycelial growth of *R. solani* on PDA plates was calculated using the following formula:

$$\text{Growth inhibition } \% = \frac{X_r - X_t}{X_r} \times 100$$

Where, X_r = Mycelium diameter of *R. solani* without *Trichoderma* (control),

X_t= Mycelium diameter of *R. solani* with *Trichoderma*

Endophytic bacteria

Bacterial isolates were collected from medicinal plants, including marigold (*Tagetes erecta*), neem (*Azadirachta indica*), and sticky nightshade (*Solanum sisymbriifolium*). Healthy stem, leaf, and root samples were subjected to bacterial isolation on Yeast Peptone Dextrose Agar (YPDA) medium following the method described by Berg et al. (2005). The cultured bacterial isolates were maintained on the same medium and preserved at −80 °C in nutrient broth supplemented with 30% glycerol.

Evaluation of antagonistic interaction between bacteria and pathogen

A five-millimeter (mm) mycelial disc from a three-day-old culture of *R. solani* was placed 2 cm away from the antagonistic bacterial isolate, which had been spread at the center of the agar plate. The antagonistic activity of the bacterial isolates was assessed by comparing the suppression of fungal growth

with that of a control plate containing *R. solani* grown alone. Reduction in fungal growth was determined by measuring colony diameter after 5 days of incubation at 25 ± 2 °C (Elkahoui *et al*., 2012). Mycelial growth of *R. solani* was recorded in three replications, and mean values were calculated using the following formula:

$$\text{Growth inhibition \%} = \frac{X_r - X_b}{X_r} \times 100$$

Where, X_r = Mycelium diameter of *R. solani* without *Trichoderma* (control),

X_b= Mycelium diameter of *R. solani* with *Trichoderma*

Molecular characterization of the microbes

The genomic DNA (gDNA) of *Trichoderma* sp. was obtained using modified CTAB techniques (Zhang *et al*., 2010). Using the conserved ribosomal ITS (Internal Transcribed Spacer) region, *Trichoderma* was molecularly identified. PCR (Polymerase Chain Reaction) was performed using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as forward and reverse primers respectively, where ITS sections between 18S rDNA and 28S rDNA as well as 5.8S rDNA were amplified. In case of bacteria, 48 hrs old culture was exposed to genomic DNA isolation using FavorPrep™ Tissue Genomic DNA Extraction Kit. Universal primer sets 8F (AGAGTTTGATCCTGGCTCAG) and 1492 R (GGTTACCTTGTTACGACTT) were used to amplify the target gene 16S rRNA by PCR of the selected bacterial isolate. A PCR thermos cycler (Applied Biosystems, 2720 Thermal Cycler) was used to do the PCR amplification. The ideal PCR conditions (denaturation- 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 minute, an annealing at 57°C for 40 seconds and an extension at 72°C for 1 minute and a final extension- 72°C for 10 minute) were maintained for both *Trichoderma* and bacteria. PCR products were maintained at 4 degrees Celsius. Thermo Scientific GeneJET PCR Purification Kit #K0701(*Trichoderma*) and #K0701(bacteria) were used to purify the PCR product in accordance with the manufacturer's instructions. Further sequencing was carried out at National Institute of Biotechnology (NIB), Dhaka. The National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST) was utilized to assess the similarity of the fungal and bacterial DNA sequences in this study with homologous sequences. NCBI accession number MW173317 and MT084037 were assigned to the fungal and bacterial isolate upon the submission of the sequencing data.

Compatibility test between the bio agents

Compatibility of *Trichoderma* and antagonistic bacterium was determined by streaking of bacterium on PDA plate followed by placing four mycelial discs of *Trichoderma* having 5 mm size at the opposite direction.

Microbial suspension and consortium preparation

On PDA medium, *Trichoderma* typically began to produce conidia (spores) within 3-5 days at 25-30^0 C. Maximum sporulation was

observed after 7-10 days incubation. At this stage, *Trichoderma* spore suspension was made by sterile distilled water being added to each petri dish's culture, lightly scraping the colony, and then filtering the spore suspension through four distinct layers of cheesecloth. Hemocytometer was the instrument used to measure the quantities of spores in the suspension (10^8 spore/mL) (Ojaghian, 2011).

The endophytic bacterium was cultured in nutrient broth for 48 hours at 28±1°C in a rotary shaker at 200 rpm. Centrifugation at 6,000 rpm for 15 minutes was used to separate bacterial cells, which were then washed two times and resuspended in sterilized distilled water. Using a spectrophotometer, the quantities of bacterial cells in the suspensions were balanced at 10^8 cells/mL (CFU/mL) (Kumar *et al*., 2015).

A consortium of *Trichoderma* and endophytic bacteria was prepared as microbial suspension containing equal volume of the fungus and bacteria. The liquid formulation was prepared at a ratio of 1:1. The mixture was mixed well before using (Wong *et al*., 2021).

Field preparation and variety selection

The entire experimental area was split into three blocks, each representing replication. 2.0 m × 1.8 m made up the unit plot. Blocks were spaced apart by 1.0 m, and plots within a block were separated by 0.50 m. Rows were set 60 cm apart and plants were spaced 25 cm apart, respectively. The most popular potato variety Diamant was chosen for this experiment. Fertilizers were applied at standard doses. When irrigation was required, the appropriate amount was delivered. Three earthing ups were performed at 20 days' intervals.

Microbial treatments

Healthy seed tubers (cv. Diamant) were treated prior to field planting by spraying with suspensions of *T. yunnanense* (1×10^8 spores mL^{-1}) and *C. citreum* (1×10^8 CFU mL^{-1}), either individually or in combination, according to the experimental treatments. The applications were performed three times at 4-h intervals. For pathogen challenge, the soil was inoculated with the test pathogen by thoroughly mixing wheat grain–based inoculum into the soil before planting.

The treatment combinations were as follows:

T_1: Soil without any treatment (control 1),

T_2: Soil inoculation with pathogen (control 2),

T_3: T_2 + seed treatment with *T. yunnanense*,

T_4: T_2 + seed treatment with *C. citreum*,

T_5: T_2 + seed treatment with consortium of *T. yunnanense* and *C. citreum*.

Data documentation

Data were noted at various phases of potato plant development. At 10 and 20 days following seed tuber sowing, seedling tuber mortality was measured before and after emergence. At 60 days following sowing, various growth data were collected. Stem canker disease incidence and percent disease

index were recorded 75 days after planting. Data on black scurf disease and yield and yield contributing attributes were recorded after harvesting of potato tubers. The following methodologies were applied to measure the percentages of disease incidence and disease severity (Tasnim *et al*., 2024).

$DI\ \% = \frac{N_i}{N_t} \times 100$, Where, DI= Disease Incidence, N_i =Number of infected stem or tubers and N_t =Total number of stem or tubers observed.

$PDI\ \% = \frac{N_r}{N_t \times S} \times 100$, where PDI= Percent Disease Index, N_r = of rating of stem or tubers observed, N_t =Total number of stem or tubers observed and S= Highest score of the scale used.

The severity of stem canker and black scurf disease was assessed using a five-point rating scale, with 0 having no symptoms, 1 = less than 15%, 2 =15-35%, 3 = 36-49%, 4 = 50-75% 5= more than 75 % of the potato stem covered in lesions and tuber with sclerotia (Hasan *et al*., 2021).

Statistical analysis

The *in vitro* studies were carried out in a Complete Randomized Design (CRD), whereas the field experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications. Using the statistical computer program Statistix 10, data on various disease variables and yield components were statistically evaluated. Following the LSD (Least Significant Difference) test, the means were compared at 5% level of significance.

Results

Selection of a virulent R. solani isolate

The most virulent pathogen was screened through a pathogenicity assay by evaluating mortality rates of potato plants inoculated with three *R. solani* isolates (isolate-1, isolate-2, and isolate-3). No pre-emergence or post-emergence mortality was observed in the uninoculated control pots. Across the tested isolates, pre-emergence mortality ranged from 6.67-20.0%, while post-emergence mortality varied from 30.0-66.67% (Fig. 1). Among the isolates, isolate-3 exhibited the highest virulence, resulting in a maximum cumulative mortality rate of 86.7%, whereas isolate-1 caused the lowest total mortality (43.3%). Based on these results, isolate-3 was selected for subsequent experiments. The identity of the causal agent was further confirmed by re-isolation of the pathogen from infected tubers, thereby fulfilling Koch's postulates.

Selection of antagonistic Trichoderma against R. solani

Five *Trichoderma* isolates including Pb-13, MYT-75, T-BU, T-Raj, and PLPTr were collected and evaluated for their antagonistic activity *in vitro* against the most virulent isolate-3 using a dual-culture plate assay. All five isolates significantly inhibited the mycelial growth, with inhibition exceeding 50% compared with the control. Among them, isolate PLPTr exhibited the strongest antagonistic effect, suppressing mycelial

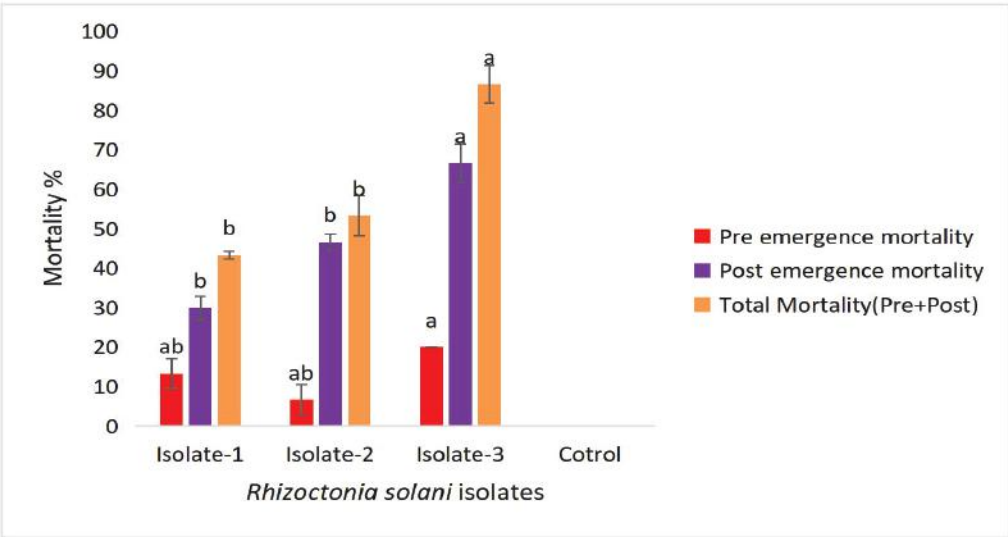


Fig. 1. Pathogenicity test revealing virulence of *Rhizoctonia solani* isolates. The bars on the columns represent the standard error of the mean (SEM). The means above the columns with distinct letters differ considerably as assessed by LSD (p = 0.05).

growth by 90.25%. Based on these screening results, isolate PLPTr was selected as the most effective antagonist and was subsequently used for consortium development (Fig. 2 and 3).

Selection of endophytic antagonistic bacterial isolate against R. solani

Eleven distinct bacterial isolates namely M-1, M-2, M-3, M-4, PLPL, M-5 K-1, K-2, K-3, N-1and N-2 were isolated from different plant parts of which six from marigold, three from sticky nightshade and two from neem. Only two of the eleven isolates, PLPL and N-1, exhibited antagonistic activity against *R. solani* (Table 1).

The growth behavior of the *R. solani* with antagonistic bacterial isolates varied considerably. Significantly, the highest 91.13% inhibition of mycelial growth of *R. solani* was found with the isolate PLPL followed by the isolate N-1 (62.09%) (Fig. 4 and 5). Based on the results of the assessment, the highly antagonistic bacterial isolate PLPL was selected for the development of consortia.

Molecular identification of Trichoderma and antagonistic bacterial isolate

The fungus isolate PLPTr and bacterial isolate PLPL were identified using molecular techniques. PCR amplification of genomic DNA from *Trichoderma* isolate PLPTr generated a 585 bp amplicon, whereas amplification of the bacterial isolate PLPL yielded a 1332 bp product. Sequence analysis revealed that *Trichoderma* strain PLPTr shared 99.65% sequence identity with *T. yunnanense* isolate Ty. Similarly, partial sequencing of

Table 1. Bacterial isolates from various plant samples and their antagonistic behavior towards *R. solani*

Isolate name	Host	Plant part	Colony color	Antagonistic activity
M-1	Marigold	Stem	Pink	-
M-2	Marigold	Stem	Off white	-
M-3	Marigold	Stem	White	-
M-4	Marigold	Root	Off-white	-
PLPL	Marigold	Stem	Yellow	+
M-5	Marigold	Leaf	White	-
K-1	Sticky nightshade	Stem	Yellow	-
K-2	Sticky nightshade	Stem	Light yellow	-
K-3	Sticky nightshade	Leaf	White	-
N-1	Neem	Leaf	Off white	+
N-2	Neem	Leaf	White	-

'+' indicates presence; '-' indicates absence.

the 16S rRNA gene of bacterial isolate PLPL showed 99.85% homology with *C. citreum* (NR_026156.1). Phylogenetic analyses were conducted using related sequences retrieved from the NCBI database. In the resulting phylogenetic trees, *Trichoderma* isolate PLPTr clustered closely with recognized *Trichoderma* species, confirming its identity as *T. yunnanense* (Fig. 6a). Likewise, the antagonistic bacterial isolate PLPL grouped within the *Curtobacterium* clade based on 16S rRNA gene sequences, confirming its affiliation with *C. citreum* (Fig. 6b).

Compatibility of the BCAs

Prior to consortium formulation, the compatibility of the biocontrol agents (BCAs) was evaluated. *T. yunnanense* and *C. citreum* exhibited complete compatibility when co-cultured on PDA. Dual-culture assays using a mixed inoculum confirmed the absence of inhibitory interactions between *T. yunnanense* and *C. citreum*, thereby demonstrating their mutual compatibility (Fig. 7). Notably, no antagonistic effects were observed between the two BCAs, indicating their suitability for consortium development.

Assessing the potential of microbial consortia on potato stem canker and black scurf disease

Disease incidence (DI) and percent disease index (PDI) of stem canker (Fig. 8a) and black scurf (Fig. 8b) were assessed under different treatments. All treatments, applied either individually or as a consortium, significantly reduced DI and PDI of both diseases compared with the pathogen-inoculated control. For stem canker, the pathogen-inoculated treatment showed the highest DI (31.74%) and PDI (14.71%), whereas the lowest DI (7.92%) and PDI (2.78%) were observed in

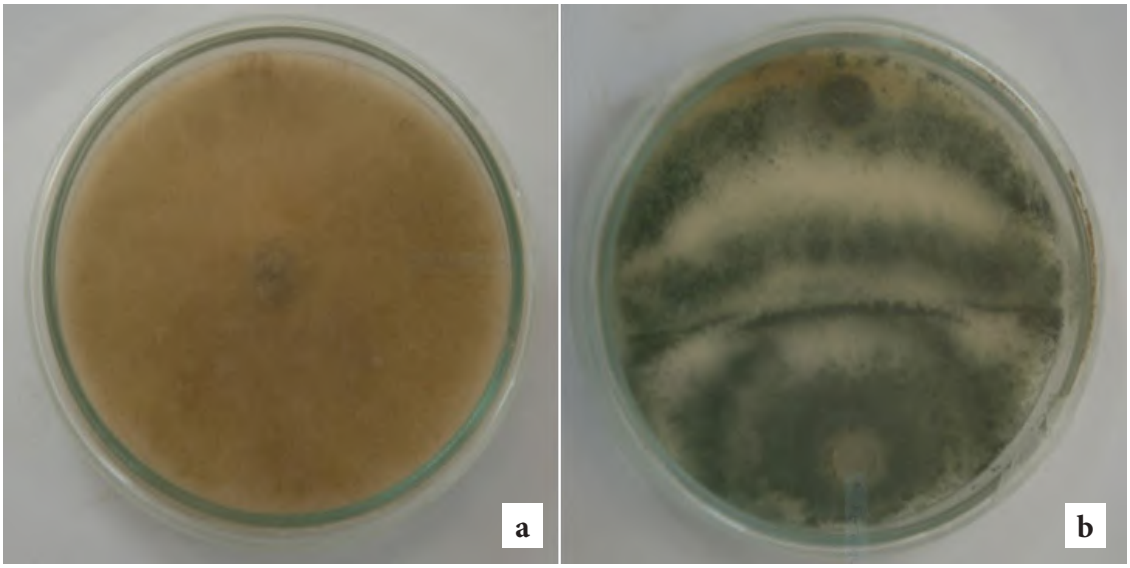


Fig. 2. Dual culture assay of *R. solani* and *Trichoderma*. (a) control (b) antagonism of *Trichoderma*

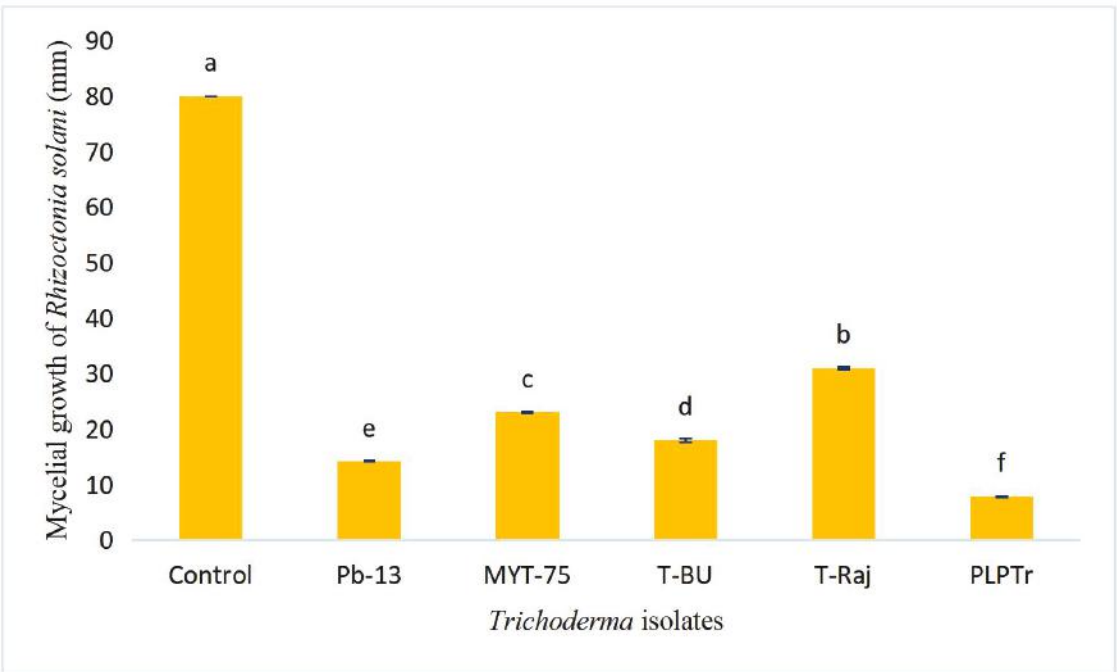


Fig. 3. Screening of *Trichoderma* isolates against *R. solani*. The bars on the columns represent the standard error of the mean (SEM). The means above the columns with distinct letters differ considerably as assessed by LSD (p = 0.05).

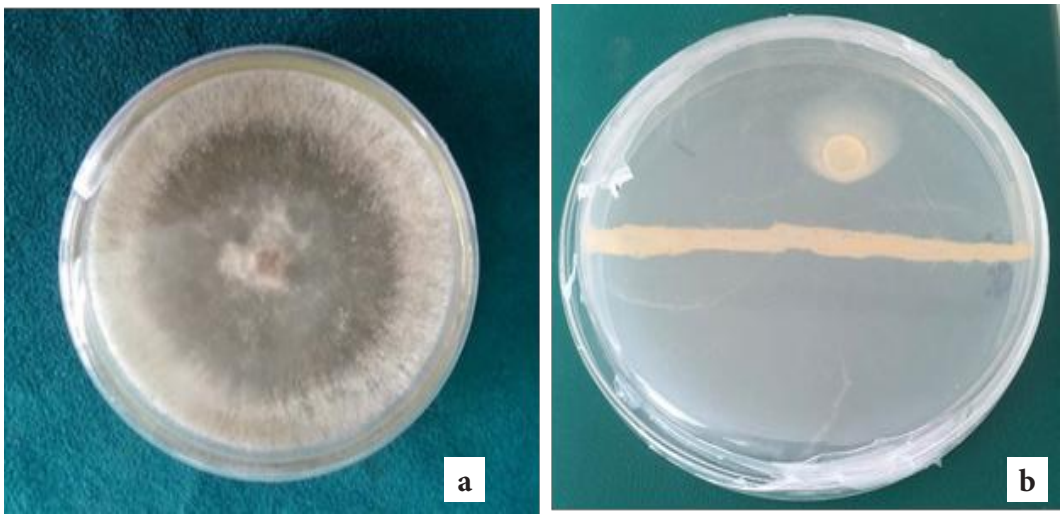


Fig. 4. Dual culture assay of *R. solani* and antagonistic bacteria. (a) Control (b) antagonism of bacterial isolate PLPL

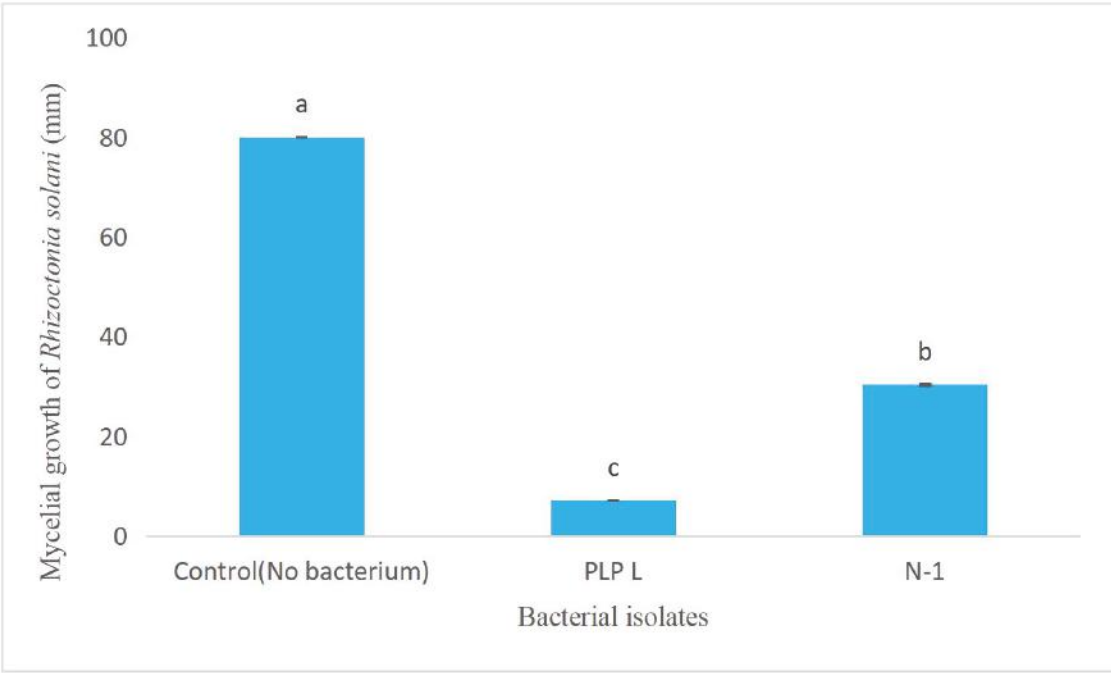


Fig. 5. Screening of bacterial isolates against *R. solani*. The bars above the columns represent the standard error of the mean (SEM). The means above the columns with distinct letters differ considerably as assessed by LSD (p = 0.05).

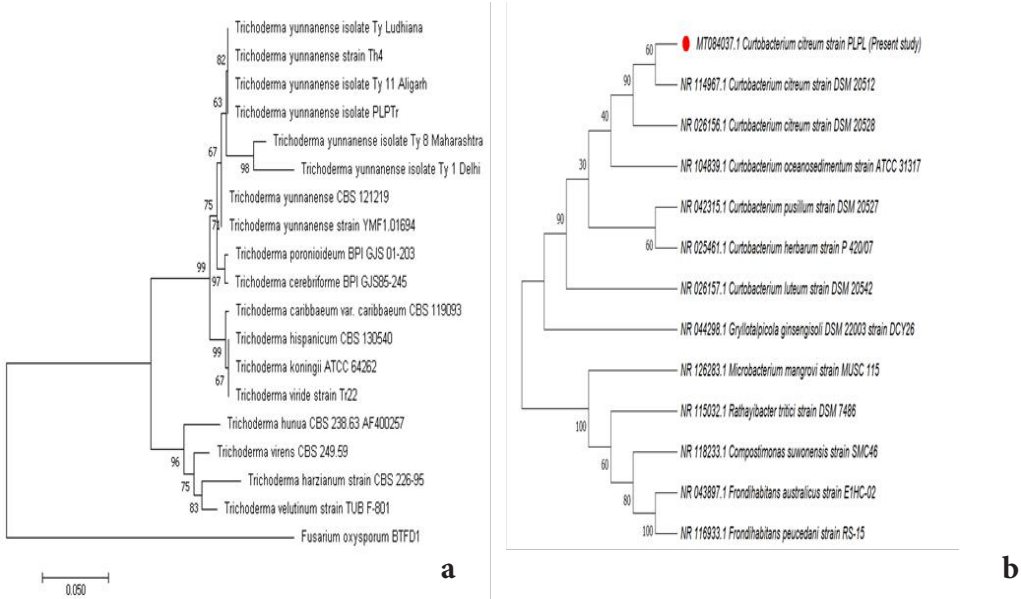


Fig. 6. Phylogenetic tree. a. *Trichoderma* spp. using ITS region, b. *Curtobacterium* spp using 16S rRNA.

treatment T_6, where seeds were treated with a consortium of *T. yunnanense* and *C. citreum* (Fig. 9a and b). This treatment resulted in the greatest reduction in DI (75.05%) and PDI (81.10%) relative to the pathogen-inoculated control. Similarly, for black scurf, soil inoculated with *Rhizoctonia solani* exhibited the highest DI (32.52%) and PDI (22.00%). In contrast, treatment T_5 showed the lowest DI (13.93%) and PDI (9.03%) (Fig. 9c and d), corresponding to the highest reductions in DI (57.26%) and PDI (58.97%) compared with the pathogen-inoculated control. Overall, application of biological control agents (BCAs), either individually or in consortium, effectively reduced disease severity. Notably, the consortium of *T. yunnanense* and *C. citreum* provided significantly better disease control than their individual applications.

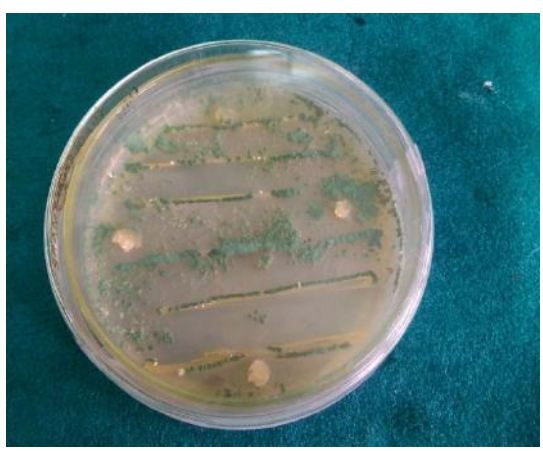


Fig. 7. Compatibility test of the biocontrol agents, *T. yunnanense* and *C. citreum*

Assessing the potential of microbial consortia on potato plant growth indicators and yield attributing traits

Plant growth–promoting (PGP) traits were significantly enhanced in all treatments except

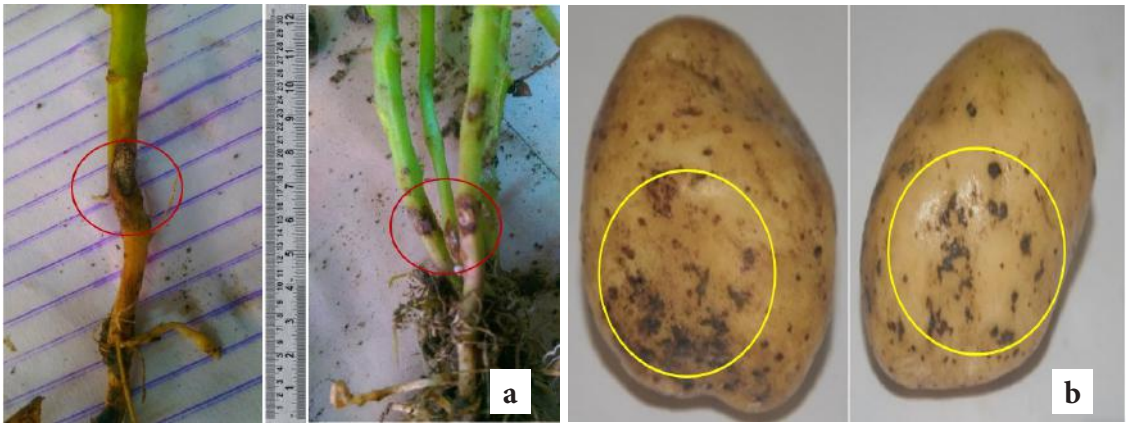


Fig. 8. Typical symptoms of stem canker (a) and black scurf (b) of potato

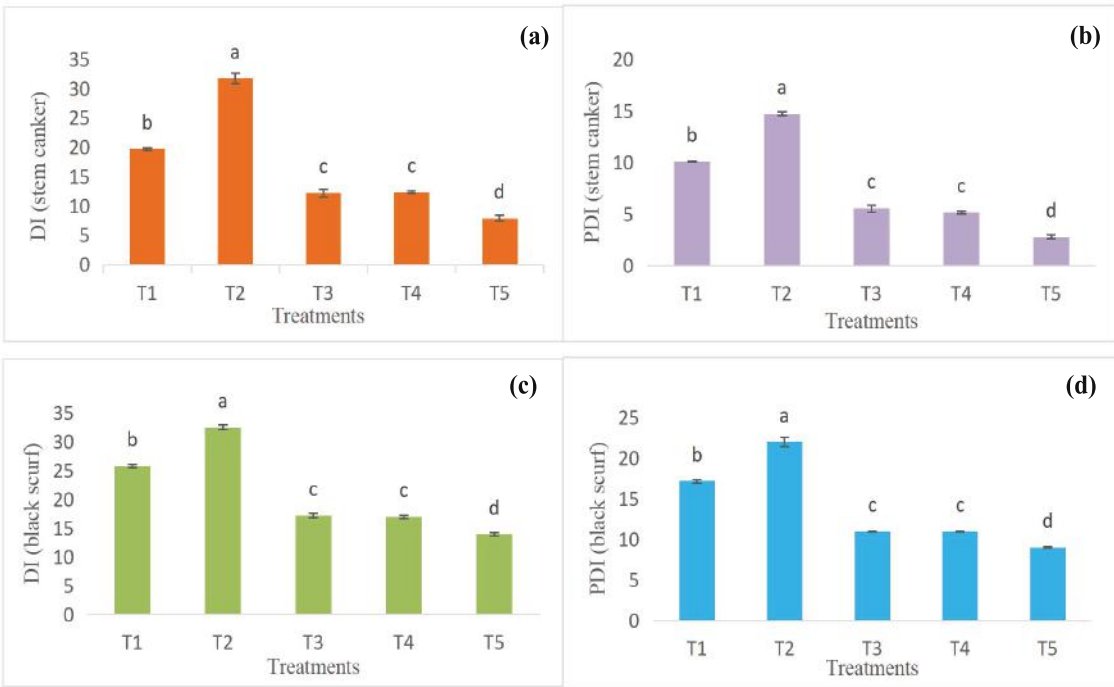
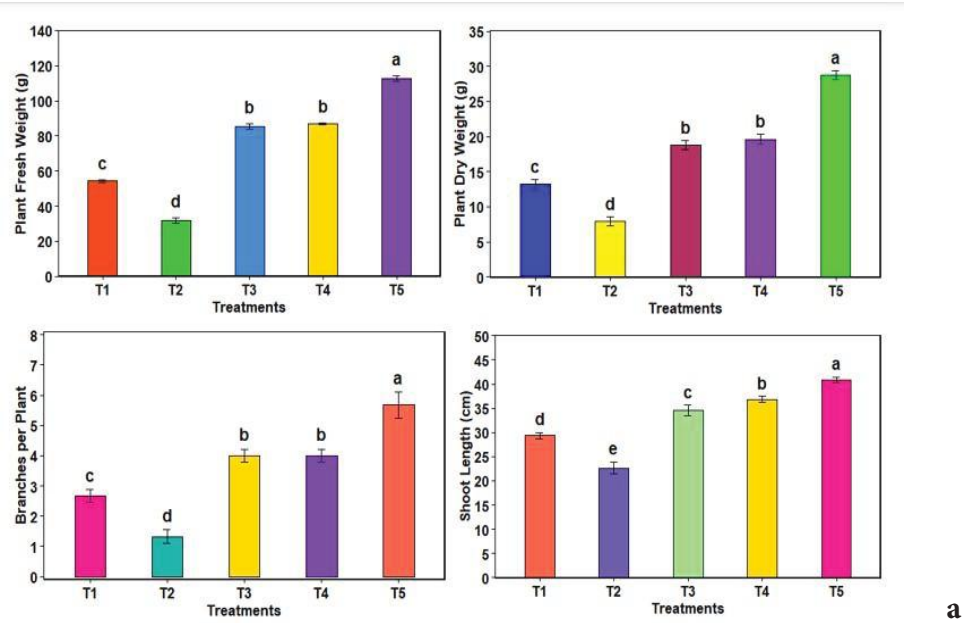
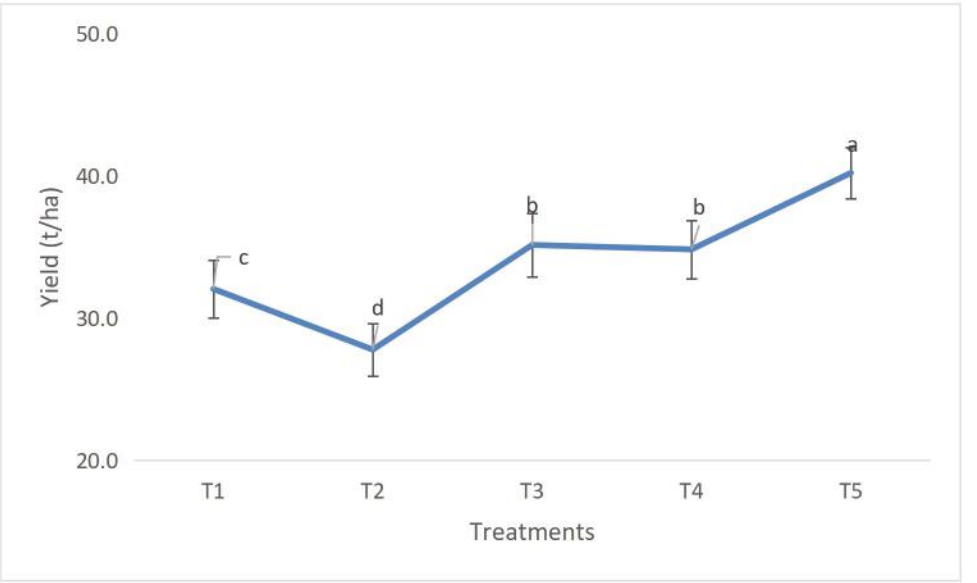


Fig. 9. Effect of microbial consortia on potato stem canker and black scurf disease. (a) Disease incidence of stem canker, (b) Percent disease index of stem canker, (c) Disease incidence of black scurf, (d) Percent disease index of black scurf. Bars on the column indicate (±) Standard Error of the Mean. The means above the columns with distinct letters differ considerably as assessed by LSD (p = 0.05). Here the treatments were; T_1: Soil without any treatment (control 1), T_2: Soil inoculation with pathogen (control 2), T_3: T_2 + seed treatment with *T. yunnanense*, T_4: T_2 + seed treatment with *C. citreum*, T_5: T_2 + seed treatment with consortium of *T. yunnanense* and *C. citreum*.



a



b

Fig. 10. Effect of microbial consortia on growth and yield of potato plants. a. growth parameters, b. yield attributes. Bars on the colums and trendline indicate (±) Standard Error of the Mean. The means above the columns and trendline with distinct letters differ considerably as assessed by LSD (p = 0.05). Here the treatments were; T_1: Soil without any treatment (control 1), T_2: Soil inoculation with pathogen (control 2), T_3: T_2 + seed treatment with *T. yunnanense*, T_4: T_2 + seed treatment with *C. citreum*, T_5: T_2 + seed treatment with consortium of *T. yunnanense* and *C. citreum*.

T_2 following application of *T. yunnanense* and *C. citreum*. Treatment T_5, in which seeds were treated with a consortium of *T. yunnanense* and *C. citreum*, produced the highest shoot length (40.67 cm), number of branches per plant (5.67), plant fresh weight (112.77 g), and plant dry weight (28.77 g). In contrast, treatment T2, where *R. solani* was introduced into the soil, resulted in the significantly lowest shoot length (22.67 cm), branches per plant (1.33), fresh weight (31.82 g), and dry weight (7.93 g) (Fig. 10a). The consortium of *T. yunnanense* and *C. citreum* was particularly effective in enhancing the growth attributes of potato plants. Moreover, evaluation of yield and yield-attributing traits revealed that application of these biological control agents (BCAs) significantly improved yield performance compared with the control. Treatment T_5 recorded the highest yield (40.19 t ha^{-1}), maximum tuber weight per plot (14.47 kg), and largest tuber diameter (5.09 cm), whereas treatment T_2 showed the lowest yield (27.77 t ha^{-1}), tuber weight per plot (10.00 kg), and tuber diameter (4.16 cm). The consortium treatment increased potato yield by 44.7% over the control (Fig. 10b). Overall, the consortium of *T. yunnanense* and *C. citreum* proved to be more effective than their individual applications.

Discussion

In agriculture, microbe-based bioinoculants are a useful tactic to counteract the need for crop production and the accelerating degradation of the environment (Yadav and Yadav, 2021). As they offer the plants numerous advantages, microbial consortia are thought to be a better bioformulation than single cultures that contain bioinoculant (Mondal *et al*., 2020). Beneficial microbial consortium is a natural and environment friendly approach for growth promotion and disease suppression in plants. In this study, we used *T. yunnanense* and *C. citreum* as a beneficial microbial consortium for the management of stem canker and black scurf diseases and boost potato yield.

In the present investigation, the beneficial microbes were isolated and screened for the ability to control the virulent isolate of *R. solani*. Isolate-3 of *R. solani* was used as test pathogen which was isolated from naturally infected crops and selected based on the pathogenicity test on potato causing 86.67% of seedling mortality. According to a previous study by Yang *et al*. (2017), black scurf and stem cankers on potatoes were indicative of the pathogenicity of the *R. solani* isolates. Our study data are consistent with them. The beneficial soil microbe *Trichoderma* was used as a BCA in this study. *Trichoderma* isolates were assessed against *R. solani* and isolate PLPTr inhibited 90.25% mycelial development of *R. solani*. According to several researchers, *Trichoderma* can successfully slow the growth of mycelial colonies (Das *et al*., 2019). *Trichoderma* can control plant pathogens through direct mycoparasitism by lysis and death of pathogens. It also acts on spore germination and mycelial growth suppression by antibiosis (Nusaibah and

Musa, 2019). A number of studies verified the significant impact of *T. harzianum* on *R. solani*, which attacks plenty of different crops in Bangladesh (Faruk and Rahman, 2016). Our results are in accordance with previous research that stated that the genus *Trichoderma* has been successfully utilized as biological control agent for *R. solani* (Das *et al*., 2014).

In the present study, the beneficial endophytic bacteria were isolated from endophytic regions of plants and screened for the ability to control *R. solani.* Bacterial isolate PLPL isolated from marigold, exhibited antagonistic activity towards *R. solani* and inhibited 91.13% of the mycelial development of *R. solani* compared to the control. Our results are consistent with prior publication indicating endophytic bacteria were effective in limiting the mycelial growth of *R. solani*. It is also claimed that several pathogenic fungi viz. *Rhizoctonia*, *Pythium*, *Fusarium*, *Aspergillus, Botrytis*, *Phytophthora*, *Pyricularia* and *Alternaria* are successfully controlled by antagonistic bacteria (Pal and Gardener, 2006). In this work, antagonistic bacteria's biocontrol strategy for inhibiting *R. solani* mycelial development in vitro was believed to involve competition, enzyme lysis, and antibiosis (Hari *et al*., 2023).

Both the fungal and bacterial isolates were molecularly analyzed and identified. DNA sequencing, physical characteristics, and phylogenetic tree analysis all confirmed the identification of the fungal and bacterial isolate. The results revealed that fungal isolate was *T. yunnanense* and the endophytic bacteria was *C. citreum.* Generally, ITS-based phylogenetic analysis is generally sufficient for fungal species identification, but multilocus analysis (e.g., *tef1*, *rpb2*) improves resolution and confidence. 16S rRNA gene analysis is widely accepted for bacterial identification, but species-level resolution may require additional genes (e.g., *gyrB*, *rpoB*) or genomic analysis in closely related taxa. Compatibility of these two BCAs did not show any antagonism. This finding complies with reports that asserted that *B. subtilis* did not exhibit any antagonistic behavior towards *Trichoderma* spp. (Ali *et al*., 2017). This result encourages the use of a mixture of these bio agents. However, the potential strains were formulated as a consortium and evaluated their effect in the field experiment.

T. yunnanense and *C. citreum* were used independently and as consortium in field experiment to control stem canker and black scurf disease of potato. The results of this study imply that single and combined use of *T. yunnanense* and *C. citreum* reduce the disease incidence and percent disease index of stem canker and black scurf diseases of potato. The highest reduction in DI (75.05% for stem canker and 57.26% for black scurf) and PDI (81.10% for stem canker and PDI (58.97%) were recorded in T_5 treatment over the pathogen inoculated plot. They also greatly boosted the growth, yield attributing factors and yield of potato. Consortia of *T. yunnanense* and *C. citreum* increased the yield up to 44.7%.

Our study results revealed that *T. yunnanense* can control stem canker and black scurf disease in field condition. Previously, it was reported that *T. yunnanense* exhibited enhanced antagonistic ability against *Alternaria brassicicola*, *A. solani,* and *Aspergillus ochraceus* (Karmakar *et al*., 2021). Numerous authors have reported that *T. harzianum* was successful in preventing soil-borne fungal infections of vegetables (Kumari *et al*., 2024). The reason behind this may be that *Trichoderma* can penetrate and consume the internal contents of plant pathogenic fungi, effectively controlling their spread and reducing their impact on the plant. It also produces various antimicrobial secondary metabolites that inhibits the growth and reproduction of plant pathogens. Additionally, it can activate the plant's defense response which protect the plant from disease infestation (Nusaibah and Musa, 2019). According to our results, *C. citreum* can effectively control stem canker and black scurf disease of potato. Our results are consistent with previous reports claiming that *C. citreum* can control plant pathogens through direct as well as indirect interactions between the bacterium and the plant (Lacava *et al*., 2007). Previously, it was reported that endophytic bacteria *Burkholderia* sp. and *Bacillus* sp. can control southern blight disease of tomato plants (Hari *et al*., 2023). Bacterial endophytes, *Burkholderia*, *Curtobacterium*, *Pseudomonas*, and *Bacillus* produce a variety of secondary metabolites, volatile compounds and antibiotics to counteract the harmful effects of pathogens through mechanisms similar to PGPR (Lodewyckx *et al*., 2002). *C. citreum* can produce antibacterial compounds that can suppress the growth of plant pathogens and can compete with them for nutrients, reducing their ability to cause disease. Additionally, *C. citreum* can induce the plant's own defense mechanisms, such as the production of phytoalexins, which are compounds that are toxic to plant pathogens. *Curtobacterium* can also colonize plant surfaces, creating a physical barrier that restricts the spread of plant pathogens for instance citrus-variegated chlorosis (Lacava *et al*., 2007). Our results are confirmed by several reports where *Trichoderma* and bacteria have influence on disease reduction of different crops. Previously researchers reported that in contrast to controls, the mixtures of *T. hamatum* + *Streptomyces griseoviridis* and *Coniothyrium minitans* + *S. griseoviridis* entirely reduced the occurrence of the white rot of beans disease and resulted in 100% plant survival (Bahkali *et al*., 2014). It is also found that potato resistance to *R. solani* was raised higher by combining *B. subtilis* and *T. virens* than by utilizing each biocontrol agent alone (Das *et al*., 2014). There is another report claiming that *B. subtilis* and *T. viride* consortium successfully controlled bacterial wilt tomato plant (Sood *et al*., 2021). According to Wong *et al*. (2021), *Pseudomonas aeruginosa* and *T. harzianum* as consortium can effectively control Fusarium wilt of banana plant. According to our findings, *T. yunnanense* and *C. citreum* reduce stem canker and black

scurf disease over the control. Our findings are in accordance with the report of Wang *et al*. (2019). They reported that common scab of potato is effectively suppressed by using a consortium of *B. subtilis* and *T. harzianum.*

Our study results revealed that *T. yunnanense* can increase the growth and yield of potato plants. It is already reported that several species of *Trichoderma* are now established to enhance plant growth as well as to increase yield significantly (Kotasthane *et al*., 2015). Also, *T. yunnanense* demonstrated a variety of antifungal and plant growth-promoting capabilities (Karmakar *et al*., 2021). Yao *et al*. (2023) stated that *Trichoderma* can not only prevent diseases but also promotes plant growth, improves nutrient utilization efficiency, enhances plant resistance, and improves agrochemical pollution of environment. Our study data are also consistent with these previous reports. Our results also pointed out that endophytic bacteria *C. citreum* improves the growth and yield of potato plants over the pathogen inoculated plot. Our findings are in accordance with the report of Bourles *et al*., (2019). They claimed that rhizosphere isolated *C. citreum* also promotes the growth of plants. Previously it is reported that bacterial endophytes benefit host plants by promoting growth and controlling diseases (Carrie *et al*., 2023). Bacterial endophyte *Burkholderia* sp. and *Bacillus* sp. can increase growth and yield of tomato plants by producing indole acetic acid (Hari *et al*., 2023). Our study results align with previous reports where growth and yield of different crops are promoted by using *Trichoderma* and bacteria. Previously it is reported that growth parameters of tomato and chilli plants are increased when they are inoculated with selected microbial consortium designed with *B. sonorensis* and *Funneliformis mosseae* (Desai *et al*., 2020). According to Bourles *et al*. (2020). plant growth is enhanced by co-inoculating *C. citreum* with arbuscular mycorrhizal fungi. There is another report claiming that *B. subtilis* and *T. viride* consortium successfully improved growth characteristics of tomato plant (Sood *et al*., 2021). The application of consortium of *P. aeruginosa* and *T. harzianum* can increase the overall growth characters of banana plants (Wong *et al*., 2021). According to our findings, *T. yunnanense* and *C. citreum* increased growth of potato plants and yield over the control. Our findings are in line with the report of Wang *et al*. (2019). They reported that potato tuber yield was increased by employing a consortium of *B. subtilis* and *T. harzianum.*

Conclusions

The present investigation indicated that *T. yunnanense* strain PLPTr and *C. citreum* strain PLPL, individually and as consortium, were efficient in suppressing stem canker and black scurf diseases along with increasing potato production. Consortium of *T. yunnanense*, and *C. citreum* was more effective in terms of disease control, growth promotion and yield of potato than their single application. This encourages the use of microbial consortia for

the sustainable management of *Rhizoctonia* diseases of potato.

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Conflict of Interest

The authors state that they have no conflicts of interest.

Author Contributions

Research implementation, data collection, analysis, drafting editing and revision of the manuscript (FT), Design and revision of the manuscript (AAK), Editing and revision of the manuscript (MTR), Design and revision of the manuscript (GKMMR), Conception, Research design, drafting and reviewing (RJ).

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