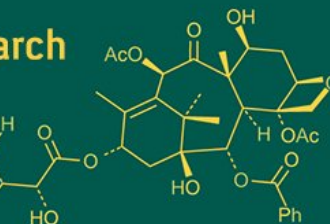
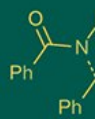


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Isolation, characterization and evaluation of native *trichoderma* isolates for biological suppression of fusarium wilt and growth promotion in pea (*Pisum sativum* L.)

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Abstract

Wilt disease of pea (*Pisum sativum* L.) caused by *Fusarium oxysporum* is a major soil-borne disease limiting pea productivity, while excessive dependence on chemical fungicides has raised concerns regarding environmental safety, pathogen resistance, and soil health degradation. Therefore, the present investigation was undertaken during 2024-25 to isolate, characterize, and evaluate native *Trichoderma* isolates for their antagonistic activity against important phytopathogens and their efficacy in suppressing pea wilt under glasshouse conditions. Thirty *Trichoderma* isolates were obtained from rhizospheric soils and characterized based on cultural, morphological, and microscopic traits. Their antagonistic potential was assessed through dual culture and inverted plate volatile assays against *Fusarium oxysporum*, *Sclerotium rolfsii*, *Alternaria solani*, *Aspergillus niger*, *Rhizoctonia solani*, and *Botrytis cinerea*. Biochemical attributes, including organic acid production and phosphate solubilization, were also evaluated. Promising isolates were further tested as seed bio-priming agents for wilt suppression and plant growth promotion in pea under glasshouse conditions. Considerable variability was observed among the isolates. In dual culture assays, isolate TDG exhibited complete inhibition (100%) of *F. oxysporum* and showed high antagonistic activity against other tested pathogens, whereas isolate TS40 recorded maximum inhibition of *R. solani* (88.14%) and *B. cinerea* (85.55%). In the volatile metabolite assay, TDG and TB1 displayed strong inhibitory effects against most pathogens, indicating the importance of volatile compounds in pathogen suppression. Biochemical characterization revealed maximum pH reduction (3.2) by TDG and TB1, suggesting enhanced organic acid production, while isolate TU3 recorded the highest phosphate solubilization (0.12 mg ml⁻¹). Under glasshouse conditions, seed bio-priming with selected isolates significantly enhanced seed germination and reduced wilt incidence compared with the untreated control. Isolate TDG recorded the highest germination (99%) and disease reduction (99%), followed by TB1 with 83.88% disease reduction, whereas the untreated control exhibited 100% disease incidence. The findings demonstrate that native *Trichoderma* isolates, particularly TDG and TB1, possess strong antagonistic, biochemical, and plant growth-promoting attributes and can serve as effective eco-friendly biocontrol agents for the management of Fusarium wilt of pea.

Keywords: *Trichoderma*, biological control, *Fusarium oxysporum*, pea wilt, seed bio-priming, phosphate solubilization, plant growth promotion

1. Introduction

Modern agricultural practices, while successful in enhancing crop productivity, have considerably disturbed the ecological balance of agro-ecosystems. The excessive and continuous use of chemical fertilizers and pesticides, coupled with mono-cropping systems, has led to soil and water contamination, increased incidence of pests and diseases, and accumulation of harmful chemical residues in agricultural produce (Bardin *et al.*, 2015) ^[1]. In view of the growing demand for safe food and environmentally sustainable farming systems, there is an urgent need to develop eco-friendly and efficient alternatives for plant disease management. Biological control has emerged as a promising strategy that employs beneficial microorganisms and their metabolites to suppress plant pathogens, thereby reducing dependence on chemical inputs (Harman *et al.*, 2021) ^[2]. Biological control agents (BCAs) serve a dual function as bio-pesticides and bio-fertilizers.

In addition to suppressing plant pathogens, they enhance soil health through bioremediation and improved nutrient availability, thus contributing to ecological balance and sustainable agricultural production (Baker and Paulitz, 1996) [3]. BCAs suppress pathogens through diverse mechanisms, including mycoparasitism, production of antibiotics and lytic enzymes, competition for nutrients and space, and induction of systemic resistance in plants (Pal and Gardener, 2006) [4]. Beneficial microorganisms, particularly plant growth-promoting fungi and bacteria, further stimulate plant growth by enhancing nutrient uptake, synthesizing phytohormones, and effectively colonizing plant roots, thereby strengthening plant defense systems (Islam *et al.*, 2016) [5].

Among the various biological control agents, species of the genus *Trichoderma* have received considerable attention owing to their adaptability and broad-spectrum antagonistic activity against numerous plant pathogens. This fungal biocontrol agent belongs to the family Hypocreaceae, are commonly found in soil and rhizosphere environments (Kubicek *et al.*, 2019) [6]. Several species, including *T. harzianum*, *T. viride*, *T. asperellum*, and *T. atroviride*, have been extensively investigated for their effectiveness in biological disease management (Sánchez-Montesinos *et al.*, 2021; Sun *et al.*, 2022) [7, 8]. They produce bioactive secondary metabolites and cell wall-degrading enzymes that suppress pathogens, enhance nutrient uptake, promote plant growth, and improve tolerance to abiotic stresses. Numerous studies have demonstrated successful management of diseases such as seed rot, damping-off, wilt, and sheath blight through application of *Trichoderma* spp. (Jogaiah *et al.*, 2013; Luo *et al.*, 2010) [29, 30].

Pea (*Pisum sativum* L.), a cool-season legume belonging to the family Fabaceae, is widely cultivated for its high nutritional value. It serves as an important source of proteins, carbohydrates, vitamins, and essential minerals, contributing significantly to human nutrition (Sharma, 2010) [9]. Globally, pea occupies millions of hectares and plays a vital role in ensuring food and nutritional security. India ranks among the leading pea-producing countries (Mohanty *et al.*, 2020) [10]. However, pea cultivation is constrained by several fungal diseases, particularly damping-off and wilt caused by pathogens such as *Rhizoctonia*, *Pythium*, *Phytophthora*, and *Fusarium*, which adversely affect seed germination, seedling establishment, and overall crop productivity (Lamichhane *et al.*, 2017) [11]. High-quality seed is fundamental for successful crop establishment and yield enhancement but biological seed treatment, especially seed bio-priming with beneficial microorganisms, has gained prominence as an environmentally safe approach for managing seed- and soil-borne pathogens while improving germination and seedling vigor (Harman and Taylor, 1988) [12]. Bio-priming ensures rapid colonization of beneficial microbes on seed surfaces, enhances plant tolerance to environmental stresses, and strengthens resistance against pathogens. Among microbial agents, *Trichoderma* species have been widely reported to enhance germination percentage, seedling growth, chlorophyll content, and overall plant health in pea and other crops (Singh *et al.*, 2015) [3].

The natural habitat of *Trichoderma* is predominantly soil and the rhizosphere, where it plays a crucial role in plant protection and growth promotion. Beyond disease suppression, these fungi produce phytohormones, solubilize

nutrients, decompose organic residues, and improve soil fertility. Owing to these multifaceted benefits, *Trichoderma* represents a major component of the global fungal biocontrol market (Swain and Mukherjee, 2020) [14]. Considering increasing environmental concerns and the necessity for sustainable disease management, exploration and utilization of native *Trichoderma* isolates are essential for developing region-specific, effective, and adaptable biocontrol strategies. Therefore, in light of the importance of eco-friendly disease management and improvement of seed quality, the present investigation was undertaken on eco-friendly and sustainable management of Fusarium wilt disease in pea by application of native *Trichoderma* spp., in mid hills of Uttarakhand.

2. Materials and Methods

The present investigation was conducted in the Biocontrol Laboratory, Department of Plant Pathology, College of Hill Agriculture, Ranichauri, Tehri Garhwal, Uttarakhand during 2024-2025. The laboratory experiments were laid out in a Completely Randomized Design (CRD) with eight treatments and three replications.

3. Collection of soil samples and isolation of *trichoderma* isolates

A total of seventy rhizospheric soil samples were collected from different farming situations of mid hill regions of Uttarakhand. From each field, soil samples were collected from 4-5 healthy plants, including one plant from the centre and four from the corners of the selected plot. Approximately 10 g of soil from each location was collected in labelled sterile sampling bags. Isolation of *Trichoderma* spp. from soil samples was carried out using the serial dilution plate technique on *Trichoderma* Selective Medium (TSM) (Waksman, 1922) [15]. The isolated cultures were characterized based on cultural, morphological and microscopic characteristics.

4. In vitro evaluation of *Trichoderma* isolates

The antagonistic potential of isolated *Trichoderma* spp. was evaluated against major plant pathogens viz., *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Botrytis cinerea*, *Aspergillus niger* and *Alternaria solani* using Dual Culture Technique on PDA. Isolated *Trichoderma* isolates were assessed against fungal pathogens by the inverted plate assay technique on PDA for antibiosis test (Dennis and Webster 1971) [16]. Percent inhibition of mycelial growth was calculated using the standard formula (Wonglom *et al.*, 2019) [17].

$$I = \frac{C - T}{C} \times 100$$

Where,

C = Mycelial growth of pathogen in control plate

T = Mycelial growth of pathogen in treatment (Test Plate)

I= Per cent mycelial growth inhibition of pathogen.

5. Organic acid production, change in pH and phosphate solubilisation

Organic acid production by different *Trichoderma* isolates was evaluated using Pikovskaya's broth supplemented with bromophenol blue indicator. Five mm mycelial discs from 72-hour-old cultures were inoculated into broth and

incubated at 28 °C for 21 days Prasad (2023) [18]. To measure pH, the electrode was rinsed with distilled water, and the meter was set to pH mode at 25 °C. The electrode was then placed in the media, and the pH reading was noted after 30 seconds of stabilization. Phosphate solubilization potential was assessed using both Pikovskaya's agar and broth media. In Pikovskaya's agar assay, isolates were inoculated on medium containing tri-calcium phosphate and halo zone formation was recorded. In broth assay, cultures were incubated for 21 days, followed by filtration, centrifugation and reaction with Bray's extract and chloromolybdc acid reagent and absorbance was measured spectrophotometrically at 660 nm with a standard curve generated using a standard K₂HPO₄ solution for reference.

6. Development of bioformulation from selected promising *Trichoderma* isolates

Selected promising *Trichoderma* isolates were mass multiplied using barnyard millet (*Echinochloa frumentacea* L.) grains. The grains were soaked in water for 12 hr (50 g/250 ml flask), autoclaved (15 lbs psi for 30 minutes), inoculated with mycelial discs of selected *Trichoderma* isolates and incubated at 28 °C for 12 days. Colonized grains by *Trichoderma* were air-dried in open shade, fine-powdered with the help of a Willy Mill grinder, sieved through a 50-mesh sieve, and mixed with sterilized talcum powder (1:3 ratio) along with 1% carboxymethyl cellulose (CMC) to prepare a talc-based bioformulation (Mohiddin *et al.*, 2017) [19].

7. Plant material

Seeds of pea (*Pisum sativum* L.) variety 'Arkel' were procured from the local market of Ranichauri, Tehri Garhwal, Uttarakhand.

8. Seed treatment

Seven promising *Trichoderma* isolates were evaluated for their effect on the seed. The details of treatments used in the present study for evaluation of *Trichoderma* isolates toward plant growth promotion activities using a pot experiment under glass house conditions are depicted in 5. Seeds were surface sterilized using 1% sodium hypochlorite solution for 3 min, then they were rinsed with sterile distilled water. Seeds were treated with a talc-based formulation of *Trichoderma* isolates @10 g/kg seeds.

9. Evaluation of *Trichoderma* isolates under glasshouse conditions for wilt disease suppression in pea caused by *Fusarium oxysporum*

Pathogen inoculum of *Fusarium oxysporum* was prepared using sterilized barnyard millet grains and incubated at 28°C for 14 days. Sterilized sandy loam soil mixed with 0.5% compost was filled in plastic pots (30 cm diameter) containing 5 kg of soil per pot. Soil was inoculated with fungal inoculum @10 g per pot and incubated for 8 days. Sand and sandy loam soils were autoclaved at 121 °C for 1 h. Sterilized soil amended with 0.5% pathogen-free matured compost was filled in sterilized plastic pots (30 cm diameter; 5 kg soil/pot). Each pot was inoculated with 10 g fungal inoculum mixed up to 5 cm soil depth and incubated under warm, humid conditions for 8 days. Seeds were bio-primed with talc-based formulations of seven *Trichoderma* isolates and ten seeds were sown per pot, while T₁ (untreated control) received non-bio-primed seeds. The

Details of treatments used for evaluation of *Trichoderma* isolates for wilt disease suppression in pea crop under glasshouse conditions are as follows

Symbol	Treatment details
T1	Untreated control
T2	Seed bio-priming with <i>Trichoderma</i> isolate TS18
T3	Seed bio-priming with <i>Trichoderma</i> isolate TS40
T4	Seed bio-priming with <i>Trichoderma</i> isolate TDG
T5	Seed bio-priming with <i>Trichoderma</i> isolate TS23
T6	Seed bio-priming with <i>Trichoderma</i> isolate TB1
T7	Seed bio-priming with <i>Trichoderma</i> isolate TS26
T8	Seed bio-priming with <i>Trichoderma</i> isolate TCH6

10. Observations

Percent disease incidence (PDI) was calculated using (Shiva *et al.* 2020) [20].

$$PDI(\%) = \frac{\text{Number of wilted plants}}{\text{Total number of plants examined}} \times 100$$

Percent disease reduction (PDR) over control was calculated as:

$$PDR(\%) = \frac{DIC - DIT}{DIC} \times 100$$

Where,

DIT=Disease incidence in treatment

DIC=Disease incidence in Untreated control

11. Statistical analysis

Experimental data were analyzed using a Completely Randomized Design (CRD). Analysis of variance (ANOVA) was performed using MS Excel and OPSTAT software at a 5% level of significance (Gomez, 1984) [21].

12. Results and Discussion

12.1 Isolation and characterization of isolates

Thirty native *Trichoderma* isolates were isolated from seventy soil samples collected from mid and high hills of Uttarakhand. Maximum isolates were obtained from Tehri district (13 isolates) followed by Chamoli (3 isolates), whereas Nainital, Pauri, Bageshwar, Uttarkashi and Almora districts yielded comparatively fewer isolates. These isolates were screened for their cultural and morphological characteristics on PDA medium (Table 1). Considerable variation was observed among the isolates with respect to colony growth rate, mycelial colour, colony pigmentation, texture, concentric ring formation, and radial growth. Significant variation was recorded in radial growth among the isolates. At 4 days after inoculation (DAI), colony diameter ranged from 56.00 mm (TS10) to 90.00 mm (TS18, TS26, TDG, TS32, TS40, TB1, and TCH6). Fast-growing isolates such as TS18, TS23, TS26, TDG, TS32, TS40, and TB1 covered the full Petri plate (90 mm) within 4 days, indicating rapid mycelial extension. At 7 DAI, all isolates attained 90.00 mm colony diameter, indicating uniform final growth potential but differing growth kinetics. The rapid radial growth observed in certain isolates may reflect higher metabolic activity and competitive ability, which are important attributes for effective rhizosphere colonization and biocontrol potential. The initial mycelial colour of most isolates was white, except TS15, TS32, TS45, TB4, and TCH3, which showed yellow or yellowish-green mycelium. With increasing incubation time, colony colour varied from white to green, dark green, yellowish

green, and pale green. Green pigmentation, characteristic of conidial production in *Trichoderma*, was prominent in several isolates such as TS26, TS22, TS29, TDG, and TCH5 (Figure 1). Similar characteristics of plant growth-promoting microorganisms from rhizospheric soils using dilution plating techniques has been reported earlier by Yeole and Dube (1997 and Hadar *et al.* (1984) [22, 23]. Significant variability was observed among isolates with respect to cultural and morphological characteristics. Eight isolates exhibited significantly faster radial growth and covered the entire Petri plate within four days (90 mm), whereas three isolates required five days and nineteen isolates required seven days. Colony colour on the upper surface includes Green, White, Yellowish green, Dark green and Light green and on the lower surface includes Off-white, Yellow, Pale green and Pale yellow in different *Trichoderma* isolates. With respect to colony texture majority of isolates exhibited smooth colony texture, while nine isolates showed coarse/ rough texture. With respect to concentric rings, 6 *Trichoderma* isolates had concentric rings type growth, whereas 24 *Trichoderma* isolates lacked concentric rings, as shown in Table 1. Under microscopic observations, isolates of *Trichoderma* were found to produce conidia of various shapes, ranging from globose to sub-globose and oval to ellipsoidal (Table 2 and Figure 2). Similar variability among *Trichoderma* isolates has been reported previously (Sharma and Singh, 2014) [24].

12.2 In-vitro evaluation of *Trichoderma* isolates

In-vitro antagonistic evaluation revealed statistically significant differences among isolates. In the dual culture assay, isolate TDG showed significantly higher mycelial inhibition against *Fusarium oxysporum* (100%), which was statistically superior to other isolates as shown in Table 3. Similarly, TDG recorded significantly higher inhibition against *Sclerotium rolfsii* (88.51%), *Alternaria solani* (88.52%) and *Aspergillus niger* (83.33%), which were statistically at par with TB1. However, isolate TS40 exhibited significantly higher inhibition against *Rhizoctonia solani* (88.14%) and *Botrytis cinerea* (85.55%). These findings corroborate earlier reports demonstrating strong antagonistic potential of *Trichoderma* spp. against soil-borne pathogens (Matarese *et al.*, 2012 and Younesi *et al.*, 2021) [25, 26]. In the inverted plate assay, isolate TDG recorded significantly higher mycelial inhibition against *F. oxysporum* (93.33%), *S. rolfsii* (85.92%), *B. cinerea* (87.45%), *A. solani* (88.52%) and *A. niger* (85.55%) and was statistically at par with TB1. However, against *R. solani*, isolate TB1 recorded significantly higher inhibition (85.55%), which was statistically similar to TDG (85.18%) as shown in Table 4. Similar inhibition of pathogens through volatile metabolite production by *Trichoderma* spp. has been reported earlier by Kumar *et al.* (2019) [27]. During biochemical evaluation, isolates TDG and TB1 recorded significantly higher pH reduction (3.2), indicating higher organic acid production potential. In phosphate solubilization assay on solid medium, seven isolates tested positive, whereas the remaining isolates showed no solubilization. In Pikovskaya broth medium, isolate TU3 recorded significantly higher phosphate solubilization (0.12 mg ml⁻¹), which was statistically superior to other isolates, whereas isolate TB4 recorded the lowest solubilization (0.07 mg ml⁻¹). Similar pressive phosphate solubilization activity among *Trichoderma* isolates has been reported earlier by Prasad *et al.* (2023) [18].

12.3 Effect of *Trichoderma* isolates on wilt disease suppression under glasshouse conditions

Evaluation of *Trichoderma* isolates applied through seed bio-priming under glasshouse conditions revealed significant differences among treatments for seed germination, percent disease incidence (PDI), and percent disease reduction (PDR) against wilt of pea caused by *Fusarium oxysporum* (Table 5 and Figure 3). Among the treatments, T4 recorded the highest seed germination (99.00%), which was significantly superior to all other treatments. This was followed by T6 (95.52%) and T8 (93.33%). The untreated control (T1) exhibited the lowest germination percentage (53.33%). With respect to disease incidence, T4 showed the minimum PDI (1.00%), which was highly significantly lower than all other treatments and resulted in the highest disease reduction (99.00%). T6 was the next best treatment, recording a PDI of 16.12% and a PDR of 83.88%. Treatments T8 (22.34% PDI; 77.66% PDR), T7 (26.33% PDI; 73.67% PDR), T2 (30.00% PDI; 70.00% PDR), T5 (33.33% PDI; 66.67% PDR) and T3 (35.55% PDI; 64.45% PDR) were statistically inferior to T4 but significantly superior to the untreated control. The untreated control (T1) recorded the highest disease incidence (100.00%) with no disease reduction (0.00%), confirming severe wilt development in the absence of bio-priming treatment. The differences among treatments were statistically significant at the 5% level of significance, as indicated by the critical difference (CD) values (CD_{0.05} = 0.788 for germination, 2.060 for PDI, and 2.650 for PDR), as shown in Table 6 and Figure 3. Disease suppression might be attributed to antagonistic, parasitic and competitive behaviour of *Trichoderma* spp., along with production of antibiotics, volatile metabolites and hydrolytic enzymes such as glucanases, chitinases and pectinases. Similar mechanisms of pathogen suppression by *Trichoderma* spp. have been reported earlier by Howell (2003) [28].

13. Conclusion

The present investigation revealed substantial variability among native *Trichoderma* isolates with respect to their cultural, morphological, microscopic, antagonistic, and plant growth-promoting attributes, highlighting the rich diversity of indigenous *Trichoderma* populations. Based on cultural characteristics and microscopic observations, the isolates were tentatively identified as belonging to different *Trichoderma* species known for their biocontrol potential against a wide range of phytopathogens. Among the isolates evaluated, TDG and TB1 exhibited superior antagonistic activity against most of the test pathogens, while TS40 demonstrated remarkable efficacy against *Rhizoctonia solani* and *Botrytis cinerea*. The enhanced inhibitory activity exhibited by these isolates may be attributed to a combination of mechanisms, including rapid colonization, competition for space and nutrients, mycoparasitism, and the production of diffusible and volatile antifungal metabolites. Furthermore, isolate TU3 displayed the highest phosphate-solubilizing ability, indicating its potential role in nutrient mobilization and plant growth enhancement. Under glasshouse conditions, seed bio-priming with selected *Trichoderma* isolates significantly improved seed germination and effectively suppressed *Fusarium* wilt of pea compared with the untreated control. Among the treatments, isolate TDG proved to be the most promising, recording the highest seed germination (99%) and disease reduction (99%), followed by TB1. The strong correspondence

between *in vitro* antagonistic performance and *in vivo* disease suppression underscores the reliability of laboratory-based screening for the identification of effective biocontrol candidates. Collectively, the findings demonstrate that native *Trichoderma* isolates, particularly TDG and TB1, possess multifunctional traits encompassing pathogen suppression, plant growth promotion, and nutrient mobilization. These isolates hold considerable promise as environmentally sustainable alternatives to chemical fungicides and warrant further validation under diverse agro-climatic field conditions. Their successful development into stable and efficient bioformulations could contribute significantly to the integrated and eco-friendly management of Fusarium wilt in pea cultivation.

14. Statements & Declarations

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- Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

- Authors' contributions: All authors contributed to the study's conception and design. Material preparation, data collection and analysis.
- Data availability: The datasets generated during and analysed during the current study are available from the first author upon reasonable request.
- Ethics approval: This research article does not contain any studies with human participants or animals performed by any authors.
- Consent to participate: This manuscript is new and not considered elsewhere for publication. All authors have approved the submission of this manuscript.
- Conflict of Interest: The authors declare no conflict of interest.

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Fig 1: Cultural and morphological growth of potential *Trichoderma* isolates

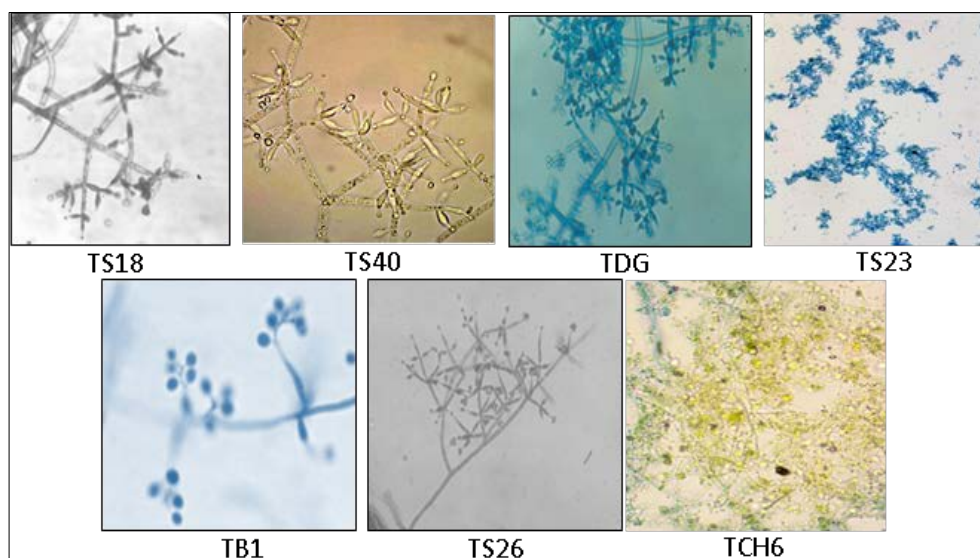


Fig 2: Microscopic observations of potential *Trichoderma* isolates



Fig 3: Germination and wilting in pea crop under glass house conditions (A) Plants raised from seeds pre-bioprimered with *Trichoderma* showed faster growth compared to plants raised from untreated seeds (control), (B) Percent disease incidence was higher in control than treated plants.

Table 1: Cultural and morphological characteristics of *Trichoderma* isolates

S. No.	<i>Trichoderma</i> isolate code	Days to cover full Petri plate	Mycelium colour	Colony colour		Colony texture	Concentric rings (P/A)	Colony diameter (mm)	
				Upper surface	Lower surface			At 4th day	At 7th day
1	TS3	7 days	White	Green	Yellow	Smooth	A	64.67	83.67
2	TS4	5 days	White	White	Off-white	Smooth	A	85.67	90.00
3	TS7	7 days	White	White	White	Smooth	A	67.67	90.00
4	TS10	7 days	White	Green	Pale green	Smooth	A	56.00	90.00
5	TS11	7 days	White	Whitish to Green	Yellow	Smooth	A	64.33	90.00
6	TS15	7 days	Yellow	Yellow to Green	Yellow	Smooth	A	68.67	90.00
7	TS18	4 days	White	Yellowish green	Yellow	Rough	P	90.00	90.00
8	TS22	5 days	White	Dark green to Light green	Dark green to Light green	Smooth	P	78.33	90.00
9	TS23	4 days	White	White	White	Rough	P	87.67	90.00
10	TS25	7 days	White	Light green	Light green	Smooth	A	66.33	90.00
11	TS26	4 days	White	Dark green	Dark green	Rough	P	90.00	90.00
12	TS29	7 days	White	Green	Green	Rough	A	59.33	90.00
13	TDG	4 days	White	Green	Green	Rough	A	90.00	90.00
14	TS32	4 days	Yellow	Green	Green	Smooth	A	90.00	90.00
15	TS33	7 days	White	Whitish to Green	Whitish to Green	Smooth	A	61.33	90.00
16	TS35	7 days	White	Yellow to Green	Yellow to green	Smooth	A	65.67	90.00
17	TS37	7 days	White	Green	Green	Smooth	A	67.33	90.00
18	TS40	4 days	White	Greenish white	Greenish white	Rough	P	90.00	90.00
19	TS43	7 days	White	Greenish white	Off-white	Smooth	A	59.00	90.00
20	TS45	7 days	Yellow	Green	Yellow	Smooth	A	61.33	90.00
21	TS48	7 days	White	Whitish to Green	Off-white	Smooth	A	63.33	90.00
22	TB1	4 days	White	Yellowish green	Pale yellow	Rough	P	90.00	90.00
23	TB4	7 days	Yellow	Green	Off-white	Smooth	A	69.00	90.00
24	TU2	7 days	White	Dark green	Green	Smooth	A	63.00	90.00
25	TU3	7 days	White	Whitish to Green	Yellow	Smooth	A	65.33	90.00
26	TA2	7 days	White	Greenish to White	Yellow	Smooth	A	63.00	90.00
27	TA5	7 days	White	Green	Yellow	Smooth	A	57.67	90.00
28	TCH3	7 days	Yellowish green	Yellowish green	Green	Smooth	A	58.67	90.00
29	TCH5	5 days	White	Green	Green	Rough	A	82.00	90.00
30	TCH6	4 days	White	White	Pale yellow	Rough	P	90.00	90.00

Table 2: Microscopic characteristics of *Trichoderma* isolates

S. No.	<i>Trichoderma</i> isolate code	Microscopic characteristics				
		Conidia shape	Conidia size (µm)	Conidia colour	Phialide size (µm)	Chlamydospores
1	TS3	Globose to subglobose	5.6×4.8	Green	11.2×3.9	Present
2	TS4	Subglobose to obovoid	2.8×2.5	Pale green	5.4×2.7	Present
3	TS7	Ellipsoidal	5.2×3.6	Dark green	12.6×3.9	Present
4	TS10	Broadly ellipsoidal	5.0×4.2	Green	12.0×4.4	Present
5	TS11	Globose to subglobose	3.8×3.1	Green	13.1×4.7	Present
6	TS15	Globose to subglobose	5.6×4.8	Green	13.8×4.9	Absent
7	TS18	Subglobose to obovoid	2.7×2.5	Pale green	6.3×3.0	Present
8	TS22	Globose to subglobose	5.1×4.2	Pale green	13.1×4.2	Present
9	TS23	Globose to subglobose	5.7×4.8	Green	13.2×4.9	Present
10	TS25	Oblong to ellipsoidal	4.3×3.7	Dark green	8.0×3.8	Absent
11	TS26	Ellipsoidal	3.9×3.1	Dark green	8.3×4.0	Present

12	TS29	Oblong to ellipsoidal	5.4×3.9	Dark green	12.5×4.1	Present
13	TDG	Subglobose to obovoid	2.6×2.5	Pale green	5.8×2.6	Present
14	TS32	Subglobose to obovoid	2.9×2.6	Pale green	6.4×2.9	Present
15	TS33	Ellipsoidal	5.8×4.0	Green	10.2×4.1	Present
16	TS35	Oblong to ellipsoidal	5.6×3.5	Dark green	11.5×3.7	Present
17	TS37	Broadly ellipsoidal	7.2×4.3	Dark green	8.9×5.0	Present
18	TS40	Globose to subglobose	4.6×3.8	Green	12.6×4.1	Present
19	TS43	Oblong to ellipsoidal	4.9×3.5	Green	12.0×4.0	Present
20	TS45	Globose to subglobose	3.8×3.1	Green	7.3×3.0	Present
21	TS48	Globose to subglobose	4.7×3.3	Green	11.6×4.1	Present
22	TB1	Subglobose to obovoid	3.0×2.6	Pale green	11.0×3.4	Present
23	TB4	Subglobose to obovoid	2.6×2.5	Pale green	8.3×2.8	Absent
24	TU2	Subglobose to obovoid	3.0×2.8	Pale green	6.2×3.1	Present
25	TU3	Broadly ellipsoidal	6.8×4.4	Dark green	6.8×2.1	Present
26	TA2	Oblong to ellipsoidal	4.8×3.7	Dark green	5.6×2.3	Present
27	TA5	Subglobose to obovoid	2.8×2.5	Pale green	5.7×3.1	Absent
28	TCH3	Globose to subglobose	5.3×3.8	Green	10.6×4.0	Present
29	TCH5	Globose to subglobose	4.3×3.7	Green	9.3×3.4	Present
30	TCH6	Globose to subglobose	5.6×4.8	Green	12.1×4.6	Present

Table 3: Effect of *Trichoderma* isolates on mycelial growth inhibition of pathogens by dual culture assay at 7th day of incubation

S. No.	<i>Trichoderma</i> isolate code	Dual culture technique											
		<i>F. oxysporum</i>		<i>S. rolfsii</i>		<i>A. solani</i>		<i>A. niger</i>		<i>R. solani</i>		<i>B. cinerea</i>	
		Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth
1	TS3	2.90	67.77	3.53	60.74	2.76	69.25	4.20	53.33	1.83	79.62	3.16	64.81
2	TS4	2.60	71.10	2.70	70.00	1.96	78.14	3.80	57.77	2.23	75.18	3.26	63.70
3	TS7	4.00	55.55	4.33	51.85	2.93	67.40	4.10	54.44	3.26	63.70	3.10	65.55
4	TS10	2.26	74.81	2.93	67.40	2.46	72.59	3.90	56.66	2.43	72.96	3.03	66.29
5	TS11	2.96	67.03	4.43	50.74	3.10	65.55	4.03	55.18	2.53	71.84	2.83	68.51
6	TS15	3.00	66.66	4.60	48.88	2.70	69.99	4.06	54.81	3.90	56.66	3.40	62.22
7	TS18	1.13	87.40	1.40	84.44	1.36	84.81	2.60	71.10	1.20	86.66	1.63	81.85
8	TS22	2.16	75.92	2.20	75.55	2.03	77.40	4.60	48.88	1.53	82.96	2.33	74.07
9	TS23	1.23	86.29	1.60	82.22	1.53	82.95	2.63	70.73	1.20	86.66	2.16	75.92
10	TS25	2.10	76.66	2.73	69.63	2.03	77.40	3.83	57.40	2.03	77.40	3.03	66.29
11	TS26	1.10	87.77	1.70	81.11	1.06	88.14	2.90	67.77	1.30	85.55	1.40	84.44
12	TS29	2.76	69.25	2.10	76.66	2.16	75.92	3.50	61.11	2.13	76.29	2.86	68.14
13	TDG	0.00	100.00	1.26	85.92	1.03	88.51	1.50	83.33	1.26	85.92	1.33	85.18
14	TS32	1.80	79.99	2.76	69.25	1.70	81.11	3.16	64.81	1.44	83.99	2.73	69.62
15	TS33	3.00	66.66	3.43	61.85	2.43	72.96	4.20	53.33	3.06	65.92	3.83	57.40
16	TS35	3.16	64.81	4.03	55.18	3.03	66.29	4.90	45.55	3.20	64.44	3.20	64.44
17	TS37	4.03	55.18	4.46	50.37	2.70	69.99	4.10	54.44	2.93	67.40	2.93	67.40
18	TS40	1.26	85.92	1.80	80.00	1.20	86.66	2.43	72.96	1.06	88.14	1.30	85.55
19	TS43	2.16	75.92	3.20	64.44	1.86	79.25	4.00	55.55	1.96	78.14	2.33	74.07
20	TS45	3.96	55.92	4.80	46.66	3.00	66.66	4.00	55.55	3.23	64.07	3.83	57.40
21	TS48	2.20	75.55	3.30	63.33	2.20	75.55	3.83	57.40	2.23	75.18	2.46	72.59
22	TB1	0.00	100.00	1.33	85.18	1.10	87.77	2.36	73.70	1.16	87.03	1.50	83.33
23	TB4	3.76	58.14	5.10	43.33	2.96	67.03	4.40	51.10	3.06	65.92	3.86	57.03
24	TU2	2.26	74.81	3.56	60.37	2.30	74.44	3.83	57.40	2.43	72.96	3.03	66.29
25	TU3	3.10	65.55	5.60	37.77	3.00	66.66	3.96	55.92	3.40	62.22	3.96	55.92
26	TA2	4.10	54.44	4.56	49.25	2.63	70.73	3.96	55.92	3.23	64.07	3.20	64.44
27	TA5	3.83	57.40	4.80	46.66	2.73	69.62	4.60	48.88	3.03	66.29	3.33	62.96
28	TCH3	2.26	74.81	2.70	70.00	2.10	76.66	4.46	50.36	2.16	75.92	2.96	67.03
29	TCH5	3.20	64.44	2.53	71.85	2.33	74.07	4.03	55.18	2.40	73.33	3.03	66.29
30	TCH6	1.06	88.14	1.40	84.44	1.13	87.40	2.83	68.51	1.10	87.77	1.40	84.81
	Control	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00
	C.D.5%		4.172		2.914		3.336		3.830		3.658		3.843
	SE(m)		1.472		1.028		1.177		1.351		1.291		1.356
	SE(d)		2.082		1.454		1.665		1.911		1.826		1.918
	C.V.		3.619		2.838		2.783		4.102		3.088		3.495

Table 4: Effect of *Trichoderma* isolates on mycelial growth inhibition of pathogens by inverted plate volatile assay at 7th day of incubation

S. No.	<i>Trichoderma</i> isolate code	Inverted plate volatile assay											
		<i>F. oxysporum</i>		<i>S. rolfsii</i>		<i>A. solani</i>		<i>A. niger</i>		<i>R. solani</i>		<i>B. cinerea</i>	
		Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth	Mycelial growth of pathogen (cm)	% inhibition of pathogen linear growth
1	TS3	6.60	26.66	3.53	60.74	3.77	58.14	4.67	48.14	2.97	67.04	3.64	59.55
2	TS4	3.66	59.25	2.70	70.00	2.47	72.59	4.87	45.92	3.43	61.85	4.48	50.22
3	TS7	5.56	38.14	4.33	51.85	4.47	50.37	4.77	47.04	3.53	60.74	4.33	51.92
4	TS10	3.96	55.92	2.93	67.40	3.11	65.49	4.87	45.92	3.30	63.33	4.33	51.85
5	TS11	4.33	51.85	4.43	50.74	4.48	50.22	4.63	48.52	3.63	59.63	4.00	55.52
6	TS15	3.93	56.29	4.60	48.88	3.04	66.26	4.97	44.81	4.30	52.22	3.93	56.34
7	TS18	1.36	84.81	1.40	84.44	1.33	85.18	2.47	72.59	1.57	82.59	1.79	80.14
8	TS22	3.20	64.44	2.20	75.55	2.64	70.67	4.77	47.03	2.40	73.33	3.12	65.35
9	TS23	2.00	77.77	1.60	82.22	2.14	76.25	3.20	64.44	1.60	82.22	2.31	74.36
10	TS25	3.50	61.11	2.73	69.63	3.63	59.67	4.70	47.78	2.93	67.41	4.59	49.00
11	TS26	2.20	75.55	1.70	81.11	2.00	77.81	3.77	58.15	1.60	82.22	2.61	71.03
12	TS29	4.30	52.22	2.10	76.66	3.46	61.58	4.60	48.89	3.57	60.37	4.56	49.34
13	TDG	0.60	93.33	1.26	85.92	1.03	88.52	1.30	85.55	1.33	85.18	1.13	87.45
14	TS32	2.76	69.25	2.76	69.25	2.22	75.30	4.23	52.96	2.13	76.29	2.84	68.40
15	TS33	4.56	49.25	3.43	61.85	3.18	64.63	4.47	50.37	4.63	48.52	4.30	52.22
16	TS35	6.80	25.18	4.03	55.18	4.48	50.26	4.43	50.74	4.40	51.11	4.20	53.37
17	TS37	6.90	23.33	4.46	50.37	4.73	47.40	4.70	47.78	4.00	55.55	4.31	52.14
18	TS40	1.76	80.37	1.80	80.00	1.56	82.68	2.80	68.89	1.50	83.33	1.41	84.32
19	TS43	3.40	62.22	3.20	64.44	3.52	60.88	4.90	45.55	2.63	70.74	3.45	61.71
20	TS45	6.63	26.29	4.80	46.66	4.51	49.88	4.67	48.15	4.37	51.48	5.39	40.06
21	TS48	3.50	61.11	3.30	63.33	3.53	60.78	4.67	48.14	3.40	62.22	3.43	61.93
22	TB1	0.73	91.85	1.33	85.18	1.08	88.01	1.40	84.44	1.30	85.55	1.47	83.66
23	TB4	4.56	49.25	5.10	43.33	4.80	46.72	3.87	57.03	3.87	57.04	4.47	50.37
24	TU2	3.43	61.85	3.56	60.37	3.77	58.12	4.40	51.11	3.03	66.29	3.81	57.62
25	TU3	4.53	49.63	5.60	37.77	4.81	46.58	4.57	49.26	4.50	50.00	4.90	45.55
26	TA2	5.43	39.63	4.56	49.25	4.15	53.85	4.80	46.66	3.90	56.66	4.20	53.38
27	TA5	4.43	50.74	4.80	46.66	4.64	48.45	5.27	41.48	3.93	56.29	4.40	51.15
28	TCH3	3.30	63.33	2.70	70.00	3.18	64.66	4.00	55.55	2.83	68.52	3.14	65.09
29	TCH5	3.80	57.77	2.53	71.85	2.99	66.74	3.47	61.48	2.80	68.89	3.58	60.24
30	TCH6	1.30	85.55	1.40	84.44	1.26	85.95	2.90	67.77	1.60	82.22	1.39	84.59
	Control	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00
	C.D.5%		2.666		2.914		4.013		2.674		3.371		2.881
	SE(m)		0.941		1.028		1.416		0.944		1.189		1.017
	SE(d)		1.331		1.454		2.003		1.334		1.681		1.438
	C.V.		2.897		2.838		3.932		3.104		3.143		2.986

Table 5: Evaluation of *Trichoderma* isolates applied through seed bio-priming for wilt disease suppression in pea caused by *Fusarium oxysporum* under glass house conditions

S. No.	Treatments	Germination (%)	PDI (%)	PDR (%)
1.	T1	53.33	100.00	0.00
2.	T2	85.33	30.00	70.00
3.	T3	80.34	35.55	64.45
4.	T4	99.00	1.00	99.00
5.	T5	83.12	33.33	66.67
6.	T6	95.52	16.12	83.88
7.	T7	86.67	26.33	73.67
8.	T8	93.33	22.34	77.66
	SE (m)	0.161	0.421	0.541
	C.D (0.05%)	0.788	2.060	2.650

Where, PDI = Percent disease incidence, PDR = Percent disease reduction

*Significant at 5% level of significance as compared to untreated control

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