JOURNAL OF AGRICULTURE AND APPLIED BIOLOGY
2026, Vol. 7, No. 1, 14 - 31
http://dx.doi.org/10.11594/jaab.07.01.02
E-ISSN: 2723-5106

Research Article

Trichoderma-Enhanced Compost: A Dual Strategy for Southern Blight Disease Suppression and Tomato Yield Improvement

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Article history:

Received 07 June 2025
Revised 28 November 2025
Accepted 15 December 2025
Published 27 April 2026

Keywords:

Disease suppression
S. rolfsii
Trichoderma-fortified compost
Tomato Production

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Abstract

Southern blight, caused by *Sclerotium rolfsii* (*Sr*), is a major constraint to tomato production. We isolated 20 Sr strains and identified Sr-4 as the most virulent based on pathogenicity assays. In parallel, 102 *Trichoderma harzianum* (*Th*) isolates were screened, with four inhibiting *Sr* growth by ≥75% in dual culture; PABT-22 showed the highest inhibition. Pot and field experiments evaluated Trichoderma-fortified compost (*TFC*; 2.0–4.0 × 10^8 spores mL^{-1}). *TFC* applied at 3.0 × 10^8 spores mL^{-1} (T_7) reduced disease incidence to 8.4% and percent disease index (PDI) to 5.2%, providing 71.4% control compared with the pathogen control. T_7 also achieved the highest yield (64.43 t ha^{-1}; +59.1%) and enhanced fruit quality, including increased β-carotene and lycopene content. These findings demonstrate that *TFC*, particularly PABT-22 at 3.0 × 10^8 spores mL^{-1}, offers an effective, residue-free strategy for suppressing southern blight while improving yield and nutritional quality in tomato.

How to cite:
Salam, M. A., Bhuiyan, M. K. A., Mia, M. I. H., Khan, M. A. A., Hossain, M. M., & Rubayet, M. T. (2026). Trichoderma-Enhanced Compost: A Dual Strategy for Southern Blight Disease Suppression and Tomato Yield Improvement. *Journal of Agriculture and Applied Biology, 7*(1): 14 - 31. doi: 10.11594/jaab.07.01.02

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetables globally, including Bangladesh, with versatile uses in both fresh markets and the processing industry. Although the cultivated area of tomatoes in Bangladesh has been increasing over recent years, but productivity remains low (BBS, 2023). This discrepancy is attributed to several constraints in tomato cultivation, including insect pests, disease infections, weed infestations, and natural calamities. Among these, disease infections significantly contribute to low tomato yields, causing an estimated 30-40% yield loss annually in Bangladesh (Rahman et al., 2001). Soil-borne diseases caused by pathogens such as *S. rolfsii*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *lycopersici*, and *Pythium* spp. are particularly severe, affecting tomatoes at both the seedling and mature stages (Rahman et al., 2024ab). Most of the growers predominantly rely on fungicide treatments for disease control during the cultivation tomato. However, fungicidal applications pose risks to human health and increase environmental pollution. Moreover, existing control measures, including cultural and chemical methods, are often inadequate for managing soil-borne diseases. The trend towards near-zero market tolerance for pesticide residues in fresh vegetables further motivates the search for non-chemical pest and disease control methods. Consequently, eco-friendly approaches for plant disease management are increasingly sought after.

Despite extensive work with *Trichoderma* and composts, there is limited field-validated evidence on dose-optimised, strain-defined Trichoderma-fortified compost for southern blight suppression with concurrent fruit-quality gains in tomato. We hypothesised that a mid-range conidial dose of a top-antagonist *T. harzianum* would maximise disease suppression and yield/quality compared with lower/higher doses and non-fortified compost. The bio-agent *Trichoderma* species (*Ts*) are common inhabitants of the rhizosphere against a wide range of soil-borne pathogens and promote plant growth (Intana et al., 2024). Some strains of *Th* establish robust colonisation on root surfaces, enhancing root growth, crop productivity, and resistance to abiotic stresses through improved mineral absorption. It's a potential biocontrol agent and growth-promoting fungus for many crop plants (Simi et al., 2019; Das et al., 2019; Roy et al., 2022; Bhuiyan and Rubayet, 2023; Rahman et al., 2024a; Chowdhury et al., 2024). Substantial research has been conducted on controlling soil-borne fungal diseases using *Trichoderma*, either individually or in combination with fungicides, cultural methods, or organic amendments (Rubayet and Bhuiyan, 2012; 2016). Combining antagonistic microbes with mature compost may be more effective in inhibiting disease than using single antagonistic strains or compost alone.

Tricho-compost, a Trichoderma-based compost fertiliser, developed by mixing a spore suspension of *Th* with various processed raw materials, has proven effective against soil-borne diseases of cabbage (Nahar et al., 2012). The determination of the appropriate dose of Trichoderma-fortified compost and understanding the population dynamics of *Ts* after field application are crucial for successful biological control of soil-borne diseases (Bhuiyan and Rubayet, 2023). This study aims to select the appropriate dose of *TFC* to manage soil-borne pathogen such as *Sr* of tomato, evaluate its potential to improve tomato yield and its quality.

2. Materials and Methods

2.1 Isolation and identification of the Sr

The pathogen was isolated from infected tomato tissues and soil samples. The samples were washed, cut into 5 mm pieces, and surface sterilised with 0.1% NaOCl. After rinsing, they were placed on sterilised water agar containing streptomycin sulphate to prevent bacterial contamination. Incubated for 3 days at 25±2°C, fungal colonies were observed, and isolates were purified using the hyphal tip technique. Twenty isolates of *Sr* were obtained using standard soil dilution techniques and stored at 10°C on PDA slants for further study.

2.2 Cultural characteristics of isolates of Sr

All isolates were cultured on PDA plates. About 5 mm mycelial agar block from the edge of a three-day-old culture was transferred to each 90 mm petri plate and incubated for 20 days at 25±1°C. Three replicate plates were used per isolate. The observations over 20 days included mycelial growth rate per 24 hours, colony structure, zonation, colony colour, sclerotia number and distribution, sclerotia colour, and size and shape of sclerotia for *Sr*.

2.3 Preparation of inoculum of Sr

The inoculum of *Sr* was prepared by soaking autoclaved wheat grains in water for 12 hours. Excess water was drained, and grains were transferred to Erlenmeyer flasks. Three-day-old mycelial discs (5-7 discs, 5 mm diameter) from PDA cultures were added to the wheat grains and incubated at 25±2°C for 21 days with intermittent shaking. The colonised wheat grains were air-dried for 48 hrs. and stored at 4°C for future use.

2.4 Pathogenicity Test

Twenty isolates of *Sr* were tested for pathogenicity in pot culture experiments using a soil infestation method (Rubayet and Bhuiyan, 2016). Each isolate was inoculated into the soil, and tomato seeds (variety 'Manik') were sown. The disease development was monitored at 10, 15, and 21 days after sowing to assess pre-emergence and post-emergence seedling mortality. The causal agent was confirmed through re-isolation from affected plant parts.

2.5 Isolation and identification of Ts from soil samples

A total of 100 isolates of *Ts* was isolated from the soil of tomato fields. Ten grams of soil from a sample was mixed with 90 ml of sterile distilled water in a sterile conical flask and the content was stirred with a magnetic stirrer for about 20 minutes. While suspension was in motion, 10 ml was withdrawn in a conical flask and 90 ml of sterile distilled water was added to make 100 ml. The flask was agitated on a vortex for two minutes for thorough mixing. The process was repeated until the four-fold serial dilution (10^{-4}) was made. Then 1 ml of each dilution was incorporated into a plate with PDA amended by 100 ppm streptomycin sulphate. The soil suspension on PDA plate was spread evenly using a turntable. The petri dishes were incubated for 3 days at 25±2°C. Fungus was purified on PDA following the hyphal tip culture technique. A total of 100 fungal isolates were identified as *Th* based on growth, colony and morphological characters following the standard key. The other isolated fungi were discarded. After purification, all the isolates were preserved in the PDA slants at 10°C as stock culture for future use.

2.6 Cultural characteristics of Ts

All the isolates were tested for their growth rate per day on PDA. A mycelial agar block (5 mm) was cut by flame-burned cork from the growing edge of three-day-old culture on PDA and transferred to the center of 90 mm diameter Petri plate of PDA and incubated for 72 hrs. at 25±1°C. Three replicated plates were used for each isolate. Data on radial colony growth was recorded at 24, 48 and 72 hrs. of incubation. The colony colour, number of zonation and mycelial density were also recorded at 7 days after inoculation.

2.7 In vitro screening of potential Trichoderma isolates against Sr

An experiment was conducted to find out the antagonistic effect of the collected each *Trichoderma* isolate against the most virulent isolates of all the selected serious pathogens following *in vitro* dual plate culture technique using PDA. Inhibition percentage of the radial growth of the pathogens was calculated after 5 days of incubation. The plates were arranged in a completely randomised design (CRD) with three replications. The antagonistic effect of all *Trichoderma* isolates was recorded based on inhibiting potentiality after 5 days of incubation. After incubation, the degree of antagonism was assessed based on the modified standard indexing scale of 1-5;

where, R_1 = antagonist overgrew at least three-fourths of the medium surface, R_2 = antagonist overgrew at least two-third of the medium surface but less than three-fourth of the medium surface, R_3 = antagonist overgrew at least one-half of the medium surface but less than two-thirds of the medium surface, R_4 = pathogen colonised more than one-half of the medium surface and appeared to withstand encroachment by antagonist, R_5 = pathogen completely overgrew the antagonist and occupied the entire medium surface.

2.8 Preparation of Trichoderma spore suspension

Spore suspension of *Th* was made by collecting the spores from a 7-day-old *Trichoderma* culture. Ten millilitres of distilled water was added per plate to make the suspension, which adjusted the concentration to $3.0x10^8$ spores ml^{-1}. Tomato seeds were soaked sufficiently with the spore suspension and air-dried before sowing.

2.9 Preparation of TFC

A selected isolate of PABT-22 was cultured in a PDA medium and kept at 25 ± 1^0 C for 2 weeks. After sporulation, 10 ml of sterile distilled water was added to each plate and scrapped with sterilised scalpel. Using a Hemacytometer, the concentration of suspension was determined and set at about 10^8 spore ml^{-1}. At last, to prepare enriched/fortified compost, a suspension of *Trichoderma* was added to the compost (Soltani et al., 2015).

2.10 Preparation of pathogen inoculum

Wheat grain colonised inoculum for the selected isolate of *Sr* was prepared following the procedure (Rubayet and Bhuiyan, 2016).

2.11 Pot experiment

A pot experiment was set up following CRD with four replications. The diameter of the pot was 20 cm. Each pot was filled with 5.0 kg soil and composts of all treatments were applied @1kg m^{-2}. Wheat grain colonised pathogen inoculum was prepared as described earlier and mixed at the time of application as the treatment. Wheat grain colonised *Trichoderma* inoculum was also prepared as the same procedure. Both pathogens and *Trichoderma* inocula were applied @ 20g per kg of pot soil. Tomato seedlings (Manik variety) were raised in the seedbed and 20-day aged three seedlings were transplanted in each pot. After establishment, uprooted the seedlings except one in each pot. The tomato seedlings were uprooted 45 days after transplanting (DAT) and data were collected.

2.12 Treatments of pot experiment:

T_1= Wheat grain colonised pathogens (Control-1), T_2= No *Trichoderma* and no compost (Control-2), T_3= Only compost (Control-3), T_4= T_1+Wheat grain colonised *Trichoderma*, T_5= T_1+*TFC* @ $2.0x10^8$ spore ml^{-1}, T_6= T_1+*TFC* @ $2.5x10^8$ spore ml^{-1}, T_7= T_1+*TFC* @ $3.0x10^8$ spore ml^{-1}, T_8= T_1+*TFC* @ $3.5x10^8$ spore ml^{-1}, T_9= T_1+*TFC* @ $4.0x10^8$ spore ml^{-1}.

2.13 Field experiment

A field experiment following RCBD design with four replications was conducted. The plot size was 3 m x 2 m and spacing was 50 cm x 50 cm. Standard doses of chemical fertilisers were used. Soil from the field was collected for analysis before transplanting of tomato. *Trichoderma*-fortified compost was also analysed before application in the field. Source of tomato seeds, seedling raising, land preparation, seedling transplanting and other intercultural operations were maintained the same as in earlier experiments. The treatments of the field experiment were the same as the pot experiment.

2.14 Assessment of the quality of tomato

Another experiment was conducted to assess the chemical properties such as ascorbic acid (mg/ 100g), beta carotene (mg/100g), lycopene (mg/100g), % TSS, reducing sugar(mg/g), total sugar (mg/g), chlorophyll a and b (mg/100g) following standard procedure.

2.15 Data record and analysis

The shoot height (cm), root length (cm), shoot and root fresh weight (g), and shoot and root dry weight (g) of tomato plants were recorded in the case of the pot experiment. Post-emergence seedling mortality and different disease incidence throughout the growing season were also recorded. Number of fruit plant^{-1} and grading of fruit by size and weight, yield of tomato after harvest (g plot^{-1} and ton ha^{-1}), chemical analysis of ripe tomato in laboratories were recorded for all the treatments. All observations were made by selecting six plants randomly from each plot.
Disease incidence and percent disease index (PDI) were assessed by the following formulae using the methods of Liu et al. (1995).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{PDI} = \frac{\Sigma \text{ of rating of plants observed}}{\text{Number of plants observed} \times \text{Highest score of the scale used}} \times 100$$

The following formula calculated percent disease control (PDC):

$$\text{PDC} = \frac{(\text{\% disease in check}) - (\text{\% disease in treatment})}{(\text{\% disease in check})} \times 100$$

Data was analysed statistically using the Statistix 10.0 computer program after proper transformation whenever necessary. The means were compared following Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1 Isolation and identification of Sr

Twenty isolates of *Sr* were identified and selected for pathogenicity test by observing colony morphology and sclerotial character (Table 1). Mycelium of all the isolates was found whitish, and fluffy with radiate growth. The mean mycelial growth rate was 29.67 to 34.33 mm/24 hrs. The highest mycelial growth was found in isolate Sr-4, which was followed by isolates Sr-15 and Sr-5, these were statistically similar, and the lowest growth was found in isolate Sr-8, which was followed by isolates Sr-13, Sr-11 and Sr-18, these were also statistically similar. Sclerotia of relatively uniform size were produced on mycelium. Sclerotia were round and white when immature and then became dark brown. Mature sclerotia resembled mustard seed-like. All the isolates produced many small sclerotia which ranged from 427 to 572. The highest number of sclerotia was produced by the isolate Sr-4, which was followed by the isolates Sr-14 and Sr-7 and the lowest number by the isolate Sr-8, which was followed by the isolates Sr-17 and Sr-3. The colour of the mature sclerotia was mostly dark brown. Sen (2010) reported about the 10 selected isolates and observed whitish fluffy with radiate growth of mycelium and light brown to dark brown sclerotia on PDA, the mycelial growth rate ranges from 26.78 to 31.56 mm/24 hours and produced many small sclerotia which ranged from 395-545. Sharma et al. (2002) reported about the 26 Indian isolates and observed fluffy mycelium and light brown to dark brown sclerotia of 1.0-1.2 mm in diameter. Although there was other sclerotia-producing fungi, the fungus is characterised by small tan to dark brown or black sclerotia with internally differentiated rind, cortex and medulla and placed in the form genus *Sclerotium*. These findings corroborate the identification of the isolate of fungus as *Sr* in the present investigation.

Table 1. Cultural characterisation of S. rolfsii

Isolates	Mean Mycelial growth (mm/day)	Colony type	No. of Sclerotia/ plate	Pattern of sclerotia formation
Sr-1	32.33 c-e	Whitish, Radiate and very Fluffy	543	Mostly in the periphery
Sr-2	32.67 b-e	Whitish, Radiate and Fluffy	531	Spread all over the plate
Sr-3	31.67 ef	Whitish, Radiate and Fluffy	448	Spread all over the plate
Sr-4	34.33 a	Whitish, Radiate and very Fluffy	572	Mostly in the periphery
Sr-5	33.33 a-c	Whitish, Radiate and Fluffy	479	Mostly in the periphery
Sr-6	31.67 ef	Whitish, Radiate and Fluffy	552	Mostly in the periphery
Sr-7	31.00 fg	Whitish, Radiate and very Fluffy	459	Spread all over the plate, preferably in margin side
Sr-8	29.67 h	Whitish, Radiate and Fluffy	427	More in the periphery
Sr-9	32.33 c-e	Whitish, Radiate and Fluffy	533	Spread all over the plate
Sr-10	32.33 c-e	Whitish, Radiate and very Fluffy	485	More in the periphery
Sr-11	30.33 gh	Whitish, Radiate and Fluffy	553	Spread all over the plate
Sr-12	31.67 ef	Whitish, Radiate and Fluffy	521	Spread all over the plate, preferably in margin side
Sr-13	30.00 gh	Whitish, Radiate and very Fluffy	476	Mostly in the periphery
Sr-14	33.00 b-d	Whitish, Radiate and Fluffy	568	Mostly in the periphery
Sr-15	33.67 ab	Whitish, Radiate and Fluffy	491	Mostly in the periphery
Sr-16	33.00 b-d	Whitish, Radiate and Fluffy	544	Spread all over the plate, preferably in the margin side
Sr-17	32.33 c-e	Whitish, Radiate and very Fluffy	433	More in the periphery
Sr-18	30.33 gh	Whitish, Radiate and Fluffy	489	Spread all over the plate
Sr-19	33.00 b-d	Whitish, Radiate and Fluffy	513	More in the periphery
Sr-20	32.00 df	Whitish, Radiate and very Fluffy	485	Mostly in the periphery

3.2 Pathogenicity test of Sr in pot culture

Pathogenicity of selected twenty isolates of *Sr* was tested to evaluate their ability to cause disease in tomato seedlings in the pot culture experiment to find a virulent isolate. The highest pre-emergence mortality (93.33%) of tomato was caused by the isolate Sr-4 followed by isolate Sr-2 (90.00%). Only 6.67% mortality was found in control pots. Isolates Sr-3 and Sr-10 showed the highest post-emergence mortality (13.33%) of tomato seedlings which was followed by Sr-15, Sr-16 and Sr-20 (10.00%). No mortality was found in control pots with sterile soil without inoculum of *Sr.* While considering total seedling mortality, the highest incidence (96.67%) was due to infection by the isolate Sr-4 followed by Sr-2 and Sr-11 (90.00%) whereas in control pots with sterile soil without inoculum of *Sr* showed only 6.67% mortality. All other isolates caused more than 56% of total mortality (Table 2). The results of the present study indicated that all the isolates are pathogenic to seed and seedlings (except Sr-2) of tomato, but the isolates' virulence was variable. The most virulent isolate Sr-4 causing the highest total mortality in tomato was selected for further study. The results of the pathogenicity test are fully agreement with Nitu et al. (2016) who found up to 93.19% seedling mortality in tomato plants due to infection by *Sr.*

Table 2. Pathogenicity test of S. rolfsii isolates on tomato variety 'Manik' in pot culture

Isolates	% seedling mortality		
	Pre-emergence	Post-emergence	Total
Sr-1	83.33	3.33	86.67 ab (1.09)*
Sr-2	90.00	00	90.00 ab (1.15)
Sr-3	60.00	13.33	73.33 c-e (0.83)
Sr-4	93.33	3.33	96.67 a (1.32)
Sr-5	80.00	6.67	86.67 bc (1.06)
Sr-6	53.33	3.33	56.67 e (0.60)
Sr-7	66.67	3.33	70.00 de (0.78)
Sr-8	76.67	6.67	83.33 b-d (0.99)
Sr-9	83.33	3.33	86.67 bc (1.06)
Sr-10	56.67	13.33	70.00 de (0.78)
Sr-11	86.67	3.33	90.00 ab (1.12)
Sr-12	80.00	3.33	83.33 b-d (0.99)
Sr-13	83.33	3.33	86.67 bc (1.06)
Sr-14	83.33	3.33	86.67 bc (1.06)
Sr-15	73.33	10.00	83.33 b-d (0.99)

Isolates	% seedling mortality		
	Pre-emergence	Post-emergence	Total
Sr-16	63.33	10.00	73.33 c-e (0.83)
Sr-17	83.33	3.33	86.67 bc (1.06)
Sr-18	80.00	6.67	86.67 bc (1.06)
Sr-19	76.67	6.67	83.33 b-d (0.99)
Sr-20	76.67	10.00	86.67 ab (1.09)
Control	6.67	00	6.67 f (0.07)

* Figures within the parentheses are the transformed (arcsine) values.

3.3 Isolation and identification of Trichoderma spp. isolates

A total of 102 isolates of *Ts* were screened against the test pathogen. Among the screened *Trichoderma* isolates, 100 isolates were collected from rhizosphere soils of tomato fields by soil dilution plate technique, and the rest of the 2 isolates were collected from the stock culture of plant pathology laboratory of BSMRAU (Table 3). *Trichoderma* isolates were identified according to their conidial morphology, colour and texture, and growth characteristics. Microscopic examination was carried out according to Bissett's (1991) classification method. Most of the isolates grew rapidly on PDA medium and produced large numbers of small green or white conidia from conidiogenous cells situated at the ends of widely branched conidiophores which allows a relatively easy identification of *Trichoderma* as a genus. *Trichoderma* isolates produced much-branched conidiophores on an artificial culture medium. The main branches of conidiophores were mostly in groups of 2-3 and stood at the right angle to the bearer and their length increased with the distance from the tip of the main branch which gave a conical or pyramidal appearance. The isolates of *Trichoderma* spp. were variable in colony colour from light green to dark green. Most of the *Ts* isolates produced profuse spores but 22 isolates were found to produce moderate spores. The mean mycelial growth of the isolates/24 hours ranged from 22.83 mm (COMT-10) to 38.83 mm (COMT-13). Based on the classification of Bissett (1991), all the *Trichoderma* isolates were identified as *Th.*

Table 3. Cultural characterisation of Trichoderma isolates on PDA medium

Isolates	Mean Mycelial growth (mm/24 hrs)	Colony color	Mycelial density*	No. of zonation	Sporulation**
COMT-1	33.67 a-g	Light green	3	2	++
COMT-2	36.67 a-e	Green	4	3	+++
COMT-3	31.50 b-i	Green	3	2	++
COMT-4	36.17 a-e	Light green	4	3	+++
COMT-5	37.00 a-e	Green	3	2	+++
COMT-6	36.67 a-e	Green	4	2	+++
COMT-7	37.00 a-e	Light green	3	3	+++
COMT-8	37.67 a-c	Green	4	3	+++
COMT-9	35.50 a-e	Green	4	2	++
COMT-10	23.17 l	Light green	4	3	+++

Isolates	Mean Mycelial growth (mm/24 hrs)	Colony color	Mycelial density*	No. of zonation	Sporulation**
COMT-11	22.83 l	Green	4	3	+++
COMT-12	37.33 a-c	Green	3	2	+++
COMT-13	38.83 a	Light green	3	2	++
COMT-14	30.17 e-k	Dark green	4	2	++
COMT-15	24.17 kl	Green	4	3	+++
PABT-16	35.67 a-e	Green	4	3	+++
PABT-17	34.67 a-f	Light green	3	2	++
PABT-18	36.00 a-e	Green	4	3	+++
PABT-19	34.17 a-f	Green	4	3	++
PABT-20	34.67 a-f	Green	4	3	+++
PABT-21	35.17 a-e	Green	3	3	+++
PABT-22	38.17 ab	Green	4	3	+++
PABT-23	34.33 a-f	Green	4	3	+++
PABT-24	35.50 a-e	Light green	4	3	++
PABT-25	35.17 a-e	Green	4	2	+++
PABT-26	34.83 a-e	Green	3	3	+++
PABT-27	35.83 a-e	Green	4	3	++
PABT-28	36.17 a-e	Light green	4	2	+++
PABT-29	37.00 a-e	Green	4	3	+++
PABT-30	35.33 a-e	Dark green	3	3	+++
PABT-31	37.33 a-c	Green	4	3	+++
PABT-32	36.67 a-e	Green	4	3	+++
PABT-33	36.33 a-e	Green	4	3	+++
PABT-34	35.50 a-e	Green	4	3	+++
PABT-35	37.50 a-c	Green	4	3	+++
GAZT-36	27.83 f-l	Green	4	2	+++
GAZT-37	33.83 a-f	Light green	3	3	++
GAZT-38	34.17 a-f	Green	4	3	+++
GAZT-39	33.83 a-f	Green	4	3	+++
GAZT-40	33.33 a-h	Light green	3	3	++
GAZT-41	36.00 a-e	Green	4	3	+++
GAZT-42	31.83 b-i	Green	4	3	+++
GAZT-43	36.00 a-e	Green	4	3	++
GAZT-44	33.17 a-h	Green	4	3	+++
GAZT-45	33.67 a-g	Green	4	3	+++
GAZT-46	32.83 a-i	Green	4	3	+++
GAZT-47	30.17 e-k	Green	4	3	+++
GAZT-48	31.17 c-j	Green	3	2	+++
GAZT-49	36.17 a-e	Green	4	3	+++
GAZT-50	35.67 a-e	Green	4	3	+++
GAZT-51	32.33 a-i	Green	4	3	+++
GAZT-52	38.00 a-c	Light green	4	3	+++
GAZT-53	31.67 b-i	Green	4	3	+++
GAZT-54	31.33 b-j	Green	4	2	++
GAZT-55	35.83 a-e	Light green	4	3	+++
GAZT-56	34.67 a-f	Green	4	3	+++
GAZT-57	32.33 a-i	Dark green	4	3	++
GAZT-58	35.67 a-e	Light green	3	2	+++

Isolates	Mean Mycelial growth (mm/24 hrs)	Colony color	Mycelial density*	No. of zonation	Sporulation**
GAZT-59	34.83 a-e	Green	4	3	+++
GAZT-60	36.33 a-e	Green	4	3	+++
NOAT-61	35.17 a-e	Green	3	2	++
NOAT-62	35.17 a-e	Green	4	3	+++
NOAT-63	32.17 a-i	Green	4	3	+++
NOAT-64	35.17 a-e	Green	4	3	+++
SATT-65	32.33 a-i	Light green	3	3	++
BBUT-66	32.67 a-i	Green	4	3	+++
BBUT-67	32.83 a-i	Green	4	3	++
BBUT-68	31.83 b-i	Green	4	3	+++
BBUT-69	26.83 g-l	Green	4	3	+++
BBUT-70	37.67 a-c	Green	4	3	+++
BBUT-71	34.83 a-e	Green	4	2	+++
BBUT-72	34.17 a-f	Light green	4	3	+++
BBUT-73	33.67 a-g	Green	4	3	+++
BBUT-74	32.67 a-i	Green	4	3	+++
BBUT-75	35.33 a-e	Light green	4	2	+++
BBUT-76	35.17 a-e	Light green	4	3	+++
BBUT-77	37.17 a-d	Green	4	3	+++
BBUT-78	34.17 a-f	Green	4	3	+++
BBUT-79	32.33 a-i	Green	4	3	+++
BBUT-80	37.17 a-d	Light green	3	3	++
BBUT-81	37.50 a-c	Green	4	3	+++
BBUT-82	26.17 i-l	Green	4	3	++
BBUT-83	37.67 a-c	Green	4	3	+++
BBUT-84	33.83 a-f	Green	4	3	+++
BBUT-85	37.67 a-c	Light green	4	3	+++
BBUT-86	37.83 a-c	Green	4	3	+++
BBUT-87	35.83 a-e	Green	4	3	+++
BBUT-88	36.50 a-e	Green	4	3	+++
BBUT-89	37.17 a-d	Green	4	3	+++
BBUT-90	37.67 a-d	Green	3	3	++
BBUT-91	34.67 a-f	Green	4	3	+++
BBUT-92	36.33 a-e	Light green	4	3	+++
BBUT-93	36.33 a-e	Green	4	3	+++
BBUT-94	36.50 a-e	Green	3	3	++
BBUT-95	36.33 a-e	Green	4	2	++
BBUT-96	36.83 a-e	Light green	4	3	+++
BBUT-97	36.83 a-e	Green	4	3	+++
BBUT-98	35.17 a-e	Green	3	3	++
BBUT-99	35.67 a-e	Green	4	3	+++
BBUT-100	37.50 a-c	Green	4	3	+++
GBRT-4	30.33 d-k	Green	4	3	+++
GBRT-31	26.67 h-l	Green	4	3	+++

* Mycelial density was evaluated by 1-4 scale. ** Sporulation was assessed by the visual rating on -, +, ++ and +++ representing no spore, very minimum spore, a moderate number of spores and profuse spores, respectively.

3.4 In vitro screening of potential Trichoderma isolates against Sr

In an *in vitro* experiment each of the isolates of *Th* showed their antagonism against *Sr* and the pattern of inhibition by them was nearly uniform. Inhibition in radial growth of *Sr* by the *Trichoderma* isolates ranged from 40.37 to 76.27%. Of the total, four isolates (3.92%) showed antagonism class 1, seven isolates (6.86%) showed antagonism class 2, eighty-seven isolates (85.30%) showed antagonism class 3, four isolates (3.92%) showed antagonism class 4 and no isolates showed antagonism class number 5 (Table 4 and Figure 1). Four isolates namely, PABT-22, BBUT-70, NOAT-63 and PABT-34 showed more than 75% inhibition (antagonism class 1). Isolate PABT-22 showed the highest mean inhibition value (76.27%) against *S. rolfsii* followed by BBUT-70 (75.37%) and NOAT-63 (75.33%) (Figure 2). The lowest inhibition in radial growth of *Sr* was obtained with the isolates GBRT-31 (40.37%) followed by GAZT-53 (49.27%). Results of the screening test revealed a highly significant interaction between *Trichoderma* and *Sr*. isolates of *Th* were able to reduce the growth of *Sr* but were variable in their antagonism ability. Only very few were found to have effective antagonism potentiality. Several researchers also reported on significant reduction of mycelial growth of *Sr* in the presence of *Th* (Ahmed et al., 2019; Liton et al., 2019; Rahman et al., 2024ab) which supports the present *in vitro* test.

Table 4. Antagonism of Trichoderma isolates against S. rolfsii in dual plate culture technique on PDA medium

Antagonism class	*Trichoderma* isolates	Number of isolates	Percentage of isolates
1	PABT-22, BBUT-70, NOAT-63, PABT-34	4	3.92
2	BBUT-77, COMT-13, BBUT-76, PABT-16, PABT-24, PABT-31, GAZT-39	7	6.86
3	BBUT-86, COMT-12, GAZT-60, BBUT-92, BBUT-100, COMT-4, PABT-26, PABT-20, BBUT-75, PABT-28, COMT-6, PABT-21, PABT-27, PABT-33, BBUT-89, BBUT-93, COMT-7, PABT-32, PABT-29, BBUT-83, BBUT-85, BBUT-94, BBUT-97, PABT-17, BBUT-88, BBUT-95, BBUT-99, PABT-18, COMT-8, PABT-23, PABT-25, GAZT-41, GAZT-56, GAZT-57, COMT-3, COMT-9, PABT-19, PABT-35, GAZT-44, GAZT-50, GAZT-55, NOAT-62, BBUT-72, BBUT-84, BBUT-96, NOAT-64, BBUT-74, BBUT-81, BBUT-91, GAZT-59, GAZT-40, GAZT-42, GAZT-59, BBUT-69, BBUT-78, BBUT-90, GAZT-58, BBUT-80, BBUT-87, COMT-2, GAZT-45, COMT-15, GAZT-43, NOAT-61, GAZT-47, BBUT-68, BBUT-79, BBUT-98, GAZT-49, BBUT-73, GAZT-38, SATT-65, BBUT-66, BBUT-67, BBUT-71, PABT-30, GAZT-46, GAZT-48, GAZT-51, COMT-1, COMT-5, GAZT-37, GAZT-54, COMT-10, GAZT-36, BBUT-82, COMT-11	87	85.30
4	COMT-14, GAZT-53, GBRT-4, GBRT-31	4	3.92
5	-	-	-
Total	-	102	-

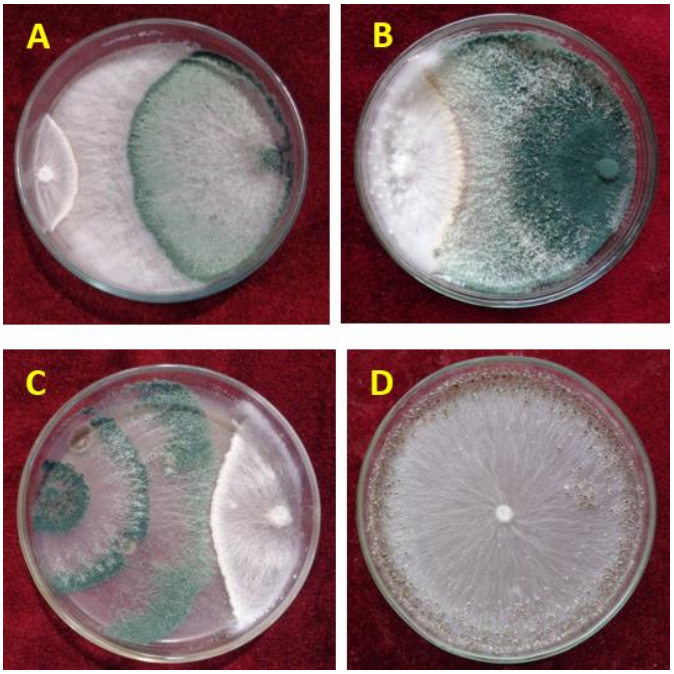

Figure 1. Antagonism of Trichoderma isolates against Sr on PDA (A. Antagonism of isolate PABT-22; B. Antagonism of isolate BBUT-70; C. Antagonism of isolate NOAT-63 D. Control)

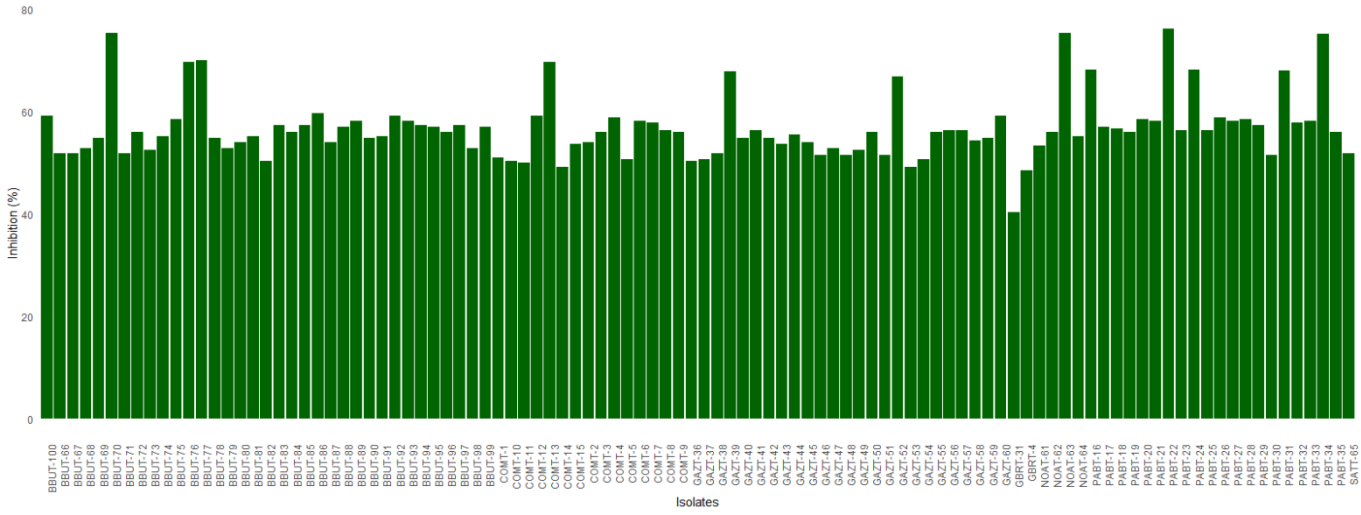

Figure 2. Screening of Trichoderma isolates against Sr in dual culture on PDA

3.5 Effect of TFC on disease suppression and yield improvement

The lowest 8.4% disease incidence (%DI) was observed in the treatments T_7 and T_6 and the highest 29.2% in the treatment T_1 followed by the treatment T_2 (20.9%) which were statistically similar but significantly different among other treatments. The lowest 5.2 percent disease index (PDI) was observed in the treatment T_7 which was statistically similar among other treatments except the treatments T_1 and T_2; the highest 25.0 PDI was observed in the treatment T_1 followed by the treatment T_2 (18.8) which were also statistically similar. The highest 71.4 percent disease control (PDC) was observed in the treatments T_7 and T_6 and the lowest 42.9% in the treatments only compost (T_3) and wheat grain colonised pathogens with *TFC* @ $4.0x10^8$ spore ml^{-1} (T_9) (Table 5).

Table 5. Effect of different treatments on collar rot and southern blight disease incidence of tomato in the field experiment

Treatments	Collar rot and southern blight		
	% DI	PDI	PDC
T_1= Wheat grain colonised pathogens (Control- 1)	29.2 a (0.30)	25.0 a (0.25)	-
T_2= No *Trichoderma* and no compost (Control- 2)	20.9 ab (0.21)	18.8 ab (0.19)	-
T_3= Only compost (Control- 3)	16.7 b (0.18)	14.6 bc (0.15)	42.9
T_4= T_1+ Wheat grain colonised *Trichoderma*	12.5 b (0.13)	10.4 bc (0.11)	57.1
T_5= T_1+*TFC* @ 2.0 x 10^8 spore ml^{-1}	12.5 b (0.13)	8.3 c (0.09)	57.1
T_6= T_1+*TFC* @ 2.5 x 10^8 spore ml^{-1}	8.4 b (0.10)	7.3 c (0.09)	71.4
T_7= T_1+*TFC* @ 3.0 x 10^8 spore ml^{-1}	8.4 b (0.10)	5.2 c (0.07)	71.4
T_8= T_1+*TFC* @ 3.5 x 10^8 spore ml^{-1}	12.5 b (0.13)	7.3 c (0.08)	57.1
T_9= T_1+*TFC* @ 4.0 x 10^8 spore ml^{-1}	16.7 b (0.17)	9.4 c (0.09)	42.9

* Figures within the parentheses are the transformed (arcsine) values. DI=disease incidence, PDI=percent disease index, PDC=percent disease control.

The highest 41.84 fruits $plant^{-1}$ were observed in the treatment wheat grain colonised pathogens with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) followed by the treatment wheat grain colonised pathogens with *TFC* @ 3.5x10^8 spore ml^{-1} (T_8) (39.08) which were statistically similar and the lowest in the treatment (T_1, control-1) (24.88). The highest 61.15 mm fruit was observed in the treatment of wheat grain colonised pathogens with *TFC* @3.0x10^8 spore ml^{-1} (T_7) followed by the treatment of wheat grain colonised pathogens with *TFC* @ 3.5x10^8 spore ml^{-1} (T_8) (56.16 mm) which were statistically different and the lowest in the treatment (T_1, control-1) (49.63 mm). The highest 92.78 g fruit was observed in the treatment of wheat grain colonised pathogens with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) followed by the treatment wheat grain colonised pathogens with *TFC* @3.5x10^8 spore ml^{-1} (T_8) (85.35 g) which were statistically similar and the lowest in the treatment (T_1, control-1) (63.21 g). Approximately regular shape and uniform size, bright red coloured fruits were observed in the treatment wheat grain colonised pathogens with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) (Table 6). The highest 38655 g $plot^{-1}$ tomato was observed in the treatment wheat grain colonised pathogens with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) followed by the treatment wheat grain colonised pathogens with *TFC* @3.5x10^8 spore ml^{-1} (T_8) (38190 g) which were statistically similar and the lowest 24300 g $plot^{-1}$ in the treatment wheat grain colonised pathogens (T_1, control-1) followed by the treatment no *Trichoderma* and no compost (T_2, control-2) (25545 g) which were also statistically similar. The highest 64.43 ton ha^{-1} tomato was also observed in the treatment wheat grain colonised pathogens with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) followed by the treatment wheat grain colonised pathogens with *TFC* @ 3.5x10^8 spore ml^{-1}(T_8) (63.65 ton ha^{-1}) which were also statistically similar and the lowest 40.50 ton ha^{-1} in the treatment wheat grain colonised pathogens (T_1, control-1) followed by the treatment no *Trichoderma* and no compost (T_2, control-2) (42.58 ton ha^{-1}) which were also statistically similar. The highest 59.07% increased yield over control (T_1) was observed in the treatment wheat grain colonised pathogens with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) followed by the treatment wheat grain colonised pathogens with *TFC* @ 3.5x10^8

spore ml^{-1} (T_8) (57.16%) and the lowest 23.95% in the treatment wheat grain colonised pathogens with wheat grain colonised *Trichoderma* (T_4) (Table 7). Increased growth promotion of different vegetable crops through the application of *Trichoderma* compost against soilborne pathogens was also reported by Rahman et al. (2020ab, 2021); Chowdhury et al. (2024), Rahman et al. (2024); Rubayet and Hossain (2025) supports the findings of the current study.

Table 6. Effect of different treatments on individual fruit characters of tomato

Treatments	Characters		
	Fruit no./plant	Fruit size (diameter) (mm)	Fruit wt.(g)
T_1= Wheat grain colonised pathogens (Control-1)	24.88 c	49.63 c	63.21 c
T_2= No *Trichoderma* and no compost (Control-2)	32.92 a-c	53.47 bc	72.32 bc
T_3= Only compost (Control-3)	34.62 ab	55.09 b	80.28 ab
T_4= T_1+ Wheat grain colonised *Trichoderma*	31.83 bc	53.44 bc	75.42 bc
T_5= T_1+*TFC* @ 2.0 x 10^8 spore ml^{-1}	34.67 ab	54.42 b	76.88 bc
T_6= T_1+*TFC* @ 2.5 x 10^8 spore ml^{-1}	36.42 ab	56.02 b	81.51 ab
T_7= T_1+*TFC* @ 3.0 x 10^8 spore ml^{-1}	41.84 a	61.15 a	92.78 a
T_8= T_1+*TFC* @ 3.5 x 10^8 spore ml^{-1}	39.08 ab	56.16 b	85.35 ab
T_9= T_1+*TFC* @ 4.0 x 10^8 spore ml^{-1}	32.25 bc	55.30 b	80.35 ab

Table 7. Effect of different treatments on the yield of tomato

Treatments	Yield of tomato		
	Per plot (g)	Per ha (tons)	% yield increased over control (T_1)
T_1= Wheat grain colonised pathogens (Control-1)	24300 c	40.50 c	-
T_2= No *Trichoderma* and no compost (Control-2)	25545 bc	42.58 bc	-
T_3= Only compost (Control-3)	35310 ab	58.85 ab	45.31
T_4= T_1+Wheat grain colonised *Trichoderma*	30120 a-c	50.20 a-c	23.95
T_5= T_1+*TFC* @ 2.0 x 10^8 spore ml^{-1}	30135 a-c	50.23 a-c	24.0
T_6= T_1+*TFC* @ 2.5 x 10^8 spore ml^{-1}	35265 ab	58.78 ab	45.11
T_7= T_1+*TFC* @ 3.0 x 10^8 spore ml^{-1}	38655 a	64.43 a	59.07
T_8= T_1+*TFC* @ 3.5 x 10^8 spore ml^{-1}	38190 a	63.65 a	57.16
T_9= T_1+*TFC* @ 4.0 x 10^8 spore ml^{-1}	31515 a-c	52.53 a-c	29.68

The highest 14.08 mg/100g ascorbic acid was found in the treatment no *Trichoderma* and no compost (T_2, Control-2) followed by the treatment wheat grain colonised pathogens (T_1, Control-1) (13.20 mg/100g) and the lowest 7.04 mg/100g was in the treatment wheat grain colonised pathogens with *TFC* @3.5x10^8 spore ml^{-1} (T_8). The highest 0.0568 mg/100g of Beta carotene was found the treatment wheat grain colonised pathogens with *TFC* @3.0x10^8 spore ml^{-1} (T_7) followed by the treatment no *Trichoderma* and no compost (T_2, Control-2) (0.0534 mg/100g) and the lowest 0.0322 mg/100g was in the treatment wheat grain colonised pathogens (T_1, Control-1). The highest 0.0978 mg/100g of Lycopene was found the treatment wheat grain colonised pathogens

with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) followed by the treatment wheat grain colonised pathogens with *TFC* @ 2.5x10^8 spore ml^{-1} (T_6) (0.0777 mg/100g) and the lowest 0.0386 mg/100g was in the treatment wheat grain colonised pathogens (T_1, Control-1). The highest 10.7% total soluble solid (TSS) was found in the treatment wheat grain colonised pathogens with *TFC* @ 2.5x10^8 spore ml^{-1} (T_6) and the lowest 8.4% was in the treatment wheat grain colonised pathogens with *TFC* @ 3.5x10^8 spore ml^{-1} (T_8). The highest 15.30 mg/g of reducing sugar was found in the treatment wheat grain colonised pathogens with *TFC* @ 4.0x10^8 spore ml^{-1} (T_9) followed by the treatment wheat grain colonised pathogens with *TFC* @ 2.5x10^8 spore ml^{-1} (T_6) (14.50 mg/g) and the lowest 10.00 mg/g was in the treatment wheat grain colonised pathogens with wheat grain colonised *Trichoderma* (T_4). The highest 24.48 mg/g of total sugar was found in the treatment wheat grain colonised pathogens with *TFC* @ 4.0x10^8 spore ml^{-1} (T_9) followed by the treatment wheat grain colonised pathogens with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) (22.81 mg/g) and the lowest 15.99 mg/g was in the treatment wheat grain colonised pathogens with wheat grain colonised *Trichoderma* (T_4). The highest 0.17092 mg/100g of chlorophyll a was found the treatment wheat grain colonised pathogens with *TFC* @ 2.5x10^8 spore ml^{-1} (T_6) followed by the treatment no *Trichoderma* and no compost (T_2, Control-2) (0.15628 mg/100g) and the lowest 0.08546 mg/100g was in the treatment wheat grain colonised pathogens with wheat grain colonised *Trichoderma* (T_4). The highest 0.19992 mg/100g of chlorophyll b was found the treatment wheat grain colonised pathogens with *TFC* @ 2.5x10^8 spore ml^{-1} (T_6) followed by the treatment wheat grain colonised pathogens with *TFC* @3.0x10^8 spore ml^{-1} (T_7) (0.17284 mg/100g) and the lowest 0.09996 mg/100g was in the treatment wheat grain colonised pathogens with wheat grain colonised *Trichoderma* (T_4). The table showed that there was no specific treatment that best for each chemical. Different treatment showed best performance on different chemical. But the treatment wheat grain colonised pathogens with *TFC* @ 3.0x10^8 spore ml^{-1} (T_7) showed the best performance on the content of Beta carotene and Lycopene, the most important elements of tomato for human health (Table 8). The *Th-based* compost not only suppressed the plant disease and increased yield but also enhanced the nutritional quality of the tomato (Khan et al., 2017). The synergistic action of multiple *Trichoderma* isolates significantly improved tomato growth while suppressing *S. rolfsii*, highlighting their potential as an environmentally sustainable approach to southern blight (Mishu et al., 2025; Cunha et al., 2025), Fusarium wilt (Awad-Allah et al., 2022), and early blight management (Imran et al., 2023).

Table 8. Effect of different treatments on chemicals present in ripe tomato

Treatments	Ascorbic acid (mg/100g)	Beta carotene (mg/100g)	Lycopene (mg/100g)	% TSS	Reducing Sugar (mg/g)	Total Sugar (mg/g)	Chlorophyll a (mg/100g)	Chlorophyll b (mg/100g)
T_1	13.20	0.0322	0.0386	10.5	10.8	16.63	0.10548	0.1364
T_2	14.08	0.0534	0.0700	10.6	12.2	19.44	0.15628	0.11768
T_3	12.32	0.0499	0.0578	10.4	11.8	17.11	0.1255	0.16348
T_4	8.80	0.0383	0.0694	9.2	10.0	15.99	0.08546	0.09996
T_5	10.56	0.0363	0.0635	10.6	13.56	18.95	0.13088	0.12704
T_6	11.44	0.0450	0.0777	10.7	14.50	20.22	0.17092	0.19992
T_7	11.44	0.0568	0.0978	10.6	13.62	22.81	0.1255	0.17284
T_8	7.04	0.0326	0.0449	8.4	10.66	16.33	0.10548	0.1364
T_9	10.56	0.0500	0.0769	10.5	15.30	24.48	0.10548	0.1364

T_1= Wheat grain colonised pathogens (Control-1), T_2= No Trichoderma and no compost (Control-2), T_3= Only compost (Control-3), T_4= T_1+ Wheat grain colonised Trichoderma, T_5= T_1+*TFC* @ 2.0x10^8 spore ml^{-1}, T_6= T_1+*TFC* @ 2.5x10^8 spore ml^{-1}, T_7= T_1+*TFC* @ 3.0x10^8 spore ml^{-1}, T_8= T_1+*TFC* @ 3.5x10^8 spore ml^{-1}, T_9= T_1+*TFC* @ 4.0x10^8 spore ml^{-1}.

4. Conclusion

Trichoderma-fortified compost, optimised at ~3.0 × 10^8 spores mL^{-1} of isolate PABT-22, effectively suppressed southern blight while enhancing tomato yield and carotenoid content, providing a low-cost, residue-free option for sustainable production. However, the present findings are limited to a single site and season, with responses influenced by strain and dose specificity. Future research should prioritise multi-location and multi-season validation, metagenomic profiling of TFC-amended soils, evaluation of shelf-life and quality control standards, and cost–benefit analyses to facilitate broader adoption in sustainable tomato farming.

Acknowledgements

The authors express their gratitude to the authority of the University Grant Commission (UGC) and the Higher Education Quality Enhancement Project (HEQEP) for providing financial support to complete the research work.

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