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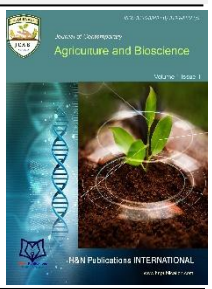

Research Article

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Use of Endophytic Bacteria in Combination of *Trichoderma* for Healthy Management of Root Rot Disease in Pea

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A b s t r a c t

Root rot disease complex, caused by multiple soil-borne pathogens, is the most destructive disease affecting pea (*Pisum sativum* L.) cultivation worldwide, often resulting in complete crop failure. This study evaluated the efficacy of fungal antagonist *Trichoderma* spp. and bacterial biofertilizer *Rhizobium* spp., both individually and in combination, for sustainable management of pea root rot. Three *Trichoderma* strains (*T. viride*, *T. harzianum*-BD, and *T. harzianum*-TH) were assessed for their in vitro antagonistic activity against *Fusarium solani*, the primary causal agent of root rot. *Rhizobium* spp. was isolated from lentil root nodules and cultured on Yeast Extract Mannitol Agar (YEMA) medium. Dual culture assays demonstrated significant mycelial growth inhibition of *F. solani* by all three *Trichoderma* strains, with inhibition percentages ranging from 65.4% to 78.9%. A field experiment was conducted using a Randomized Complete Block Design (RCBD) with seven treatments: T_0 (Control), T_1 (*T. viride*), T_2 (*T. harzianum*-BD), T_3 (*T. harzianum*-TH), T_4 (*Rhizobium*), T_5 (*Trichoderma* + *Rhizobium*), and T_6 (Chemical fungicide). The combined application of *Trichoderma* and *Rhizobium* (T_5) showed superior performance in reducing disease incidence and enhancing plant growth parameters compared to individual treatments. This integrated biocontrol approach offers a promising eco-friendly alternative to chemical fungicides for sustainable pea production.

Keywords : Pea root rot, *Fusarium solani*, *Trichoderma* spp., *Rhizobium*, Biocontrol, Integrated disease management and Seed treatment.

INTRODUCTION

Pea (*Pisum sativum* L.) is an economically important pulse crop cultivated worldwide for its high nutritional value and contribution to sustainable agriculture through biological nitrogen fixation. However, root rot disease complex remains the most devastating constraint to pea production, causing yield losses exceeding 60% in severely affected fields (Kaur et al., 2024). This multipathogenic disease involves several soil-borne pathogens including *Fusarium solani*, *Fusarium oxysporum*, *Aphanomyces euteiches*, and *Pythium* spp., which act synergistically to damage root systems, impair nutrient uptake, and cause premature plant death (Karim et al., 2024). The facultative saprophytic nature of these pathogens enables long-term survival in soil, making disease management particularly challenging through conventional approaches. Traditional control strategies relying on resistant varieties have shown limited success due to the complex pathogen assemblage, while chemical fungicides, though effective, pose environmental concerns, disrupt beneficial soil microbiomes, and leave chemical residues that compromise food safety and ecosystem health (Nadarajah, 2024). The growing global emphasis on sustainable agriculture and organic production systems necessitates the development of eco-friendly disease management alternatives that can effectively suppress pathogens while enhancing plant health and soil fertility. Biological control using beneficial microorganisms represents a promising sustainable approach. *Trichoderma* species have emerged as versatile biocontrol agents with multiple mechanisms of action including mycoparasitism, antibiosis, competition, and induction of systemic resistance in plants (Dutta et al., 2023, Karim et al., 2024). Similarly, *Rhizobium* species, beyond their established role in biological nitrogen fixation, have demonstrated potential in disease suppression and plant growth promotion (Sharma et al., 2023). Recent research suggests that combining fungal antagonists with beneficial bacteria may provide synergistic effects, offering superior disease control and yield enhancement compared to single-agent applications (Imran et al., 2023). However, limited

information exists on the integrated use of *Trichoderma* and *Rhizobium* for managing pea root rot disease complex. Therefore, this study was designed with the following objectives: (1) to evaluate the in vitro antagonistic activity of different *Trichoderma* strains against *Fusarium solani* using dual culture technique; (2) to assess the field efficacy of *Trichoderma* and *Rhizobium*, both individually and in combination, in reducing root rot disease incidence and severity in pea; and (3) to evaluate the effects of these biocontrol treatments on plant growth, development, and yield components of pea under field conditions.

MATERIALS AND METHODS

Experimental Site and Duration: The present investigation was conducted during the growing season of 2024-2025 at two locations: the Plant Pathology Laboratory and the Agronomy Experimental Field, Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi, Bangladesh. The experimental site is located at approximately 24°22'N latitude and 88°36'E longitude, with an elevation of about 20 meters above sea level. The region experiences a subtropical monsoon climate with distinct seasonal variations.

Collection and Maintenance of Fungal Cultures: Three strains of *Trichoderma* with documented biocontrol potential were obtained from different sources. *Trichoderma viride* was procured from the Plant Pathology Laboratory, Department of Agronomy and Agricultural Extension, University of Rajshahi, Bangladesh. *Trichoderma harzianum*-BD was obtained from the Disease Resistance Laboratory, Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. *Trichoderma harzianum*-TH was acquired from Thailand through Dr. Jate Sathornkich, Researcher at the Horticulture Innovation Lab, Regional Center, Kasetsart University, Thailand. Potato Dextrose Agar (PDA) medium was prepared following standard procedures using 200g potato (peeled and sliced), 20g dextrose, 15-20g agar, and 1000ml distilled water. Potato slices were boiled in 500ml distilled water for approximately 20-30 minutes until soft, then the extract was filtered through cheesecloth. Dextrose and agar were added to the filtrate, and the volume was adjusted to 1000ml with distilled water. The medium was autoclaved at 121°C and 15 psi pressure for 20 minutes. After cooling to approximately 45-50°C, the medium was poured into sterile Petri plates (90mm diameter) under aseptic conditions in a laminar airflow cabinet. *Trichoderma* strains were inoculated onto freshly prepared PDA plates using sterile inoculation loops under aseptic conditions. The inoculated plates were sealed with parafilm and incubated at 25±2°C in a temperature-controlled incubator. Growth was monitored daily, and plates were maintained for 7 days until characteristic green conidia of *Trichoderma* appeared, indicating sporulation. After 7 days of incubation, conidia were harvested by adding 10ml sterile distilled water to each plate and gently scraping the mycelial mat with a sterile glass rod. The conidial suspension was filtered through sterile cheesecloth to remove mycelial fragments. The concentration of conidia was determined using a hemocytometer and adjusted to approximately 1×10^7 colony-forming units per milliliter (CFU/ml). The harvested conidial suspensions were stored in sterile tubes at 4°C in a refrigerator for subsequent use in seed treatment and bioassays.

Isolation and culturing of *Rhizobium*: Healthy, actively growing lentil (*Lens culinaris* Medik.) plants at 45 days after sowing were selected from the experimental field of the Department of Agronomy and Agricultural Extension, University of Rajshahi. Lentil was chosen as the source of *Rhizobium* due to its close taxonomic relationship with pea, both belonging to the family Fabaceae, and the cross-inoculation group compatibility of their associated rhizobia. Plants were carefully uprooted using a grubber (hand tool) to minimize damage to root systems and nodules. Entire root systems with intact nodules were collected and immediately transported to the laboratory in sterile polythene bags to prevent desiccation and contamination. Upon arrival at the laboratory, roots were thoroughly washed under running tap water for 15-20 minutes to remove adhering soil particles, then gently blotted with sterile filter paper to remove excess water. Healthy, unbroken, brown to pink-colored nodules were carefully detached from roots using sterile forceps and selected for isolation procedures. To eliminate surface contaminants while maintaining viability of internal *Rhizobium* cells, selected nodules were immersed in sodium hypochlorite solution (3% available chlorine) for 2 minutes with gentle agitation, then immediately transferred to sterile distilled water and washed 7 times (2 minutes each wash) to completely remove traces of sodium hypochlorite. Following water rinses, nodules were immersed in 70% ethanol for 30 seconds, then final washing was performed with sterile distilled water (7 rinses, 2 minutes each) to remove all sterilant residues. All sterilization steps were performed under aseptic conditions in a laminar airflow cabinet using sterile glassware and instruments. Yeast Extract Mannitol Agar (YEMA) medium, specific for culturing *Rhizobium* species, was prepared using 10g mannitol, 0.5g K_2HPO_4, 0.2g $MgSO_4\cdot7H_2O$, 0.1g NaCl, 1g yeast extract, 15g agar, and 1000ml distilled water. The pH was adjusted to 6.8-7.0 using 0.1N HCl or 0.1N NaOH. All ingredients were dissolved in distilled water, and the medium was autoclaved at 121°C and 15 psi for 20 minutes. After cooling to approximately 45-50°C, the medium was poured into sterile Petri plates under aseptic conditions. Three different nodule preparation methods were employed to maximize isolation success. In the whole nodule technique, surface-sterilized intact nodules were directly placed on YEMA plates and gently pressed to establish contact with the medium. In the nodule piece technique, surface-sterilized nodules were aseptically cut into 2-3 small pieces using a sterile scalpel blade, and pieces were placed on YEMA plates. In the crushed nodule technique, surface-sterilized nodules were crushed individually in sterile test tubes containing 1ml sterile distilled water using sterile glass rods, then the resulting suspension was streaked onto YEMA plates using sterile inoculation loops. All inoculated plates were sealed with parafilm and incubated at 25±2°C in a temperature-controlled incubator. Plates were examined daily for bacterial growth. Isolated colonies exhibiting typical *Rhizobium* morphology (white to cream-colored, circular, raised, mucoid, and translucent) were selected and sub-cultured on fresh YEMA plates through repeated streaking to obtain pure cultures. Pure cultures were maintained on YEMA slants at 4°C and sub-cultured every 2-3 weeks to maintain viability.

Pathogen isolation and identification: Naturally infected pea plants exhibiting characteristic root rot symptoms (browning/blackening of roots, reduced root mass, wilting, stunting) were collected from various pea-growing areas in Rajshahi district. Sampling was conducted during the peak disease incidence period to ensure pathogen recovery. Diseased root tissues showing typical symptoms were selected, and sections were cut from the border between diseased and healthy tissue (approximately 5-10mm pieces)

using a sterile scalpel blade. Root pieces were surface sterilized by immersion in 70% ethanol for 30 seconds, transfer to 1% sodium hypochlorite solution for 1 minute, triple washing with sterile distilled water (1 minute each), and blotting dry on sterile filter paper. Surface-sterilized root pieces (4-6 pieces per plate) were placed equidistantly on PDA plates supplemented with streptomycin sulfate (100µg/ml) to suppress bacterial growth. Plates were sealed with parafilm and incubated at room temperature (25±2°C) for 5-7 days, with emerging fungal colonies observed daily. Fungal colonies emerging from plated root pieces were purified using the hyphal tip culture technique. Under a dissecting microscope, actively growing hyphal tips from the periphery of colonies were excised using a sterile needle and transferred to fresh PDA plates. This process was repeated 2-3 times to ensure pure cultures. Pure cultures were identified as *Fusarium solani* based on macroscopic characteristics (white to cream colony color becoming pale orange to brown with age, moderate to fast growth rate of 6-7cm diameter in 7 days at 25°C, abundant fluffy to cottony aerial mycelium, and tan to brown pigment diffusing into medium) and microscopic characteristics observed under compound microscope at 400× magnification including slightly curved to almost straight macroconidia (3-5 septate, measuring 25-45×4-6µm, with blunt to papillate apical cell and foot-shaped basal cell), ellipsoidal to oval microconidia (0-1 septate, measuring 8-16×3-5µm), and thick-walled spherical to sub-spherical chlamydospores (8-12µm diameter, produced terminally or intercalary). Identification was confirmed through comparison with authenticated cultures and published descriptions. Pure cultures were maintained on PDA slants at 4°C for subsequent experiment

In vitro antagonistic activity assessment: The antagonistic potential of *Trichoderma* strains against *Fusarium solani* was evaluated using the dual culture technique on PDA medium following standard protocols. Fresh PDA plates (90mm diameter) were prepared and allowed to solidify. Six-millimeter diameter mycelial discs were cut from the periphery of actively growing 7-day-old cultures of *F. solani* and *Trichoderma* spp. using sterile cork borers. One disc of *F. solani* and one disc of *Trichoderma* spp. were placed on opposite sides of the same PDA plate, approximately 70mm apart (measured center to center). Control plates contained only *F. solani* disc placed at the center of the plate. Each treatment combination was replicated four times. Plates were sealed with parafilm and incubated at 25±2°C in a temperature-controlled incubator. Radial mycelial growth of both fungi was measured daily using a transparent ruler until the control plates were fully colonized by *F. solani*. The percent inhibition of *F. solani* mycelial growth by *Trichoderma* strains was calculated using the formula: Inhibition (%) = [(C - T) / C] × 100, where C = radial growth of *F. solani* in control plate (cm) and T = radial growth of *F. solani* toward *Trichoderma* in dual culture plate (cm).

Seed Treatment Procedures: For *Trichoderma* treatment, harvested conidia were suspended in sterile distilled water and the concentration was adjusted to 1×10^7 CFU/ml by appropriate dilution. Healthy, disease-free pea seeds were placed in sterile beakers, immersed in the respective *Trichoderma* conidial suspension (ratio: 10ml suspension per 100g seeds), and thoroughly mixed by gentle stirring for 5 minutes to ensure uniform coating. Excess liquid was drained, and treated seeds were spread on sterile aluminum foil and air-dried under laminar airflow for 2-3 hours until surface dry. Dried treated seeds were stored in sterilized, airtight polythene bags at 15°C until sowing (maximum storage: 24 hours). For *Rhizobium* seed treatment, a 48-hour-old *Rhizobium* culture in YEMA broth was used as inoculum with bacterial cell density adjusted to approximately 10^9 cells/ml. Pea seeds were first coated with 10% sugar solution (10ml per 100g seeds) to enhance adherence of *Rhizobium* cells to seed surface, then *Rhizobium* suspension was added (10ml per 100g seeds) and mixed continuously for 5 minutes. Treated seeds were air-dried in shade for 30-45 minutes and sown within 2 hours of treatment to maintain bacterial viability. For combined treatment (T_5), seeds were first treated with *Trichoderma* conidial suspension, air-dried for 1-2 hours, then subsequently treated with *Rhizobium* suspension and sown within 2 hours of final treatment. Seeds assigned to chemical treatment (T_6) were treated with Carbendazim (12% + Mancozeb 63% WP) prepared at 0.25% concentration (2.5g per kg seeds) by thoroughly mixing with moistened seeds in a closed container. Control seeds (T_0) were treated only with sterile distilled water following the same procedure as bioagent treatments.

Field experiment: The field experiment was conducted using a Randomized Complete Block Design (RCBD) with seven treatments and three replications, resulting in 21 experimental plots. These treatments include- T_0=Untreated control (seeds soaked in sterile distilled water), T_1 = *Trichoderma viride* seed treatment, T_2=*Trichoderma harzianum*-BD seed treatment, T_3=*Trichoderma harzianum*-TH seed treatment, T_4=*Rhizobium* seed treatment, T_3=Combined *Trichoderma* + *Rhizobium* seed treatment and T_6=Chemical fungicide (Carbendazim 0.25%) seed treatment. Plot size was 2m × 2m (4m²) with 4 rows per plot, row spacing of 40cm, plant spacing of 10cm, between plot spacing of 50cm, and between replication spacing of 1m. The experimental field was ploughed thoroughly 2-3 times using a power tiller to achieve a fine tilth. All weeds, crop stubbles, and plant debris were removed manually, and the field was leveled using a ladder to ensure uniform water distribution. Fertilizers were applied according to recommended doses for pea cultivation: Urea 40kg/ha, Triple Super Phosphate (TSP) 85kg/ha, Muriate of Potash (MoP) 40kg/ha, Gypsum 55kg/ha, Zinc Sulfate 3kg/ha, and Boric Acid 1.5kg/ha. All fertilizers except urea were applied during final land preparation and thoroughly incorporated into soil. Half of the urea was applied as basal, and the remaining half was top-dressed at 30 days after sowing. Treated seeds were sown using the line sowing method at a depth of approximately 5cm with approximately 60 seeds per row to ensure a target plant population of 240 plants per plot. A light irrigation was applied immediately after sowing to ensure adequate moisture for germination, with subsequent irrigations provided based on crop requirement. Manual weeding was performed at 20 and 40 days after sowing to maintain weed-free conditions throughout the growing period.

Data collection: Disease incidence (percentage of infected plants) was recorded at 15-day intervals starting from 30 days after sowing until harvest. At each observation, all plants in each plot were examined for root rot symptoms and disease incidence was calculated as: Disease Incidence (%) = (Number of diseased plants / Total number of plants) × 100. Disease severity was assessed using a 0-5 rating scale based on visual symptoms of root rot (0 = No visible symptoms; 1 = 1-10% root system affected; 2 = 11-25% affected; 3 = 26-50% affected; 4 = 51-75% affected; 5 = >75% root system affected). Ten plants were randomly selected from each plot, carefully uprooted, and

roots were washed for assessment. The disease severity index (DSI) was calculated using the formula:

$$\text{Disease Severity Index (\%)} = \frac{\Sigma(n \times v)}{\Sigma(N \times V)} \times 100$$

where n = number of plants in each disease category, v = disease rating value (0-5), N = total number of plants assessed, and V = maximum disease rating value (5).

Plant Growth Parameters: Germination percentage and seedling vigor were recorded at 10 days after sowing (DAS). At 45 DAS, plant height (cm) was measured from ground level to the tip of the main stem, number of branches per plant, root length (cm) from crown to tip of tap root, root fresh weight (g/plant), root dry weight (g/plant) after oven drying at 70°C for 48 hours, number of root nodules per plant (for *Rhizobium*-treated plants), and fresh nodule weight (mg/plant) were recorded.

Yield and Yield Components: At physiological maturity, number of pods per plant (counted from 10 randomly selected plants), number of seeds per pod (average of 20 randomly selected pods), 100-seed weight (g) of 100 randomly selected seeds adjusted to 12% moisture, seed yield per plot (kg), seed yield per hectare (t/ha) calculated from plot yield, biological yield (kg/plot) as total above-ground biomass at harvest, and harvest index (%) = (Seed yield / Biological yield) × 100 were recorded.

Statistical Analysis: All data collected were subjected to statistical analysis using MSTAT-C software. Analysis of Variance (ANOVA) was performed for all parameters following the RCBD model. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% level of significance ($p \leq 0.05$).

RESULTS

In Vitro Antagonistic Activity of *Trichoderma* spp. Against *Fusarium solani*: All three *Trichoderma* strains exhibited significant antagonistic effects on mycelial growth of *Fusarium solani* compared to control. The analysis revealed highly significant differences among treatments ($p \leq 0.05$). *Trichoderma harzianum*-TH demonstrated the highest inhibition of *F. solani* mycelial growth with 78.94% reduction, which was statistically similar to *T. harzianum*-BD (74.71%) but significantly superior to *T. viride* (65.41%). In control plates, *F. solani* achieved maximum radial growth of 8.50cm, completely colonizing the plate surface. In dual culture with *T. harzianum*-TH, *F. solani* growth was restricted to only 1.79cm, representing the lowest pathogen growth among all treatments. *T. viride* showed the least antagonistic activity among the three strains but still maintained significant inhibition (65.41%) compared to control. All *Trichoderma* strains exhibited rapid growth rates and eventually overgrew *F. solani* colonies within 5-7 days of co-culture, with *T. harzianum*-TH showing the most vigorous growth and sporulation upon contact with the pathogen (**Table 1**).

Isolation of *Rhizobium*: *Rhizobium* bacteria were successfully isolated from lentil root nodules using three different nodule preparation methods, with varying success rates. The crushed nodule method yielded the highest success rate of 90% (18 successful isolations from 20 nodules processed), which was superior to the nodule pieces method at 80% (16 from 20) and the whole nodule method at 60% (12 from 20).

Table 1. In vitro inhibition of *Fusarium solani* mycelial growth by different *Trichoderma* strains

Treatment	Mycelial growth of *F. solani* (cm)	Inhibition over control (%)
Control (*F. solani* alone)	8.50 ± 0.12 a	-
T. viride	2.94 ± 0.18 b	65.41 b
T. harzianum-BD	2.15 ± 0.22 c	74.71 a
T. harzianum-TH	1.79 ± 0.16 c	78.94 a
Level of significance	***	***

Values represent mean ± standard error of three replications. Means followed by the same letter within a column are not significantly different as per DMRT. DAS = Days after sowing.

The crushed nodule method also resulted in faster appearance of visible colonies, with growth observed within 2-3 days compared to 3-4 days for the whole nodule method. Isolated *Rhizobium* colonies on YEMA plates exhibited characteristic features including white to cream color initially becoming slightly translucent with age, circular shape with smooth margins, raised to convex elevation, mucoid and glistening texture due to exopolysaccharide production, colony size of 1-3mm diameter after 3-4 days incubation, and sticky consistency when touched with inoculation loop. Pure cultures were successfully obtained after 2-3 successive transfers on fresh YEMA plates.

Table 2. Effect of seed treatments on germination parameters of pea

Treatment	Germination (%)	Seedling vigor index	Speed of germination
T_0 (Control)	76.33 ± 2.08 d	892.5 ± 28.4 d	6.82 ± 0.21 c
T_1 (*T. viride*)	84.67 ± 1.53 bc	1156.8 ± 35.6 c	8.45 ± 0.18 b
T_2 (*T. harzianum*-BD)	87.33 ± 1.15 ab	1284.2 ± 41.2 b	8.92 ± 0.15 ab
T_3 (*T. harzianum*-TH)	89.00 ± 1.00 ab	1325.6 ± 38.9 ab	9.18 ± 0.17 ab
T_4 (*Rhizobium*)	82.67 ± 1.53 c	1098.4 ± 32.7 c	8.15 ± 0.22 b
T_5 (Combined)	91.67 ± 0.58 a	1456.3 ± 42.5 a	9.67 ± 0.14 a
T_6 (Chemical)	88.33 ± 1.53 ab	1298.7 ± 39.8 b	9.02 ± 0.19 ab
Level of significance	***	***	***

Values represent mean ± standard error of three replications. Means followed by the same letter within a column are not significantly different as per DMRT. DAS = Days after sowing.

Field experiment results

Seed Germination: Treatment effects on germination parameters showed highly significant differences ($p \leq 0.05$). The combined treatment (T_5) resulted in the highest germination percentage of 91.67%, which was significantly superior to all other treatments and represented a 20.08% increase over the control (76.33%). Among individual bioagent treatments, *T. harzianum*-TH (T_3) produced the second-highest germination at 89.00%, which was statistically similar to *T. harzianum*-BD (87.33%) and chemical treatment (88.33%), but all were significantly better than *T. viride* (84.67%) and *Rhizobium* alone (82.67%). The control treatment recorded the lowest germination percentage at 76.33%. Seedling vigor index followed a similar pattern, with T_5 achieving the maximum value of 1456.3, representing 63.17% improvement over control (892.5). Speed of germination was also fastest in the combined treatment (9.67), showing 41.79% enhancement compared to

control (6.82). The coefficient of variation for germination percentage, seedling vigor index, and speed of germination was 3.18%, 4.56%, and 3.84% respectively, indicating good experimental precision (**Table 2**).

Root Rot Disease Incidence: Disease incidence varied significantly among treatments at all observation periods (45, 60, and 75 DAS) with $p \leq 0.05$. At 45 DAS, the combined treatment (T_5) recorded the lowest disease incidence of 5.67%, which was significantly lower than all other treatments and represented a 77.02% reduction compared to control (24.67%). Among individual *Trichoderma* treatments, *T. harzianum*-TH (T_3) showed the best performance with 8.33% disease incidence, followed by *T. harzianum*-BD (9.67%) and *T. viride* (12.33%). *Rhizobium* alone (T_4) showed moderate disease suppression with 17.33% incidence. The chemical treatment (T_6) produced 7.00% disease incidence, which was statistically similar to T_3. Disease incidence increased progressively from 45 to 75 DAS across all treatments, reflecting natural disease development dynamics. At 60 DAS, T_5 maintained the lowest incidence at 9.33%, while control reached 38.33%, representing a 75.65% reduction. At 75 DAS, similar trends continued with T_5 recording 14.00% disease incidence compared to 52.67% in control, showing 73.42% disease suppression. Throughout the growing season, the combined treatment consistently outperformed all other treatments including the chemical fungicide. The coefficient of variation ranged from 8.65% to 10.52% across different observation periods, indicating acceptable experimental precision (**Table 3**).

Table 3: Effect of treatments on root rot disease incidence (%) of pea at different growth stages

Treatment	45 DAS	60 DAS	75 DAS
T_0 (Control)	24.67 ± 1.53 a	38.33 ± 2.08 a	52.67 ± 2.52 a
T_1 (*T. viride*)	12.33 ± 1.15 c	18.67 ± 1.53 c	26.00 ± 1.73 c
T_2 (*T. harzianum*-BD)	9.67 ± 1.15 cd	15.00 ± 1.00 cd	21.33 ± 1.53 cd
T_3 (*T. harzianum*-TH)	8.33 ± 0.58 d	13.33 ± 1.15 d	19.00 ± 1.00 d
T_4 (*Rhizobium*)	17.33 ± 1.53 b	26.00 ± 2.00 b	36.67 ± 2.08 b
T_5 (Combined)	5.67 ± 0.58 e	9.33 ± 0.58 e	14.00 ± 1.00 e
T_6 (Chemical)	7.00 ± 1.00 de	11.67 ± 1.15 de	17.33 ± 1.53 d
Level of significance	***	***	***

Values represent mean ± standard error of three replications. Means followed by the same letter within a column are not significantly different as per DMRT. DAS = Days after sowing.

Disease Severity Index: Treatment effects on disease severity were highly significant ($p \leq 0.05$). The combined treatment (T_5) recorded the lowest disease severity index of 15.00%, which was significantly lower than all other treatments and represented a 78.07% reduction compared to control (68.40%). Among individual *Trichoderma* treatments, *T. harzianum*-TH (T_3) showed the best performance with 21.60% DSI (68.42% reduction over control), followed by *T. harzianum*-BD at 25.00% (63.45% reduction) and *T. viride* at 31.60% (53.80% reduction). *Rhizobium* alone (T_4) demonstrated moderate disease severity reduction with 43.40% DSI (36.55% reduction over control). The chemical treatment (T_6) produced 18.40% DSI (73.10% reduction), which was statistically similar to T_5 but numerically higher. The control treatment exhibited the highest disease severity index at 68.40%, corresponding to a mean disease rating of 3.42 on the 0-5 scale, indicating severe root damage. The combined treatment achieved the lowest mean disease rating of 0.75, indicating minimal root damage and healthy plant growth. Disease severity patterns closely followed disease incidence trends, with strong positive correlation between these two parameters. The coefficient of variation for disease severity index was 7.85%, confirming good experimental precision (**Table 4**).

Table 4: Effect of treatments on disease severity index (DSI) of pea root rot

Treatment	Mean disease rating (0-5 scale)	Disease Severity Index (%)
T_0 (Control)	3.42 ± 0.15 a	68.40 ± 2.95 a
T_1 (*T. viride*)	1.58 ± 0.12 c	31.60 ± 2.38 c
T_2 (*T. harzianum*-BD)	1.25 ± 0.10 cd	25.00 ± 2.05 cd
T_3 (*T. harzianum*-TH)	1.08 ± 0.09 d	21.60 ± 1.82 d
T_4 (*Rhizobium*)	2.17 ± 0.14 b	43.40 ± 2.75 b
T_5 (Combined)	0.75 ± 0.08 e	15.00 ± 1.58 e
T_6 (Chemical)	0.92 ± 0.09 de	18.40 ± 1.85 de
Level of significance	***	***

Values represent mean ± standard error of three replications. Means followed by the same letter within a column are not significantly different as per DMRT. DAS = Days after sowing.

Plant Growth Parameters: Treatment effects on all plant growth parameters were highly significant ($p \leq 0.05$). The combined treatment (T_5) produced significantly taller plants at 58.35cm, representing a 51.76% increase over control (38.45cm) and outperforming all other treatments. Among individual *Trichoderma* treatments, *T. harzianum*-TH (T_3) achieved the maximum plant height of 51.92cm (35.01% increase over control), followed by *T. harzianum*-BD at 49.85cm (29.66% increase) and *T. viride* at 46.78cm (21.67% increase). *Rhizobium* treatment (T_4) produced plant height of 48.25cm (25.49% increase over control), while chemical treatment (T_6) resulted in 52.68cm (36.98% increase). The number of branches per plant followed similar trends, with T_5 recording the maximum of 5.42 branches (73.72% increase over control's 3.12), significantly superior to all other treatments. Root length was also maximized in T_5 at 22.48cm, showing 74.94% improvement over control (12.85cm). Root fresh weight was highest in T_5 (5.45g/plant), representing 91.90% increase compared to control (2.84g/plant), followed by T_3 (4.58g/plant, 61.27% increase) and T_6 (4.72g/plant, 66.20% increase). Root dry weight pattern mirrored fresh weight trends, with T_5 achieving 0.82g/plant (95.24% increase over control's 0.42g/plant), demonstrating substantial enhancement in root biomass production. All bioagent treatments significantly improved vegetative growth parameters compared to control, with the combined treatment consistently outperforming individual applications. The coefficient of variation for plant height, number of branches, root length, root fresh weight, and root dry weight was 5.42%, 8.15%, 6.78%, 7.25%, and 9.52% respectively, indicating satisfactory experimental precision (Table 5).

Nodulation in Rhizobium-Treated Plants: Treatment effects on nodulation parameters were highly significant ($p \leq 0.05$). The combined treatment (T_5) produced the highest number of nodules per plant at 34.85, representing a 312.43% increase over control (8.45) and a 21.55% increase over *Rhizobium* alone (T_4, 28.67). Control plants showed natural nodulation from indigenous soil rhizobia but with poor effectiveness, as evidenced by

Table 5: Effect of treatments on plant growth parameters of pea

Treatment	Plant height (cm)	No. of branches/plant	Root length (cm)	Root fresh weight (g/plant)	Root dry weight (g/plant)
T_0 (Control)	38.45 ± 1.25 d	3.12 ± 0.18 d	12.85 ± 0.65 d	2.84 ± 0.15 d	0.42 ± 0.03 d
T_1 (*T. viride*)	46.78 ± 1.42 c	4.28 ± 0.22 c	16.92 ± 0.78 c	3.95 ± 0.18 c	0.58 ± 0.04 c
T_2 (*T. harzianum*-BD)	49.85 ± 1.38 bc	4.68 ± 0.24 bc	18.45 ± 0.82 bc	4.32 ± 0.20 bc	0.64 ± 0.04 bc
T_3 (*T. harzianum*-TH)	51.92 ± 1.45 b	4.92 ± 0.26 ab	19.58 ± 0.88 ab	4.58 ± 0.22 b	0.68 ± 0.05 b
T_4 (*Rhizobium*)	48.25 ± 1.35 c	4.45 ± 0.23 c	17.68 ± 0.75 c	4.12 ± 0.19 c	0.61 ± 0.04 c
T_5 (Combined)	58.35 ± 1.52 a	5.42 ± 0.28 a	22.48 ± 0.95 a	5.45 ± 0.25 a	0.82 ± 0.05 a
T_6 (Chemical)	52.68 ± 1.48 b	5.05 ± 0.25 ab	20.15 ± 0.85 ab	4.72 ± 0.21 b	0.70 ± 0.04 b
Level of significance	***	***	***	***	***

Values represent mean ± standard error of three replications. Means followed by the same letter within a column are not significantly different as per DMRT. DAS = Days after sowing.

predominantly white to pale green nodule interiors indicating inactive nitrogen fixation. *Rhizobium* inoculation (T_4) significantly increased nodule number to 28.67 per plant (239.29% increase over control) with good effectiveness, as most nodules exhibited pink to red interior coloration characteristic of active leghemoglobin and nitrogen fixation. Nodule fresh weight followed similar trends, with T_5 achieving the maximum of 192.45mg/plant (354.48% increase over control's 42.35mg/plant), significantly higher than T_4 at 156.82mg/plant (270.37% increase over control). Nodule dry weight was also highest in T_5 at 62.38mg/plant (385.45% increase over control's 12.85mg/plant), followed by T_4 at 48.52mg/plant (277.59% increase over control). The synergistic effect of *Trichoderma* and *Rhizobium* in the combined treatment not only enhanced nodule number but also increased nodule size and biomass compared to *Rhizobium* alone, suggesting improved nodulation efficiency and nitrogen fixation potential. Nodules from both T_4 and T_5 treatments were predominantly pink to red in color, indicating excellent nodule effectiveness and active nitrogen fixation, whereas control nodules were mostly white to pale green, indicating poor effectiveness. The coefficient of variation for nodule number, fresh weight, and dry weight was 10.25%, 9.78%, and 11.42% respectively (**Table 6**).

Table 6: Effect of *Rhizobium* treatment on nodulation parameters of pea

Treatment	No. of nodules/plant	Nodule fresh weight (mg/plant)	Nodule dry weight (mg/plant)	Nodule effectiveness
T_0 (Control)	8.45 ± 0.85 c	42.35 ± 3.25 c	12.85 ± 1.15 c	Poor
T_4 (*Rhizobium*)	28.67 ± 2.15 b	156.82 ± 8.65 b	48.52 ± 3.85 b	Good
T_5 (Combined)	34.85 ± 2.45 a	192.45 ± 9.85 a	62.38 ± 4.25 a	Excellent
Level of significance	***	***	***	-

Values represent mean ± standard error of three replications. Means followed by the same letter within a column are not significantly different as per DMRT. DAS = Days after sowing.

Yield and Yield Components: Treatment effects on all yield parameters were highly significant ($p \leq 0.05$). The combined treatment (T_5) produced the highest number of pods per plant at 18.92, representing a 129.33% increase over control (8.25) and significantly superior to all other treatments. Among individual *Trichoderma* treatments, *T. harzianum*-TH (T_3) achieved 15.18 pods per plant (83.88% increase over control), followed by *T. harzianum*-BD at 14.25 (72.73% increase) and *T. viride* at 12.58 (52.48% increase). Chemical treatment (T_6) produced 15.85 pods per plant (92.12% increase over control). Number of seeds per pod was maximum in T_5 at 8.15, showing 39.32% improvement over control (5.85), followed by T_6 (7.68 seeds/pod, 31.28% increase) and T_3 (7.52 seeds/pod, 28.55% increase). The lowest seeds per pod was recorded in control (5.85). The 100-seed weight was highest in T_5 at 25.28g, representing a 37.02% increase over control (18.45g), followed by T_6 (24.12g, 30.73% increase) and T_3 (23.45g, 27.10% increase). The control recorded the lowest 100-seed weight at 18.45g. Seed yield per plot followed similar trends, with T_5 achieving maximum yield of 1.152kg (137.53% increase over control's 0.485kg), significantly outperforming all other treatments including chemical fungicide T_6 (0.985kg, 103.09% increase over control). Seed yield per hectare in T_5 reached 2.88t/ha compared to 1.21t/ha in control, representing a 138.02% increase. Among individual bioagent treatments, *T. harzianum*-TH produced the highest yield at 2.31t/ha (90.91% increase over control). Biological yield was also maximized in T_5 at 5.52kg/plot (93.68% increase over control's 2.85kg/plot), indicating enhanced overall plant biomass production. Harvest index ranged from 17.02% in control to 20.87% in T_5, demonstrating improved partitioning of assimilates toward economic yield in biocontrol treatments. The coefficient of variation for pods per plant, seeds per pod, 100-seed weight, seed yield per plot, seed yield per hectare, biological yield, and harvest index was 7.82%, 8.45%, 6.28%, 5.92%, 5.92%, 7.15%, and 8.35% respectively, confirming good experimental precision (**Table 7**).

DISCUSSION

The present study conclusively demonstrated that integrated seed treatment with *Trichoderma* spp. and *Rhizobium* provides highly effective and sustainable management of root rot disease complex in pea while simultaneously enhancing plant growth and yield. The superior antagonistic activity of *Trichoderma harzianum*-TH against *Fusarium solani* (78.94% inhibition) can be attributed to multiple mechanisms operating synergistically, including mycoparasitism through production of cell wall-degrading enzymes such as chitinases and β-1,3-glucanases, antibiosis via secretion of secondary metabolites that disrupt pathogen growth, and competition for nutrients and space in the rhizosphere. The variation in antagonistic efficacy among *Trichoderma* strains reflects genetic diversity and differences in enzyme production profiles, with *T. harzianum* strains generally producing a broader spectrum of antifungal compounds compared to *T. viride* (Jambhulkar et al., 2024, Philip et al., 2024 and Jalal et al., 2025), consistent with recent findings by Dutta et al. (2023) who reported strain-specific differences in biocontrol mechanisms. The geographic origin of *T. harzianum*-TH from Thailand may have

Table 7: Effect of treatments on yield and yield components of pea

Treatment	Pods/plant	Seeds/pod	100-seed weight (g)	Seed yield (kg/plot)	Seed yield (t/ha)	Biological yield (kg/plot)	Harvest index (%)
T_0 (Control)	8.25 ± 0.45 d	5.85 ± 0.28 c	18.45 ± 0.52 d	0.485 ± 0.028 e	1.21 ± 0.07 e	2.85 ± 0.15 d	17.02 ± 0.85 d
T_1 (*T. viride*)	12.58 ± 0.55 c	6.92 ± 0.32 b	21.35 ± 0.58 c	0.748 ± 0.035 d	1.87 ± 0.09 d	3.92 ± 0.18 c	19.08 ± 0.92 cd
T_2 (*T. harzianum*-BD)	14.25 ± 0.62 bc	7.28 ± 0.35 ab	22.68 ± 0.62 bc	0.865 ± 0.038 c	2.16 ± 0.10 c	4.35 ± 0.20 bc	19.89 ± 0.95 bc
T_3 (*T. harzianum*-TH)	15.18 ± 0.65 b	7.52 ± 0.36 ab	23.45 ± 0.65 ab	0.925 ± 0.040 bc	2.31 ± 0.10 bc	4.68 ± 0.22 b	19.76 ± 0.88 bc
T_4 (*Rhizobium*)	13.45 ± 0.58 bc	7.08 ± 0.34 b	21.95 ± 0.60 bc	0.812 ± 0.036 cd	2.03 ± 0.09 cd	4.15 ± 0.19 bc	19.57 ± 0.90 bc
T_5 (Combined)	18.92 ± 0.75 a	8.15 ± 0.38 a	25.28 ± 0.68 a	1.152 ± 0.045 a	2.88 ± 0.11 a	5.52 ± 0.25 a	20.87 ± 0.98 a
T_6 (Chemical)	15.85 ± 0.68 b	7.68 ± 0.37 ab	24.12 ± 0.66 ab	0.985 ± 0.042 b	2.46 ± 0.11 b	4.85 ± 0.23 b	20.31 ± 0.95 ab
Level of significance	***	**	**	***	***	**	**

Values represent mean ± standard error of three replications. Means followed by the same letter within a column are not significantly different as per DMRT. DAS = Days after sowing.

contributed to its superior performance, as tropical strains often exhibit enhanced metabolic versatility and stress tolerance developed through adaptation to diverse pathogen pressures. The successful isolation of *Rhizobium* from lentil nodules and its effective establishment of symbiosis with pea validates the cross-inoculation group compatibility between these legumes, both belonging to the tribe Fabeae. The crushed nodule technique's superior success rate (90%) can be explained by the liberation of large numbers of viable *Rhizobium* cells from disrupted bacteroid tissue, facilitating efficient plating and isolation (Almihyawi et al., 2024). The field performance results revealed remarkable synergistic effects when *Trichoderma* and *Rhizobium* were combined, achieving 77.02% reduction in disease incidence and 78.07% reduction in disease severity, substantially exceeding the performance of either agent applied individually. This synergy operates through complementary mechanisms wherein *Trichoderma* provides direct antagonism against fungal pathogens (Li et al., 2025) while *Rhizobium*-mediated biological nitrogen fixation enhances plant vigor and stress tolerance (Nauanova et al., 2025). The enhanced nodulation observed in the combined treatment (34.85 nodules per plant) compared to *Rhizobium* alone (28.67 nodules per plant) suggests that *Trichoderma* may suppress nodule parasites, produce plant growth regulators that stimulate nodule initiation, or improve root health thereby providing more favorable sites for nodulation. Furthermore, both biocontrol agents are known to induce systemic resistance in plants through recognition of microbe-associated molecular patterns, priming defense responses for rapid activation upon pathogen attack, as recently documented by Nadarajah (2024) in comprehensive reviews of plant-pathogen interactions. The superior performance of the combined treatment over chemical fungicide (Carbendazim) is particularly noteworthy, as it demonstrates that biological control strategies can match or exceed the efficacy of synthetic chemicals while offering additional benefits including absence of toxic residues (Curk and Trdan, 2024), enhancement of beneficial soil microorganisms, and improvement of long-term soil health. The substantial plant growth promotion observed, particularly in the combined treatment showing 51.76% increase in plant height and 74.94% increase in root length compared to control, extends beyond mere disease suppression and reflects multiple plant growth-promoting attributes of the biocontrol agents. *Trichoderma* species produce auxin-like compounds that stimulate root elongation and lateral root formation, solubilize phosphate and micronutrients through organic acid secretion, and enhance nutrient uptake efficiency through improved root architecture, as extensively demonstrated by Sharma et al. (2023) in studies on plant-microbe interactions in Himalayan soils. The biological nitrogen fixation by *Rhizobium*, evidenced by effective pink-colored nodules, provided sustained nitrogen nutrition throughout the growing season, reducing dependency on synthetic nitrogen fertilizers while enhancing protein synthesis and overall plant vigor. The remarkable yield enhancement of 138.02% in the combined treatment compared to control resulted from comprehensive improvements across all yield components including 129.33% increase in pods per plant, 39.32% increase in seeds per pod, and 37.02% increase in 100-seed weight, indicating that the integrated biocontrol approach enhanced both source capacity (photosynthetic biomass) and sink strength (reproductive structures). Recent economic analyses by Imran et al. (2023) have demonstrated favorable benefit-cost ratios for integrated biological control approaches in vegetable production systems, supporting their commercial viability. The consistency of disease suppression throughout the growing season from 45 to 75 DAS indicates successful establishment and persistence of biocontrol agents in the rhizosphere, a critical factor for sustained disease management. These findings align with the growing body of literature advocating integrated biocontrol strategies that combine multiple beneficial microorganisms with complementary modes of action as superior alternatives to single-agent applications or chemical-intensive management, particularly in the context of organic agriculture and sustainable intensification of food production systems.

CONCLUSION

The combined application of *Trichoderma harzianum*-TH and *Rhizobium* as seed treatment demonstrated superior efficacy in managing pea root rot disease, achieving 77-78% disease reduction while enhancing yield by 138% compared to control, significantly outperforming individual bioagent applications and chemical fungicide treatment. This integrated biocontrol approach offers a sustainable, eco-friendly, and economically viable alternative for pea production systems, warranting large-scale field validation and farmer adoption.

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