



OPEN ACCESS

EDITED BY

Matteo Balderacchi,
Independent Researcher, Piacenza, Italy

REVIEWED BY

Surasak Khankhum,
Maharakham University, Thailand
Abayeneh Girma,
Mekdela Amba University, Ethiopia
Mingzheng Duan,
Zhaotong University, China

*CORRESPONDENCE

Ram Dutta

✉ rddutta.icar@gmail.com

K. Jayalakshmi

✉ jayalakshmipat@gmail.com

Satish Kumar

✉ satseemayadav@gmail.com

RECEIVED 10 July 2025

ACCEPTED 04 September 2025

PUBLISHED 24 September 2025

CITATION

Dutta R, Jayalakshmi K, Kumar S, Soumia PS,
Auji R, Mahajan V and Singh M (2025)
Evaluation of potent *Trichoderma* strains
against anthracnose-twister disease and
enhancement of crop health and bulb yield in
kharif onion.
Front. Sustain. Food Syst. 9:1663405.
doi: 10.3389/fsufs.2025.1663405

COPYRIGHT

© 2025 Dutta, Jayalakshmi, Kumar, Soumia,
Auji, Mahajan and Singh. This is an
open-access article distributed under the
terms of the [Creative Commons Attribution
License \(CC BY\)](#). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Evaluation of potent *Trichoderma* strains against anthracnose-twister disease and enhancement of crop health and bulb yield in *kharif* onion

Ram Dutta*, K. Jayalakshmi*, Satish Kumar*, P. S. Soumia,
Radhakrishna Auji, Vijay Mahajan and Major Singh

ICAR-Directorate of Onion and Garlic Research, Pune, Maharashtra, India

In India, the productivity of onions during the *kharif* season (June–October) is quite low compared to the *Rabi* season (December–April). The yield losses in *kharif* onion are primarily attributed to the increased incidence of anthracnose-twister disease, which is often exacerbated by concurrent monsoon rains and high relative humidity during the *kharif*. To address this issue, a field study was conducted over three consecutive years (2021–2023) to assess the performance of eleven different *Trichoderma* strains in managing anthracnose-twister disease and improving plant growth and bulb yield in *kharif* onion. The strains were initially selected based on their ability to inhibit the growth of the anthracnose pathogen *Colletotrichum gloeosporioides* (*in vitro* confrontation assay) and HCN production; amylase, and protease production. The selected strains were also screened *in vitro* for PGPA traits (IAA and siderophore production; ability to grow in N₂ free media, PO₄^{2−}, Zn, and K solubilization). The antagonistic activity of *Trichoderma* strains against *C. gloeosporioides* ranged from 18.8% to 70.0% with *T. longibrachiatum* (OGRDT2) recording the highest inhibition (70%). Application of GRDT2 and OGRDT2 reduced the PDI to 36.00 ± 0.98 and 37.06 ± 1.38, respectively, compared to 62.77 ± 0.14 in the untreated control. The *T. longibrachiatum* (OGRDT2) and *T. harzianum* (GRDT2) treated plots registered an increased plant height (54.97 ± 0.80 and 53.29 ± 0.79 cm) and pseudostem diameter (13.96 ± 0.18 and 13.76 ± 0.20 mm) compared to the control (42.68 ± 1.90 and 10.55 ± 0.04 mm), respectively, under field conditions. Additionally, these treatments supported higher biomass, as reflected in the increased normalized dry weights of the shoot (10.82 ± 0.89% and 10.39 ± 1.76 %), root (12.27 ± 0.11 % and 11.86 ± 2.67 %), and bulb (12.90 ± 0.65 % and 12.21 ± 0.81 %). The chlorophyll contents were again higher (4.1 ± 1.44 and 4.1 ± 0.83 mg/ml) in OGRDT2 and GRDT2 treated plants than 3.3 ± 0.80 mg/ml in the untreated control. The GRDT2 and OGRDT2 treated plots supported higher bulb yield of 20.66 ± 1.42 t/ha and 19.21 ± 0.87 t/ha compared to control (16.52 ± 0.69 t/ha). Our study demonstrated that *T. harzianum* (GRDT2) and *T. longibrachiatum* (OGRDT2) effectively reduced anthracnose severity and therefore can be explored for management of Anthracnose-twister disease of onion.

KEYWORDS

anthracnose-twister, *Colletotrichum gloeosporioides*, *kharif* onion, *Trichoderma*, bulb yield

1 Introduction

Onion (*Allium cepa*) holds a central place in Indian agriculture, as it is a staple and indispensable ingredient in almost all Indian cuisines, enhancing taste and flavor while offering several health benefits (Kalyani et al., 2014; Benke et al., 2022). India has emerged as the leading onion-producing country, with an overall production of 31.7 Mt in 2022 and 30.2 Mt in 2023, surpassing China, which produced 24.5 and 24.9 Mt in the respective years (FAO, 2025). Despite surplus production, onion remains a critical area of focus for the Government due to their significant impact on rural economic growth and livelihoods (Kale et al., 2024). In India, onion is cultivated across three cropping seasons: *kharif* (crop sown with monsoon rains and harvested as they end during June–October), late *kharif* (post monsoon crop taken during September–December), and *rabi* (crop sown in winter and harvested before summer during December–April) (Sharma and Chauhan, 2024). *Kharif* onion is crucial in maintaining a steady supply and providing fresh produce when *rabi* onion stocks decline (Srinivas and Lawande, 2007; Gopal, 2015). However, *kharif* onion production is affected by several factors, including climatic conditions, soil health, and biotic stresses. Among the biotic stresses, several diseases caused by viruses, bacteria, fungi, and insect infestation can significantly reduce productivity (Paibomesai et al., 2012). During the *kharif* season, anthracnose-twister is the most devastating disease, causing estimated yield losses up to 80–100% (Chawda and Rajasab, 1996; Alberto and Aquino, 2010; Salunkhe et al., 2022; Dutta et al., 2024a).

The development of Anthracnose-twister disease of onion crop can be attributed either to a single fungal pathogen or to a complex of closely related fungal species acting synergistically, which include complex interplay of fungal pathogens such as *Colletotrichum gloeosporioides*, *Glomerella cingulata*, and *Fusarium acutatum*, *Gibberella moniliformis* (Guarro et al., 1998; Schwartz and Mohan, 2007; Dutta et al., 2022, 2024a; Saini et al., 2024). Anthracnose disease typically begins as water-soaked, whitish, sunken lesions on leaf blades that later darken into oval or elliptical spots, leading to chlorosis and eventually necrosis (Schwartz and Mohan, 2007; Dutta et al., 2022, 2024a). The disease causes leaf curling, disrupts vascular function, and reduces photosynthesis, leading to major yield losses (Alberto, 2014; Dutta et al., 2022). In case of severe anthracnose infection, the symptoms may also appear on onion bulbs (Schwartz et al., 2015).

Management of anthracnose in *Kharif* onions is rather more challenging due to the frequent rainfall during the monsoon season and high humidity, which is conducive for spore germination and the splashing of fungal spores from infected plant debris onto healthy tissues, thereby spreading the disease rapidly (Salunkhe et al., 2022). The frequent rains during *kharif* wash away fungicides, reducing their effectiveness against anthracnose pathogens. The problem is further aggravated by the simultaneous increase in the number of insect vectors (Lorini et al., 1986; Liu, 2004), which may contribute further to the spread of fungal spores during the *kharif* season (McKenzie et al., 1993). Such challenges make the *kharif* onion cultivation less remunerative to farmers due to the lower yields and higher production costs, which adversely affect the onion supply chain and thereby increase onion prices during

the lean period (August–October). Efforts have been undertaken to address the challenges of *kharif* onion cultivation through various strategies like developing anthracnose resistant/tolerant varieties through conventional breeding or biotechnological intervention, usage of effective chemical fungicides and dosage optimization, improved agronomic practices, and other integrated disease and pest management practices. The development of anthracnose-resistant onion varieties through traditional breeding is particularly difficult due to the lack of novel resistance gene(s) in the onion gene pool, as well as challenges related to gene introgression and viability of the crosses. To date, no complete resistance gene has been reported in onion; however, partial resistance has been observed in the shallot cultivar “Sumenep,” which too lacks flowering, leading to practical difficulties in its use for introgression (Suhardi, 1993; Bui et al., 2019). Two more onion cultivars, “Barreiro” and “Texas Early Grano” have exhibited resistance against *C. gloeosporioides*, suggesting that resistance is controlled by multiple genes, making it difficult to incorporate into a traditional breeding programme (Melo and Costa, 1983). Similar challenges exist in breeding *Allium fistulosum*, *A. galanthum*, and *A. roylei*, which have shown only partial resistance to anthracnose (Galván et al., 1997). These factors further complicate disease management, forcing the farmers to rely solely on chemical fungicides, which may lead to resistance development and other environmental concerns (Jaiswal et al., 2020).

To overcome these issues, an eco-friendly use of beneficial microbes having plant growth-promoting and bio-control potential is highly effective, as it has no negative impact on agroecosystems or human health. Beneficial microbes can influence plant growth parameters either directly (Direct mechanism) or through indirect mechanism (El-Saadony et al., 2022). The direct processes include the microbe-facilitated nutrient acquisition (nitrogen fixation, phosphate and other mineral solubilization) and phytohormone production [indole-3-acetic acid, gibberellins, and cytokinins, and the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase]. The indirect processes include antibiosis, mycoparasitism, competition for niches, and induction of systemic resistance that primarily operate through biocontrol activities that mitigate the deleterious effects of pathogens and adverse environmental factors. Several bacteria and fungi have been identified as antagonistic to *Glomerella cingulata*, *Colletotrichum gloeosporioides*, *G. moniliformis*, and *Fusarium* spp., and they can be utilized to manage the anthracnose sustainably. These include *Paenibacillus polymyxa* APEC128, *Bacillus* spp., *Trichoderma piluliferum*, and *Trichoderma* spp., *Cryptococcus nemorosus*, *Rhodotorula toruloides* and *Nakaseomyces glabratus* (Han et al., 2013; Kim et al., 2016; Musheer and Ashraf, 2017; da Costa et al., 2021; Ezebo and Okigbo, 2024; Li et al., 2025).

Biocontrol agents play a dual role: they exhibit antagonistic activity (antibiosis, parasitism, and secondary metabolites production) and promote plant growth by solubilization of phosphate and production of IAA (Indole acetic acid) and ammonia (Jaiswal et al., 2020). Among fungi, *Trichoderma* is the most extensively studied genus as a biocontrol agent. Several species, including *T. asperellum*, *T. atroviride*, *T. harzianum*, *T. longibrachiatum*, *T. virens*, and *T. viride*, were frequently used as biocontrol agents (Guzman-Guzman et al., 2019, Li

et al., 2025). Unlike chemical pesticides and fertilizers, which often leave harmful residues and results in loss of soil microbial diversity, the application of *Trichoderma*-based formulations offer a safer alternative for sustaining crop yields and managing crop diseases without any adverse impact on soil ecology (Abdullah et al., 2021; Siddiqui et al., 2025). Various investigators have documented that the secondary metabolites from *T. harzianum* and *T. virens*, such as viridins, azaphilones, heterocyclic nitrogen compounds, and volatile terpenes, were pivotal in the biocontrol of fungal pathogens (Chethana et al., 2012; Priya et al., 2015; Manzar et al., 2022; Ramzan et al., 2023). Recently, da Silva Pereira et al. (2025) demonstrated that bioactive proteins such as glutaminase, metalloproteinase, chitinase, and peptide hydrolase from *Trichoderma* exhibit strong antagonistic activity against *Fusarium* spp., highlighting their biocontrol potential. While *Trichoderma* is well-known for antagonism and plant growth promotion, very few studies have correlated their PGP traits and *in vitro* antagonism with actual reduction in PDI and yield enhancement in onion. Whereas disease incidence and yield losses are higher in *kharif* onion, the published reports are more skewed on *rabi* onion crop as majority of onion production comes from *rabi* crop. Our study assumes significance because all *Trichoderma* strains do not perform equally and strain-specific evaluations are required to bridge the knowledge gap concerning identification of region-specific, potent strains that can be recommended for sustaining yield and for integrated management of anthracnose–twister disease of *kharif* onion crop.

Considering these factors, the current study was undertaken to assess the potent *Trichoderma* strains that can serve as dual-purpose biocontrol and growth-promoting agents for linking the *in vitro* traits with *in vivo* performance, thereby reducing the disease severity and increasing the bulb yield in *kharif* onion crop.

2 Materials and methods

2.1 Trichoderma strains

In this study, the *Trichoderma* strains were isolated from the soil samples collected from the experimental plots of ICAR-Directorate of Onion and Garlic Research, Pune, and ICAR-Directorate of Groundnut Research, Junagadh, Gujarat, India. *Trichoderma* selective medium (TSM) and Potato dextrose agar (PDA) were used for the isolation, and putative colonies were identified and purified by the single spore isolation method as described in our previous study (Dutta et al., 2024b). The pure cultures obtained were preserved at 4°C for further study.

2.2 Morphological and DNA-based identification

The morphology of *Trichoderma* strains was studied based on colony and spore morphology following the guidelines of the International Sub-Commission on *Trichoderma* and *Hypocrea* as given by Kumar and Sharma (2016). Genomic DNA was isolated from the 5 days old fresh mycelium grown on Potato dextrose broth (PDB) using the CTAB method (cetyl-trimethyl ammonium

bromide) as Kumar et al. (2013). DNA-based identification of all *Trichoderma* isolates was carried out using the ITS (Internal Transcribed Spacer) and *Tef1α* (translation elongation factor) with the following primers pairs (5'TCCGTAGGTGAACCTGCGG3') and (5'TCCTCCGCTTATTGATATGC3') and (5'CATCGAGAA GTTCGAGAAGG3') and (5'TACTTGAAGGAACCCCTTACC3'), respectively synthesized by Eurofins Genomics (Bengaluru, India). The obtained sequences of ITS gene (NCBI accessions: OR048743-OR048753) and *Tef1α* gene (NCBI accessions: OR102865-OR102875) were analyzed using the BLAST algorithm against the NCBI GenBank database to confirm the identity of the strains. The colony morphology and microscopic images of the strains used in the current study are provided in the Supplementary Figure S1. Phylogenetic analysis was conducted using MEGA version 11 (Tamura et al., 2021). The evolutionary history was inferred employing the Maximum Likelihood method based on the Jukes-Cantor model (Jukes and Cantor, 1969).

2.3 Confrontation assays

A confrontation assay was performed for all eleven *Trichoderma* strains on potato dextrose agar plates (HiMedia Laboratories, Mumbai, India). Five mm mycelial disks taken from the margin of 5 days old culture of the *Trichoderma* were tested against the onion Anthracnose pathogen, *Colletotrichum gloeosporioides*, previously described by Dutta et al. (2024a). The potential test *Trichoderma* strains were inoculated at the margin of the Petri plate, placed opposite each other. Observations were recorded up to 7 days of incubation at 28°C. The *Colletotrichum gloeosporioides* plates without *Trichoderma* served as control. The treatments were replicated five times. The percent inhibition of the pathogen growth was calculated according to formula given by Vincent, 1927 as described below

$$\text{Percent Inhibition (I)} = \frac{C - T}{C} \times 100 =$$

Radial growth of the pathogen in control (mm)

T = Radial growth of the pathogen (mm) in presence of antagonist *Trichoderma* strain used.

I = Percentage inhibition of pathogen growth

C = growth (radial mycelial growth) in control.

2.4 Biochemical characterization

All eleven *Trichoderma* strains were subjected to various biochemical characterizations for PGP activities using qualitative or quantitative assays. Indole acetic acid (IAA) production was tested by growing isolates in tryptophan-supplemented broth, where IAA reacts with Salkowski's reagent to produce a pink coloration the intensity of which is proportional to the amount of IAA secreted into the medium. The absorbance of the developed color was measured at 540 nm using spectrophotometer (SpectroNanoStar, BMG LABTECH, Ortenberg, Germany) as described by Glickmann and Dessaux (1995). The siderophore production was assessed on Chrome Azurol S (CAS) medium based on the principle of

iron chelation in which the siderophores chelate Fe^{3+} from the CAS-dye complex, leading to a blue-to-orange color change. The *Trichoderma* isolates were inoculated onto CAS agar plates and plates were incubated at 30°C. The siderophore production was indicated by the development of a distinct orange halo around colonies against the blue background of the medium. The diameter of the halo was used as a qualitative measure of siderophore production (Schwyn and Neilands, 1987; Machuca and Milagres, 2003; Loudon et al., 2011). The phosphate solubilization was determined on Pikovskaya's agar, containing insoluble tricalcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$] as the only phosphorus source, which makes the medium opaque. The phosphate solubilizer strains can release organic acids that dissolve the insoluble phosphate, resulting in a development of clear halo zone around the test colony (Promwee et al., 2014). Zinc solubilization was evaluated on mineral salt medium supplemented with insoluble zinc compounds, with solubilization indicated by halo zone formation (Sharma et al., 2012). Potassium solubilization was tested on Aleksandrov medium containing insoluble mica or feldspar, where solubilization is visualized as halo zones around colonies (Dhaked et al., 2017). Hydrogen cyanide (HCN) production was assayed by culturing isolates on glycine-supplemented medium, where volatile HCN reacts with picrate-impregnated filter paper placed in the lid, causing a color change from yellow to brown or reddish brown (Bakker and Schippers, 1987; Meera and Balabaskar, 2012).

Enzymatic activities, including amylase and protease, were analyzed using GYP (Glucose Yeast extract Peptone) medium with 1% soluble starch (Abdel and Shearer, 2002) and 0.4% gelatin (Sathish et al., 2012). For amylase activity, plates containing 1% soluble starch were inoculated with *Trichoderma* strains and incubated. After growth, the plates were flooded with iodine solution; amylase-producing isolates hydrolyse starch into simple sugars, resulting in clear zones around colonies against the blue-black background. The nitrogen-fixing ability of the *Trichoderma* strains was evaluated using Jensen's media devoid of nitrogen source, where the growth of *Trichoderma* strain indicated its ability to grow in N_2 -free medium by fixing the nitrogen (Zhang et al., 2017).

2.5 Field application of *Trichoderma* strains

Field experiments were conducted during *kharif* season for three consecutive years viz., 2021, 2022, and 2023, at the experimental research farm of ICAR-Directorate of Onion and garlic Research (DOGR), Pune, Maharashtra, India. The weather data of 2021, 2022, and 2023 *kharif* onion season were obtained from the ICAR-DOGR automatic weather station installed 50 m away from the experimental field. The potent *Trichoderma* strains, identified through *in vitro* screening, were evaluated under field conditions using a randomized complete block design comprising eleven treatments of *Trichoderma* and untreated control, each replicated thrice. For this study, the *Bhima Super* variety released from ICAR-DOGR, recommended for the *kharif* season, was used. Each *Trichoderma* strain was cultured in potato dextrose broth at $25 \pm 2^\circ\text{C}$ for 36 h. A 100 mL aliquot of the liquid culture, adjusted to 108 cfu/mL, was thoroughly mixed with 50 kg of farmyard

manure (FYM) of nearly uniform composition, achieved through vigorous mixing. The mixture was placed in a rectangular pit ($100 \times 100 \times 100$ cm; $l \times b \times h$) and covered with a polythene sheet, allowing the *Trichoderma* strains to multiply in the FYM over a period of 12 days (Dutta et al., 2024b). The *Trichoderma*-enriched FYM was applied to each plot at a rate of 250 g per plot (8 m^2), corresponding to 250 kg FYM per hectare, as a soil amendment prior to transplanting onion seedlings. Subsequent applications were made at 35, 65, and 95 days after transplanting (DAT). Control plots (T9) received similarly processed FYM without *Trichoderma* inoculation, applied at the same rate and time intervals. A mineral fertilizer dose of 100:50:50:30 kg NPKS per hectare was applied, and standard recommended practices were followed throughout the crop production cycle.

2.6 Anthracnose disease severity, relative water content (RWC), chlorophyll content, biomass, and bulb yield

Anthracnose disease severity was assessed using the 0–9 grade scale for scoring the disease (Dutta et al., 2022), and the Percent Disease Index (PDI) was calculated as described by Wheeler (1969). At 60 days after treatment application, the crop growth parameters, viz., number of leaves, plant height (cm), and pseudostem diameter (mm), were recorded. Total biomass production was also determined from each replicate at 60 DAT (Dutta et al., 2024b). The bulb yield per plot was expressed in t/ha.

At 60 DAT, Leaf relative water content was measured using the following formula;

$$\text{Relative Water Content (RWC)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

The Chlorophyll content was also estimated at 60 DAT using the procedure as described by Arnon (1949). Total biomass production was calculated using the fresh and dry weights of the shoot, root, and bulb at 60 DAT. Total biomass was determined by expressing the normalized weight as described by Dutta et al. (2024b).

2.7 ICBR (incremental cost-benefit ratio) calculation

The incremental cost benefit ratio (ICBR) was calculated for all treatments using formula described by Chejara (2013), i.e., $\text{ICBR} = \frac{\text{Additional income received (from the particular treatment)}}{\text{Additional cost incurred for the particular treatment}}$. The additional income and cost were estimated based on the prevailing market rates.

2.8 Statistical analysis

Analysis of variance (ANOVA) was performed on the dataset to assess the efficacy of *Trichoderma* strains using pooled analysis. The differences in treatment means at $p \leq 0.05$ were computed

using Dunken's Multiple Range Test (DMRT) in SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA). The effect of *Trichoderma* on the suppression of anthracnose-twister disease and supporting bulb yield was studied through Linear Regression and Pearson's correlation analysis using SPSS software. Data from 3 years were pooled, and SD (Standard deviation) values were figured for each treatment to reflect the variability across years and replications. The Canonical Correspondence Analysis (CCA analysis) was performed to link biological responses like PDI with weather parameters using PAST software.

3 Results

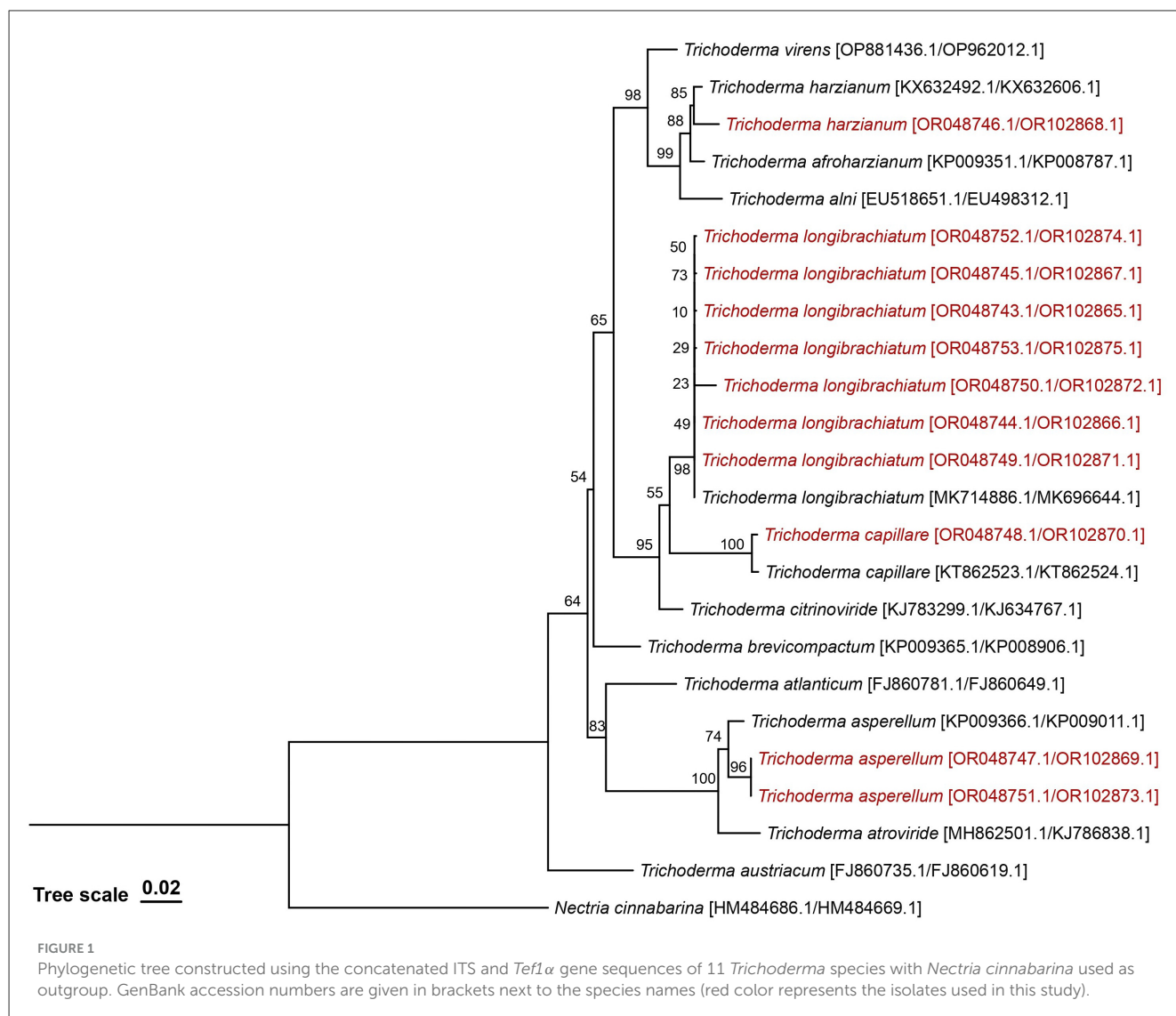
3.1 Morphological and molecular characterization of *Trichoderma* strains

Based on the morphological and molecular characterization, seven out of eleven strains were identified as *T. longibrachiatum* (OGRDT1, OGRDT2, GRDT1, GRDT4, GRDT5, GRDT7, and GRDT8), two as *T. asperellum* (GRDT3 and GRDT6), one as *T. harzianum* (GRDT2) and one as *T. capillare* (OGRDT3)

(Supplementary Tables S1, S2). The ITS and *Tef1 α* gene sequences of eleven strains have been submitted to NCBI GenBank, with the corresponding ITS accessions (OR048743–OR048753) and *Tef1 α* accessions (OR102865–OR102875) listed in Supplementary Table S2. A phylogenetic tree (Figure 1) was constructed using concatenated ITS and *Tef1 α* gene sequences from 11 isolated *Trichoderma* strains, along with reference sequences retrieved from NCBI GenBank. The sequence of *Nectria cinnabarina* was used as outgroup to root the phylogenetic tree. The pure cultures of all eleven species used in this study have been deposited in the International Repository of the ICAR- National Agriculturally Important Microbial Culture Collections (NAIMCC), Mau, Uttar Pradesh, India, and presented in Supplementary Table S2.

3.2 Confrontation assay

All eleven *Trichoderma* strains exhibited antagonistic activity against the plant pathogen *C. gloeosporioides*, ranging from 18.8 to 70.0% (Figure 2). Based on the inhibition percentage,



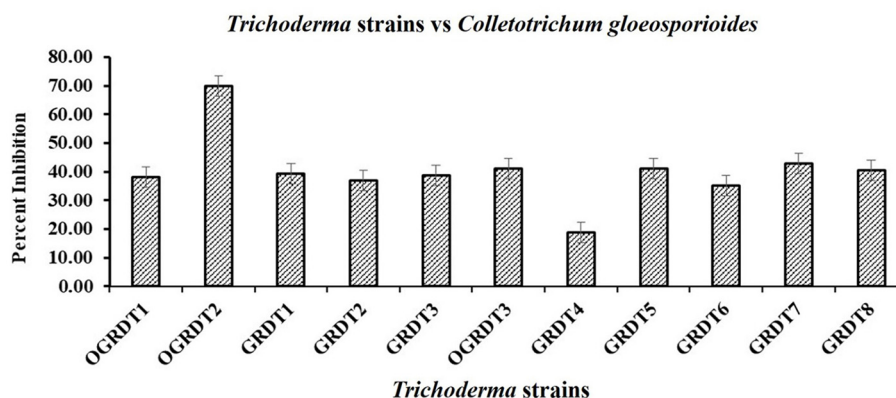


FIGURE 2

Antagonist potential of eleven *Trichoderma* strains against *C. gloeosporioides* with inhibition percentage (7 days after incubation).

the antagonistic activity followed a decreasing trend as *T. longibrachiatum* (OGRDT2) exhibited the highest inhibition (70.0%), followed by *T. longibrachiatum* (GRDT7) (42.9%). A set of strains comprising *T. capillare* (OGRDT3), *T. longibrachiatum* (GRDT5, GRDT8, GRDT1, OGRDT1), and *T. asperellum* (GRDT3) exhibited moderate antagonistic activity, with inhibition percentages ranging between 38.24 and 41.18%. Two strains, *T. harzianum* (GRDT2) and *T. asperellum* (GRDT6) exhibited lower inhibition (36.0%), whereas *T. longibrachiatum* (GRDT4) showed the lowest antagonistic activity (18.82%). Conclusively, the strain *T. longibrachiatum* (OGRDT2) outperformed all other *Trichoderma* strains effectively inhibiting the growth of *C. gloeosporioides* by 70% (Supplementary Figure S2).

3.3 Biochemical characterization

Biochemical analysis for plant growth promotion activity (PGPA) of the *Trichoderma* strains was done, and the strains were assessed for IAA, siderophore, and HCN production; phosphate, zinc, and potassium solubilization; enzymatic activity of amylase and protease; and N₂ fixation (Table 1). Qualitative and quantitative analyses were conducted to determine IAA and siderophore production, and significant differences were observed among all eleven *Trichoderma* strains. A high amount of IAA was produced by OGRDT3 (45.65 µg/mL), whereas GRDT5 produced the lowest (23.52 µg/mL). Similarly, the production of siderophore also differed among the tested *Trichoderma* strains, with the highest siderophore production recorded for OGRDT3 (40.45%), and the lowest for GRDT4 (30.00%). The presence of transparent zones on media containing zinc indicated that the strains could solubilize Zn. The results showed that *T. asperellum* and *T. longibrachiatum* strains formed a prominent transparent zone around the culture, confirming their Zn solubilizing ability. Likewise, three *T. longibrachiatum* strains (OGRDT1, GRDT7, and GRDT8) demonstrated the ability to solubilize phosphorus by forming transparent circles in the medium. In contrast, the remaining strains failed to produce transparent zones around the culture. However, all strains tested negative for potassium

solubilization. On the contrary, all strains tested positive for HCN production as indicated by the change of filter paper color from yellow to orange or reddish brown. All *Trichoderma* strains also showed positive results for amylase and protease activity, as evidenced by the formation of clear zones due to starch and gelatin degradation, respectively. Among *Trichoderma* isolates, nine strains, including *T. longibrachiatum*, *T. harzianum*, *T. capillare* and *T. asperellum*, showed nitrogen fixation in N-free medium.

3.4 Effect of *Trichoderma* strains on growth attributes, physiological responses, biomass and yield parameters

3.4.1 Effect of *Trichoderma* strains on growth attributes

Different *Trichoderma* strains exhibited variable effects on plant growth parameters, including plant height, pseudostem diameter, and number of leaves per plant. Among the *Trichoderma* strains used, maximum plant height (54.97 ± 0.80 and 53.29 ± 0.79 cm), number of leaves per plant (7.89 ± 0.99 and 7.62 ± 0.57), pseudostem diameter (13.96 ± 0.18 and 13.76 ± 0.20 mm) were recorded in OGRDT2 and GRDT2 strains, respectively (Table 2). The *Trichoderma* strain OGRDT2 consistently performed best in terms of plant height, pseudostem diameter, and number of leaves/plant in each experimental season of 2021–23 (The year wise data presented in Supplementary Table S3).

Application of different *Trichoderma* strains significantly influenced root, shoot and bulb biomass accumulation in onion plants. The highest shoot biomass accumulation was recorded for GRDT2 (and OGRDT2 treated plants (Table 2). The pooled analysis of 3 year normalized dry weights of shoot, root, and bulb revealed significantly higher weight for *T. harzianum* (GRDT2) treated plots with normalized dry weight values of $10.82 \pm 0.89\%$, $12.27 \pm 0.11\%$, and $12.90 \pm 0.65\%$ whereas *T. longibrachiatum* (OGRDT2) showed at par results in terms of normalized dry weight (Table 2). Both the strains (OGRDT2 and GRDT2) consistently performed superior in all experimental years

TABLE 1 Evaluation of PGPA traits for *Trichoderma* isolates.

Strain ID	IAA ($\mu\text{g/ml}$)	Siderophore (%)	Phosphate solubilization (mm)	Zinc solubilization (mm)	HCN production	Amylase	Protease	N fixation
OGRDT1	26.96	36.22	3.10	1.10	+	+	+	–
OGRDT2	38.45	38.43	0	1.50	+	+	+	+
GRDT1	45.54	39.20	0	1.05	+	+	+	+
GRDT2	32.10	38.51	0	2.7	+	+	+	+
GRDT3	37.44	36.45	0	4.75	+	+	+	+
OGRDT3	45.65	40.45	1.40	1.70	+	+	+	+
GRDT4	28.84	30.00	0	1.45	+	+	+	+
GRDT5	23.52	31.22	0	1.35	+	+	+	+
GRDT6	33.52	39.40	0	5.60	+	+	+	+
GRDT7	31.17	33.22	3.59	2.35	+	+	+	–
GRDT8	27.13	34.15	2.15	1.50	+	+	+	+

TABLE 2 Effect of application of *Trichoderma* strains on the plant growth of onion (pooled mean of Kharif 2021–2023).

<i>Trichoderma</i> isolates	Plant height (cm)	Pseudostem diameter (mm)	No. of leaves/plant	Normalized dry weight (%)		
				Shoot	Root	Bulb
OGRDT1	46.37 ^{cd} $\pm$ 0.68	11.52 ^b $\pm$ 0.40	6.77 ^a $\pm$ 0.68	7.69 ^b $\pm$ 2.36	7.96 ^b $\pm$ 0.82	9.99 ^b $\pm$ 1.55
OGRDT2	54.97 ^a $\pm$ 0.80	13.96 ^a $\pm$ 0.18	7.89 ^a $\pm$ 0.99	10.39 ^a $\pm$ 1.76	11.86 ^a $\pm$ 2.67	12.21 ^a $\pm$ 0.81
GRDT1	49.93 ^{abc} $\pm$ 2.52	11.98 ^b $\pm$ 0.67	6.90 ^a $\pm$ 0.26	8.03 ^b $\pm$ 0.63	8.72 ^b $\pm$ 0.77	10.47 ^a $\pm$ 0.87
GRDT2	53.29 ^a $\pm$ 0.79	13.76 ^a $\pm$ 0.20	7.62 ^a $\pm$ 0.57	10.82 ^a $\pm$ 0.89	12.27 ^a $\pm$ 0.11	12.90 ^a $\pm$ 0.65
GRDT3	51.37 ^{abc} $\pm$ 1.17	12.56 ^a $\pm$ 0.60	7.23 ^a $\pm$ 0.43	8.88 ^a $\pm$ 0.34	9.94 ^b $\pm$ 0.54	11.11 ^a $\pm$ 0.52
OGRDT3	52.26 ^{ab} $\pm$ 2.89	13.03 ^a $\pm$ 0.73	7.38 ^a $\pm$ 0.47	9.40 ^a $\pm$ 1.47	10.74 ^a $\pm$ 0.23	11.50 ^a $\pm$ 0.73
GRDT4	48.34 ^{bc} $\pm$ 1.83	11.96 ^b $\pm$ 0.38	6.83 ^a $\pm$ 0.11	8.00 ^b $\pm$ 0.06	8.32 ^b $\pm$ 1.45	10.02 ^a $\pm$ 0.92
GRDT5	52.60 ^{ab} $\pm$ 3.11	13.21 ^a $\pm$ 0.56	7.57 ^a $\pm$ 0.10	9.90 ^a $\pm$ 1.94	11.43 ^a $\pm$ 1.05	11.82 ^a $\pm$ 1.49
GRDT6	45.05 ^d $\pm$ 1.96	11.17 ^b $\pm$ 0.49	5.93 ^b $\pm$ 0.13	7.53 ^b $\pm$ 0.67	7.89 ^b $\pm$ 0.30	9.54 ^b $\pm$ 1.05
GRDT7	50.32 ^{abc} $\pm$ 1.57	12.11 ^b $\pm$ 0.96	7.11 ^a $\pm$ 0.23	8.45 ^a $\pm$ 0.95	9.12 ^b $\pm$ 0.43	10.42 ^a $\pm$ 0.58
GRDT8	50.89 ^{abc} $\pm$ 0.76	12.37 ^b $\pm$ 0.56	7.18 ^a $\pm$ 0.71	8.64 ^a $\pm$ 0.49	9.38 ^b $\pm$ 0.56	10.64 ^a $\pm$ 0.98
Untreated control	42.68 ^c $\pm$ 1.90	10.55 ^c $\pm$ 0.04	5.78 ^b $\pm$ 0.15	7.08 ^b $\pm$ 0.87	7.35 ^c $\pm$ 0.44	7.38 ^c $\pm$ 0.34
LSD ($p = 0.05$) (year)	1.27	0.45	0.36	0.76	0.60	0.83
LSD ($p = 0.05$) (treatments)	2.55	0.90	0.71	1.53	1.19	1.66
LSD ($p = 0.05$) (years $\times$ treatments)	4.41	1.55	1.23	2.65	2.06	2.88

Superscript alphabets signify statistically significant grouping of the treatments.

(2021–23) in terms of shoot, root, and bulb biomass accumulation (Supplementary Table S4).

3.4.2 Effect of *Trichoderma* application on physiological responses

A three-year comparison of cumulative RWC values revealed that the *Trichoderma* application enhanced the RWC in the leaves as compared to the untreated control (Table 3). The highest RWC values ($\sim 71.36.0\%$) were observed in plots treated with

GRDT2, OGRDT2, GRDT5, OGRDT3, GRDT3, and GRDT8, which were statistically at par with each other, whereas the lowest ($57.86 \pm 3.07\%$) was recorded in the untreated control. Strains GRDT2 and OGRDT2 consistently performed best across all three experimental years (2021–2023) (Supplementary Table S5). Similarly, substantial differences were recorded in chlorophyll content. Plots treated with OGRDT2 and GRDT2 strains recorded higher total chlorophyll contents (4.1 ± 0.83 mg/mL and 4.1 ± 1.44 mg/mL, respectively), whereas the control exhibited the lowest value (3.3 ± 0.84 mg/mL). All strains induced an increase

TABLE 3 Effect of *Trichoderma* strains on RWC and Chlorophyll in onion leaves (pooled mean of *kharif* 2021–2023).

<i>Trichoderma</i> isolates	Relative water content	Chlorophyll a (mg/mL)	Chlorophyll b (mg/mL)	Total Chlorophyll (mg/mL)
OGRDT1	61.32 ^b ± 3.46	2.8 ± 1.11	0.6 ± 0.09	3.5 ± 1.29
OGRDT2	68.77 ^a ± 3.80	3.2 ± 1.10	0.9 ± 0.22	4.1 ± 0.83
GRDT1	63.91 ^a ± 2.78	3.1 ± 0.55	0.7 ± 0.18	3.7 ± 0.35
GRDT2	71.36 ^a ± 5.41	3.2 ± 0.66	0.8 ± 0.34	4.1 ± 1.44
GRDT3	68.13 ^a ± 3.85	3.1 ± 0.54	0.8 ± 0.14	3.9 ± 0.18
OGRDT3	68.70 ^a ± 3.87	3.1 ± 1.13	0.8 ± 0.03	3.9 ± 1.38
GRDT4	62.41 ^b ± 2.76	2.9 ± 0.55	0.7 ± 0.20	3.6 ± 0.73
GRDT5	68.34 ^a ± 3.77	3.2 ± 0.83	0.8 ± 0.23	4.0 ± 1.05
GRDT6	61.56 ^b ± 3.10	2.7 ± 0.75	0.7 ± 0.06	3.3 ± 0.80
GRDT7	64.83 ^a ± 2.86	3.0 ± 0.30	0.7 ± 0.03	3.8 ± 0.58
GRDT8	65.61 ^a ± 2.87	3.1 ± 0.21	0.8 ± 0.24	3.9 ± 0.62
Untreated control	57.86 ^b ± 3.07	2.7 ± 0.72	0.6 ± 0.09	3.3 ± 0.84
LSD ($p = 0.05$) (year)	2.20	NS	NS	NS
LSD ($p = 0.05$) (treatments)	4.40	NS	NS	NS
LSD ($p = 0.05$) (years × treatments)	7.63	NS	NS	NS

Superscript alphabets signify statistically significant grouping of the treatments.

in total chlorophyll content, indicating a clear positive effect on the chlorophyll levels in the leaf. Moreover, the total chlorophyll content showed a stronger correlation with chlorophyll a than with chlorophyll b.

3.4.3 Effect of *Trichoderma* strains on bulb yield

The *Trichoderma* strains exerted significant and variable effects on bulb yield across three consecutive years, with the highest yield (21.77 t/ha) recorded in 2022 for strain GRDT2, whereas the control plots produced only 16.71 t/ha in the same year (Supplementary Table S7). The results of the pooled analysis presented in Table 4, showed that the highest yield (20.66 ± 1.42 t/ha) was recorded in plots treated with *T. harzianum* (GRDT2) which were statistically at par with *T. longibrachiatum* OGRDT2 (19.21 ± 0.87 t/ha), GRDT5 (18.95 ± 0.61 t/ha), OGRDT3 (18.66 ± 0.36 t/ha), GRDT3 (18.50 ± 0.44 t/ha), GRDT8 (18.35 ± 1.49 t/ha), GRDT7 (18.24 ± 0.74 t/ha) and GRDT1 (18.10 ± 1.14 t/ha) indicating an overall increase of 19.89, 14.04, 12.81, 11.36, 10.62, 9.88, 9.33 and 8.63 %, respectively, over the untreated control (Table 4).

3.5 Effect of *Trichoderma* strains against anthracnose disease of onion

The field efficacy of *Trichoderma* strains against anthracnose of onion was assessed over three consecutive *kharif* seasons (2021–2023) (Supplementary Table S6), and the pooled analysis results are presented in Table 4 indicating the strain-wise PDI recorded.

Overall, all *Trichoderma* strains effectively reduced the disease severity compared to the control. The most effective treatments were *T. harzianum* (GRDT2) and *T. longibrachiatum* (OGRDT2 and GRDT5), which recorded the lowest PDI (36.00 ± 0.98 , 37.06 ± 1.38 and 39.73 ± 1.54 pooled mean) and the highest disease inhibition (42.65, 40.96, and 36.70%). This was followed by OGRDT3, GRDT3, GRDT8, GRDT7, GRDT1, GRDT4 with PDI of 40.81 ± 1.66 , 42.67 ± 0.57 , 45.61 ± 0.91 , 46.59 ± 0.87 , 49.04 ± 3.21 , 49.28 ± 1.14 and corresponding reductions of 34.98, 32.03%, 27.35, 25.77, 21.88, and 21.48%, respectively. In contrast, the highest disease (62.77 ± 0.14 PDI) was observed in the untreated (control) plot.

3.6 Correlation and linear regression analysis between anthracnose-twister disease severity and bulb yield

Correlation between anthracnose-twister disease severity and bulb yield for pooled analysis mean data of three consecutive years showed a strong negative correlation with $r = -0.92$. Linear regression line graphs for each individual year (2021–23) are presented in Supplementary Figure S3. Additionally, the data were exposed the linear regression analysis to determine the influence of disease incidence on bulb yield. The coefficient of determination (R^2) for the pooled data is $R^2 = 0.8643$, showing a high goodness of fit (Figure 3). This indicates that each 1% increase in disease severity corresponded to a reduction of approximately 0.07–0.12 t/ha in bulb yield.

TABLE 4 Effect of *Trichoderma* strains on anthracnose disease severity and yield of onion (pooled mean of *kharif* 2021–2023).

<i>Trichoderma</i> strains	Percent disease index (PDI)	Disease reduction over control (%)	Bulb yield (t/ha)	Increase in yield over control (%)	ICBR
OGRDT1	53.31 (46.92) ^{*c} ± 0.95	15.07	17.12 ^{bc} ± 0.65	3.54	3.00
OGRDT2	37.06 (37.52) ^a ± 1.38	40.96	19.21 ^{ab} ± 0.87	14.04	13.45
GRDT1	49.04 (44.47) ^b ± 3.21	21.88	18.10 ^{ab} ± 1.14	8.63	7.90
GRDT2	36.00 (36.89) ^a ± 0.98	42.65	20.66 ^a ± 1.42	19.89	20.70
GRDT3	42.67 (40.80) ^b ± 0.57	32.03	18.50 ^{ab} ± 0.44	10.62	9.90
OGRDT3	40.81 (39.73) ^b ± 1.66	34.98	18.66 ^{ab} ± 0.36	11.36	10.70
GRDT4	49.28 (44.61) ^b ± 1.14	21.48	17.30 ^{bc} ± 0.55	4.55	3.90
GRDT5	39.73 (39.09) ^a ± 1.54	36.70	18.95 ^b ± 0.61	12.81	12.15
GRDT6	55.97 (48.45) ^c ± 0.57	10.83	16.99 ^{bc} ± 0.17	2.77	2.35
GRDT7	46.59 (43.07) ^b ± 0.87	25.77	18.24 ^{ab} ± 0.74	9.33	8.60
GRDT8	45.61 (42.50) ^b ± 0.91	27.35	18.35 ^{ab} ± 1.49	9.88	9.15
Untreated control	62.77 (52.42) ^a ± 0.14	–	16.52 ^c ± 0.69	–	–
CD @ 5% for years	1.83	–	0.75	–	–
CD @ 5% for treatments	3.65	–	1.49	–	–
CD @ 5% for years × treatments	6.32	–	2.58	–	–

*Figures in parentheses are arcsine-transformed values. Superscript alphabets signify statistically significant grouping of the treatments.

3.7 Effect of weather parameters on anthracnose-twister disease severity

Disease severity varied across cropping seasons, ranging from 21.33 to 42.00 PDI in 2021, 45.19 to 80.00 PDI in 2022, and 41.48 to 66.30 PDI in 2023 (Supplementary Table S6). Rainfall during the *kharif* (July–October) was 275 mm in 2021, 402 mm in 2022, and 266 mm in 2023 (Supplementary file 2). Canonical correspondence analysis (CCA) revealed differential associations of *Trichoderma* strains with total rainfall, relative humidity, and temperature. Strains OGRDT2 and GRDT2 showed strong affinity for high rainfall, humidity, and lower temperatures, indicating their niche preference and proliferation under moderate relative humidity (67–80%) and moderately lower temperatures (17–26°C) conditions (Figure 4). The rainfall obtained during the *kharif* from July to October was 275 mm in 2021, 402 mm in 2022, and 266 mm in 2023 (Supplementary file 2). Interestingly, although rainfall and humidity are known to increase anthracnose-twister incidence, these conditions simultaneously favored OGRDT2 and GRDT2 proliferation (Figure 4), thereby reducing disease pressure and enhancing their biocontrol efficacy. Thus, efficacy of OGRDT2 and GRDT2 was highest in 2021 due to the concurrent presence of moderate disease pressure and favorable conditions for *Trichoderma* strains (OGRDT2 and GRDT2) establishment, since year 2021 received lesser rainfall (275) compared to 2022 (402 mm). Although the recorded rainfall in 2023 was even lower (266 mm), the higher average relative humidity (90%) may have contributed to the inferior performance of OGRDT2 and GRDT2 during that year.

In CCA plot, the years 2021–2023 were positioned near the origin, with year 2022 slightly inclined toward rainfall and humidity, reflecting relatively wetter conditions during

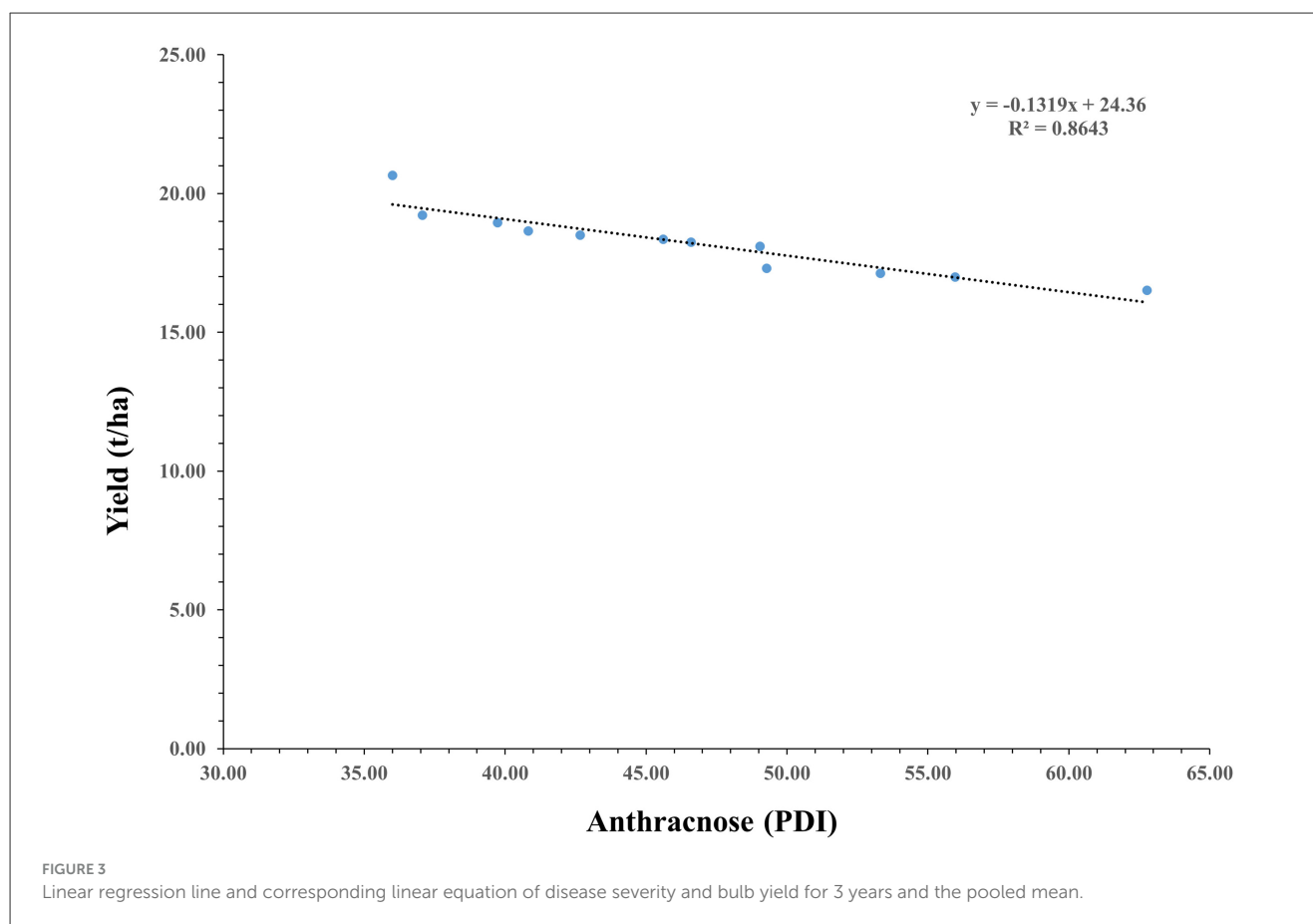
this year (2022) and consequently more PDI during this year. The multivariate analysis (CCA analysis) also revealed correlation amongst weather parameters where average minimum temperature, relative humidity, rainfall, and wind velocity clustered on the right-hand side of the CCA plot, indicating their strong association with higher anthracnose incidence, as elevated humidity and rainfall are known to favor *Colletotrichum* growth (Figure 4). Wind velocity can also be a critical determinant, as higher wind speeds during infestation may facilitate greater spore dissemination. Contrary to this, the weather conditions like higher temperature, more sunshine hours, and lower relative humidity/rainfall are typically unfavorable for anthracnose development.

3.8 Cost-economic analysis of bioagent

Pooled mean data from 2021 to 2023 revealed that the treatments *T. harzianum* (GRDT2) achieved the highest incremental cost-benefit ratio (ICBR) of 20.70, followed by *T. longibrachiatum* (OGRDT2) (13.45). However, the lowest ICBR was recorded for *T. asperellum* (GRDT6) (2.35) due to the lower yields (Table 4). Notably, the input cost of bioagents was lower than that of chemicals, highlighting their economic advantage, particularly for the resource-poor farmers.

4 Discussion

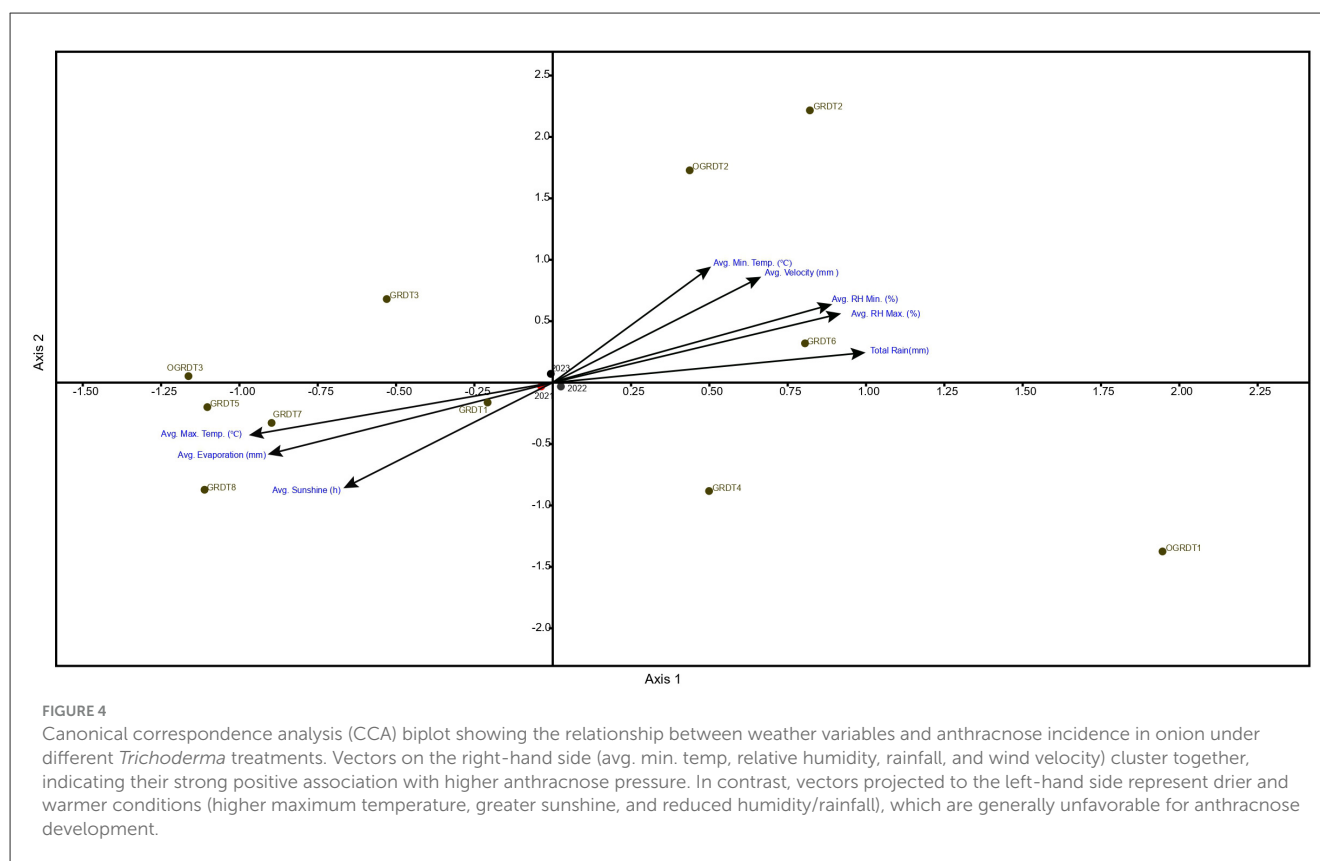
Trichoderma is extensively used in agriculture for its mycoparasitic (direct attack on pathogen hyphae), antibiosis (production of antifungal compounds), and Plant Growth



Promoting Potential (PGPP). This work was aimed to screen the potential of eleven *Trichoderma* strains of Indian origin against the devastating Anthracnose-twister disease of onion. Our results showed that the eleven *Trichoderma* strains tested in this study displayed variable PGPA potential and varying disease reduction efficacy against the Anthracnose pathogen *Colletotrichum gloeosporioides*. The *Trichoderma* strains identification was confirmed initially through morphological (Singh et al., 2015) methods and then through molecular methods (Samuels et al., 2012). Accurate species identification is crucial because different *Trichoderma* species vary significantly in their biocontrol efficacy and PGPA potential, and enzyme production. Based on *Tef1α* gene sequences and morphological characters, seven out of eleven strains were identified as *Trichoderma longibrachiatum*, two as *T. asperellum*, and *T. harzianum*, and *T. capillare*, one each. In this study, *Trichoderma longibrachiatum* emerged as the most frequently isolated species, likely owing to their saprotrophic competitiveness rendered by cellulolytic, chitinolytic, and proteolytic activity and secretion of effector proteins like *Cpe1* and its efficiency as a root colonizer which favors their persistence in rhizosphere/ bulk soils (Wang et al., 2025). The repeated isolation of *Trichoderma longibrachiatum* can also be attributed to its high level of adaptation to edaphic stresses, which supports its persistence even under unfavorable soil conditions (Liu et al., 2023). Samuels et al. (2012) and Alwadai et al. (2024) used the polyphasic approach involving a combination of molecular (ITS

and *Tef1α* gene sequences) and morphological characters to identify all strains of *T. longibrachiatum* due to existing concerns related to the *longibrachiatum* clade. While the ITS region is widely used for fungal identification, it has limitations in resolving closely related species within *Trichoderma*, as ITS sequences often lack sufficient variability to distinguish between phylogenetically proximate taxa (Chaverri et al., 2015). On the other hand, the translation elongation factor 1- α (*Tef1α*) gene provides greater discriminatory power at the species level and is frequently used for higher-resolution phylogenetic inference. Alwadai et al. (2024) further emphasized the use of a polyphasic approach combining morphology (e.g., spore size, colony structure), and molecular phylogenetics (ITS and *tef1α* gene sequences) for identification of *T. longibrachiatum* and *T. harzianum* strains. Furthermore, molecular phylogenetic analyses have revealed that *T. longibrachiatum*, while traditionally considered a single morphological species, represents a genetically diverse complex with cryptic species (Samuels et al., 2012; Alwadai et al., 2024).

The bio-efficacy of *Trichoderma* against plant pathogens has been well documented. To evaluate its antagonistic potential against *C. gloeosporioides*, a dual culture (confrontation) assay was conducted using eleven *Trichoderma* strains. Among them, *T. longibrachiatum* (OGRDT2) showed stronger *in vitro* inhibition, achieving 70% suppression of *C. gloeosporioides* mycelial growth (Figure 2 and Supplementary Figure S2). The results of confrontation assay between *Trichoderma* strains and



Colletotrichum gloeosporioides (the causal agent of anthracnose), clearly indicated that certain *Trichoderma* strains possessed strong inhibitory activity, while others showed only moderate to weak suppression. The strain OGRDT2 exhibiting the highest inhibition (~70%), can also be explored for production of hydrolytic enzymes (chitinases, glucanases), and secretory antifungal metabolites, which rapidly inhibit mycelial growth of *Colletotrichum gloeosporioides* for effective management of anthracnose disease. The other strains showing lower inhibitory activity may lack strong antifungal metabolite production or competitive ability, making them less suitable as stand-alone biocontrol agents. Still, the strain GRDT2 which showed moderate inhibitory activity (40%) against *Colletotrichum gloeosporioides*, in the dual culture assay, it proved equally effective under field conditions, which may be due to triggering the Induced Systemic Resistance (ISR), enabling plants to resist infection more effectively instead of directly inhibiting the mycelial growth (Khan et al., 2023).

The strain specific variations in biocontrol efficacy is known from previous findings, which have highlighted broad spectrum antagonistic potential of *T. longibrachiatum*, *T. asperellum*, and *T. harzianum* against wide range of onion pathogens including *C. gloeosporioides*, *S. cepivorum*, *A. porri*, *S. vesicarium*, *F. oxysporum* f. sp. *cepae*, *F. proliferatum* and several post-harvest pathogens (Cos-kuntuna and Ozer, 2008; Kumar et al., 2012; Prakasam and Sharma, 2012; Ghanbarzadeh et al., 2016; Helman et al., 2019; Rivera-Méndez et al., 2020; Pawar et al., 2020; Camacho-Luna et al., 2023). Likewise, Mishra and Gupta (2012) also highlighted the

antagonistic activity of *T. harzianum*, *T. viride*, *T. hamatum*, *T. koningii*, and *T. virens* against the *S. vesicarium*. The antagonistic activity of these *Trichoderma* strains has been validated under both *in vitro* and *in vivo* conditions, indicating their potential for use in integrated disease management strategies in onion cultivation. The varying potential among *Trichoderma* strains is largely attributed to differences in the production of extracellular cell wall-degrading enzymes (like chitinases, glucanases, and proteases), which play a key role in degrading the cell walls of the fungal pathogens. Additionally, differential production of secondary metabolites, particularly triterpenes, is linked to the activity of the ERG-1 gene encoding squalene epoxidase, an essential enzyme in the sterol/triterpene biosynthesis pathway. These mechanisms collectively contribute to the strain specific efficacy observed in *Trichoderma* mediated fungal suppression (Rai et al., 2016).

In this study, all eleven *Trichoderma* isolates were evaluated for plant growth-promotion, including the direct mechanism based attributes like production of IAA, siderophore, HCN, and phosphate, zinc, potassium solubilization, N fixation and indirect mechanism traits like activity of amylase, protease, and cellulase. The strains classified as *T. longibrachiatum*, varied in IAA and siderophore production (Table 1), confirming that PGP traits are strain-dependent. These findings underscore the importance of selecting specific strains for optimal crop benefits (Pedrero-Mendez et al., 2021). All *Trichoderma* strains demonstrated the ability to solubilize zinc however, none showed potassium solubilization activity. Notably, only three strains of *T. longibrachiatum* (OGRDT1, GRDT7, and GRDT8) were

capable of solubilizing phosphate. Additionally, all tested strains exhibited positive reactions for HCN production and Nitrogen fixation, highlighting their plant growth-promoting potential (Singh et al., 2017, 2019; Singh et al., 2020; Chouhan, 2022; Alwadai et al., 2024). All *Trichoderma* strains used in this study produced protease and amylase lytic enzymes, enabling them to degrade pathogen cell walls. The production of these enzymes by *Trichoderma* spp. has been previously documented (Harman and Shores, 2007; Singh et al., 2013; Keswani et al., 2014; Singh et al., 2019) and contributes to improved disease management.

In this study, *Trichoderma* application significantly enhanced overall crop biomass (Table 2) and bulb yield (Table 4), with *T. harzianum* and *T. longibrachiatum* strains showing the most pronounced effects. These treatments performed significantly better than the untreated control and were at par with each other in pooled data, particularly improving plant height and pseudostem diameter (Table 2), which are the key indicators of onion health and productivity. These results align with previous findings by Metwally and Al-Amri (2020) who reported increased plant height and neck diameter of onion plants due to the application of *Trichoderma viride*. Similarly, Prakasam and Sharma (2012) also reported that *T. harzianum* and *T. viride* application enhanced Plant height and leaf number in onion. A related study by Vojnović et al. (2023) found that T3 significantly increased bulb yield by approximately 24.3% over the control with application of *Trichoderma* sp. through foliage spray.

In addition to growth parameters, this study also assessed the canopy traits, including leaf number per plant (Table 2), relative water content, and chlorophyll content (Table 3). A normal increase in leaf number was recorded after *Trichoderma* application, which might be attributed to enhanced nutrient uptake, stimulation of growth hormones, and improved root health (Harman et al., 2004). RWC improved across treatments, with notable increases observed in the pooled data (Table 3). The strains *T. harzianum* and *T. longibrachiatum* were particularly effective in improving chlorophyll content, surpassing the levels observed in the control treatment. Among the *Trichoderma* strains tested, *T. harzianum* and *T. longibrachiatum* significantly enhanced chlorophyll content, resulting in higher levels than the untreated control. Since chlorophyll is vital for photosynthesis and biomass accumulation (Simkin et al., 2022), this increase suggests improved physiological performance. These findings align with previous studies demonstrating *T. asperellum* application improved chlorophyll and carotenoid contents in onions (Helman et al., 2019; Metwally and AL-AMri 2020; Hernandez et al., 2023). These cumulative results support the role of *Trichoderma* in enhancing photosynthetic activity, ultimately contributing to improved plant growth and productivity under field conditions (Zhou et al., 2020; Mergawy et al., 2022).

In our study, *Trichoderma* strain OGRDT2 not only inhibited the growth of *C. gloeosporioides* but also enhanced plant growth parameters by attaining better root, shoot, and leaf biomass accumulation (Table 2). Even though, the strain OGRDT2 was positive for HCN production, amylase and protease activity which might render this strain biocontrol potential but the strain was not adjudged best in terms of other PGPA traits

like IAA production (38.45 µg/ml), % siderophore production (38.43%), and Zinc solubilization (1.5 mm) along with the other better performing strain GRDT2 (Table 1). Our data pointed out that the strain that showed the best PGPA traits *in vitro* did not perform best in the actual field experiments in terms of plant biomass and bulb yield enhancement. Our findings are in agreement with those of Alwadai et al. (2024) who tested eight different *Trichoderma* strains (*T. lixii*, *T. harzianum*, *T. koningii*, *T. virens*, and *T. longibrachiatum*) for plant-growth-promoting traits *in vitro* but the presence of growth-promoting traits *in vitro* did not show direct correspondence to plant growth promotion of tomato plants. The absence of a direct correspondence between PGPA traits and field performance of the *Trichoderma* strains can be attributed to multiple factors, including strain-specific variation in root colonization efficiency, environmental stability, rhizosphere competence, and interactions with the native soil microbiota. Root colonization ability is both strain- and host-dependent, with different *Trichoderma* strains showing variable persistence and root association across plant species (Cripps-Guazzone et al., 2025). Consistent with this, several strains in our study that displayed strong PGPA traits *in vitro* may have failed to effectively colonize onion roots, thereby limiting their performance under field conditions. Therefore, employing the population density-based assessments (colony forming units per gram of rhizosphere soil), confocal laser scanning microscopy (CLSM) or scanning/transmission electron microscopy (SEM/TEM) based approaches may help to link rhizosphere competence more directly with field performance of *Trichoderma* strains. Furthermore, the edaphic factors like soil temperature, aeration, moisture, pH, salinity, and nutrient regime also strongly influence the growth, sporulation, metabolite secretion, and reproductive potential of the strains (Poppeliers et al., 2023). Field microclimates are often quite different from lab culture condition (25–35°C, moderate pH), influencing the root colonization of the strains. The interaction of applied *Trichoderma* strains with native soil microbiota remains an important determinant of strain successful determinant as native communities may outcompete the applied strain leading to inoculant functional attenuation (Causevic et al., 2024; Cavalcante et al., 2025).

Our findings revealed that the increased bulb yield observed with *T. harzianum* was positively influenced by root development. The fungus is known to produce phytohormones such as indole-3-acetic acid and auxin-like metabolites and also the reduced ethylene levels through ACC deaminase activity, which stimulate root elongation (Wang et al., 2024). Improved root systems not only facilitate more efficient acquisition of water and nutrients but also contribute to higher biomass accumulation and ultimately greater yield of the crop. Over three consecutive experimental years, *T. harzianum* GRDT2 consistently improved onion yield, averaging 20.66 ± 1.42 t/ha, being statistically at par with *T. longibrachiatum* OGRDT2 (19.21 ± 0.87 t/ha) against control plants (16.52 ± 0.69 t/ha), confirming the beneficial effects of *Trichoderma* application. Furthermore, cost economics (ICBR) revealed that the *Trichoderma* application provided higher returns due to its low cost and effective protection (Table 4). *T. harzianum* (GRDT2) supported the highest incremental cost-benefit and marginal rate

of return, highlighting its economic advantage, particularly for resource-poor farmers. These findings are in agreement with earlier studies highlighting the cost effectiveness of *Trichoderma* based biocontrol strategies in various cropping systems (Sharma et al., 2011; Prasad and Anwar, 2020; Sultana et al., 2021). The integration of *Trichoderma* not only improves crop productivity and health but also contributes to sustainable and economically feasible disease management.

Our field trials demonstrated that anthracnose-twister disease reduction was highest in *T. harzianum* (GRDT2) and *T. longibrachiatum* (OGRDT2) treated plots, identifying these as the most effective strains among those tested (Table 4). Beyond disease suppression, these strains significantly promoted plant growth. Our results align with those of Alberto and Perez (2020), who reported that *Trichoderma* spp. effectively reduce anthracnose-twister disease incidence. Similarly, Manthasha et al., 2022 also demonstrated effective control of onion twister disease using a combination of *Trichoderma* and *Pseudomonas* spp., while Prakasam and Sharma (2012) highlighted the efficacy of *T. harzianum* against onion purple blotch and basal rot. The disease suppressive effects of *Trichoderma* are attributed to its multifaceted mechanisms, including antifungal metabolites, mycoparasitism, competition for nutrients and space, and induced systemic resistance in the host (Elad, 2000; Solanki et al., 2011). Few other studies have identified *T. virens* as highly effective against *A. porri* (Imtiaj and Lee, 2008) and *T. asperellum* against *S. cepivorum* (Alvarado-Marchena and Rivera-Mendez, 2016; Helman et al., 2019).

The disease severity, in general, is negatively correlated with bulb yield; a trend was also observed in our study (Figure 3). Over 3 years, the *Trichoderma* strains GRDT2 and OGRDT2 consistently showed a strong negative correlation between anthracnose-twister disease severity and bulb yield. As the disease advanced, a significant decline in bulb yield was recorded. Linear Regression analysis quantified this relationship, indicating a yield reduction of 0.07 to 0.12 tons for every one percent increase in disease severity (Tanya et al., 2020; Dias et al., 2016; Rajput et al., 2022). Collectively, these results confirm *T. harzianum* and *T. longibrachiatum* as the most potent biocontrol agents against anthracnose-twister of onion under field conditions.

Our findings also highlight the impact of weather on anthracnose-twister development, with notable variations in disease progression among *Trichoderma* strains. Disease progression was consistently lowest in strains GRDT2 and OGRDT2 treated plots, followed by GRDT5 and OGRDT3. The disease typically peaks between August and September, coinciding with favorable weather conditions, the crop's vulnerable stage, and peak pathogen activity, resulting in sustainable yield loss. This highlights the importance of timely disease management strategies that interrupt the overlap between peak disease pressure and the crop's most susceptible stage. The onset and severity of anthracnose-twister are strongly influenced by the interaction of three key factors: conducive environmental conditions, a susceptible host, and a virulent pathogen. Notably, the 2022 season experienced more favorable conditions for disease development compared to 2021 and 2023, leading to significantly higher disease severity and yield loss that year.

5 Conclusion

Our study highlights the beneficial effects of *Trichoderma* on onion crop health, bulb yield, and anthracnose-twister disease management, reinforcing its role as a valuable bio-stimulant. Among the evaluated strains in our study, GRDT2 (*T. harzianum*) showed highest bulb yield and thereby higher incremental cost-benefit ratio (ICBR) of 20.70 adjudged to be best for *kharif* onion cultivation. The strain OGRDT2 (*T. longibrachiatum*) exhibited the highest potential for enhancing green onion production and reducing the anthracnose-twister supporting ICBR (13.45). Therefore, based on the above findings these two *Trichoderma* strains namely OGRDT2 and GRDT2 could be exploited for the use by farming community as these strains recorded higher green onion (OGRDT2) and bulb yield (GRDT2) production during the *kharif* season while managing the anthracnose-twister disease.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

RD: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. KJ: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. SK: Visualization, Writing – original draft, Writing – review & editing. PS: Visualization, Writing – review & editing. RA: Visualization, Writing – review & editing. VM: Funding acquisition, Visualization, Writing – review & editing. MS: Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This research was supported by the Indian Council of Agricultural Research, Department of Agricultural Research and Education, Government of India through its in-house projects (IXX16540 & IXX16074) implemented at ICAR-Directorate of Onion and Garlic Research, Pune, MH, India.

Acknowledgments

The authors gratefully acknowledge the Indian Council of Agricultural Research and ICAR-DOGR, Pune, for funding support and providing the necessary facilities to conduct the research. The authors are also grateful to those who directly or indirectly supported this work and the development of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

References

- Abdel, R. A., and Shearer, C. A. (2002). Extracellular enzyme production by freshwater ascomycetes. *Fungal Div.* 11, 1–19.
- Abdullah, N. S., Doni, F., Mispan, M. S., Saiman, M. Z., Yusuf, Y. M., Oke, M. A., et al. (2021). Harnessing *Trichoderma* in agriculture for productivity and sustainability. *Agronomy* 11:2559. doi: 10.3390/agronomy11122559
- Alberto, R. T. (2014). Pathological response and biochemical changes in *Allium cepa* L. (bulb onions) infected with anthracnose-twister disease. *Pl. Pathol. Quar.* 4, 23–31. doi: 10.5943/ppq/4/1/4
- Alberto, R. T., and Aquino, V. M. (2010). Characterization of *Colletotrichum gloeosporioides* (Penzig) Penzig and Sacc. (anthracnose) and *Gibberella moniliformis* Wineland (twister) infecting onions in the Philippines. *Asia Life Sci.* 19, 23–58.
- Alberto, R. T., and Perez, P. M. (2020). Development of integrated disease management program against Anthracnose-Twister (*Colletotrichum gloeosporioides*-*Gibberella moniliformis*) disease of onion (*Allium cepa*). *Pl. Pathol. and Quar.* 10, 111–119. doi: 10.5943/PPQ/10/1/13
- Alvarado-Marchena, L., and Rivera-Mendez, W. (2016). Molecular identification of *Trichoderma* spp. in garlic and onion fields and in vitro antagonism trials on *Sclerotium cepivorum*. *Rev. Brasil. Ciênc. Solo* 40:e0150454. doi: 10.1590/18069657rbcs20150454
- Alwadai, A. S., Al Wahibi, M. S., and Alsayed M. F. (2024). Molecular characterization of plant growth-promoting *Trichoderma* from Saudi Arabia. *Sci. Rep.* 14:23236. doi: 10.1038/s41598-024-73762-5
- Arnon, D. L. (1949). A copper enzyme is isolated chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24, 1–15. doi: 10.1104/pp.24.1.1
- Bakker, A. W., and Schippers, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and pseudomonas SPP-mediated plant growth-stimulation. *Soil Bio. Biochem.* 19, 451–457. doi: 10.1016/0038-0717(87)90037-X
- Benke A. P., Mahajan, V., Manjunathagowda D. C., and Mokat D. N. (2022). Interspecific hybridization in *Allium* crops: status and prospectus. *Gen. Resou. Crop Evol.* 69, 1–9. doi: 10.1007/s10722-021-01283-5
- Bui, T. T., Nguyen, D. K., and Tran, Q. T. (2019). Identification of shallot pathogens in Vinh Châu town of Sóc Trăng province. *J. Innov. Sust. Develop.* 11, 11–18. doi: 10.22144/ctu.jen.2019.033
- Camacho-Luna, V., Pizar-Quiroz, A. M., Rodríguez-Hernández, A. A., Rodríguez-Monroy, M., and Sepúlveda-Jiménez, G. (2023). *Trichoderma longibrachiatum*, a biological control agent of *Sclerotium cepivorum* on onion plants under salt stress. *Biol. Cont.* 180:105168. doi: 10.1016/j.biocontrol.2023.105168
- Causevic, S., Dubey, M., Morales, M., Salazar, G., Sentchilo, V., Carraro, N., et al. (2024). Niche availability and competitive loss by facilitation control proliferation of bacterial strains intended for soil microbiome interventions. *Nat. Commun.* 15:2557. doi: 10.1038/s41467-024-46933-1
- Cavalcante, A. L. A., Negreiros, A. M. P., Melo, N. J. D. A., Santos, F. J. Q., Soares Silva, C. S. A., Pinto, P. S. L., et al. (2025). Adaptability and sensitivity of *Trichoderma* spp. isolates to environmental factors and fungicides. *Microorganisms* 13:1689. doi: 10.3390/microorganisms13071689
- Chaverri, P., Branco-Rocha, F., Jaklitsch, W., Gazis, R., Degenkolb, T., and Samuels, G. J. (2015). Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. *Mycologia* 107, 558–590. doi: 10.3852/14-147
- Chawda, H. T., and Rajasab, A. H. (1996). Onion anthracnose disease symptoms: a review. *Onion Newslitt. Trop.* 7, 82–84.
- Chejara, B. K. (2013). *Studies on Gram Pod Borer, Helicoverpa armigera (hub.) on Chickpea and its Control With Insecticides and Biopesticides* (M.Sc. Thesis). Jabalpur: Jawaharlal Nehru Krishi Vishwavidyalaya.
- Chethana, B., Ganeshan, G., Rao, A. S., and Bellishree, K. (2012). *In vitro* evaluation of plant extracts, bioagents and fungicides against *Alternaria porri* (Ellis) Cif., causing purple blotch disease of onion. *Pest. Manag. Hortic. Ecosyst.* 18, 194–198.
- Chouhan, G. K. (2022). “Exploration of plant growth-promoting rhizobacteria (PGPR) for improving productivity and soil fertility under sustainable agricultural practices,” in Singh, U. B., Rai, J. P., Sharma, A. K., editors. *Re-visiting the Rhizosphere Eco-system for Agricultural Sustainability*. Rhizosphere Biology (Singapore: Springer).
- Cos-kuntuna, A., and Ozer, N. (2008). Biological control of onion basal rot disease using *Trichoderma harzianum* and induction of antifungal compounds in onion set following seed treatment. *A. Crop Prot.* 27,330–336. doi: 10.1016/j.cropro.2007.06.002
- Cripps-Guazzone, N., Ridgway, H. J., Condron, L. M., McLean, K. L., Stewart, A., and Jones, E. E. (2025). Isolate and plant host specificity of rhizosphere competence in *Trichoderma* species. *Fungal Biol.* 129:101554. doi: 10.1016/j.funbio.2025.101554
- da Costa, A. C., de Miranda, R. F., Costa, F. A., and Ulhoa, C. J. (2021). Potential of *Trichoderma piluliferum* as a biocontrol agent of *Colletotrichum musae* in banana fruits. *Biocat. Agric. Biotechnol.* 34:102028. doi: 10.1016/j.bcab.2021.102028
- da Silva Pereira, L., Salomón, A. L., Bermudez, V. A. C., and Rius, S. P. (2025). Bioactive proteins from *Trichoderma* for the control of Fusarium: evaluation of antagonist efficacy and potential for agronomic application. *Biocat. Agric. Biotechnol.* 67:103628. doi: 10.1016/j.bcab.2025.103628
- Dhaked, B. S., Triveni, S., Subhash, R., and Padmaja, G. (2017). Isolation and screening of potassium and zinc solubilizing bacteria from different rhizosphere soil. *Int. J. Curr. Microbiol. App. Sci.* 6, 1271–1281. doi: 10.20546/ijcmas.2017.608.154
- Dias, M. D., Valdeci, F. P., and Adalberto, C. C. (2016). Impact of anthracnose on the yield of soybean subjected to chemical control in the north region of Brazil. *Summa Phytopathol.* 42, 18–23. doi: 10.1590/0100-5405/2114
- Dutta, R., Jayalakshmi, K., Nadig, S. M., Manjunathagowda, D. C., Gurav, V. S., and Singh, M. (2022). Anthracnose of onion (*Allium cepa* L.): a twister disease. *Pathogens* 11:884. doi: 10.3390/pathogens11080884
- Dutta, R., Jayalakshmi, K., Satish, K., Radhakrishna, A., Manjunathagowda, D. C., Nadig, S. M., et al. (2024a). Insights into the cumulative effect of *Colletotrichum gloeosporioides* and *Fusarium acutatum* causing anthracnose-twister disease complex of onion. *Sci. Rep.* 14:9374. doi: 10.1038/s41598-024-59822-w
- Dutta, R., Kumar, S., Jayalakshmi, K., Radhakrishna, A., Bhagat, K., Manjunathagowda, D. C., et al. (2024b). Potential of *Trichoderma* strains to positively modulate plant growth processes and bulb yield in Rabi onion. *Front. Sustain. Food Syst.* 8:1427303. doi: 10.3389/fsufs.2024.1427303

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2025.1663405/full#supplementary-material>

- Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protec.* 19, 709–714. doi: 10.1016/S0261-2194(00)00094-6
- El-Saadony, M. T., Saad, A. M., Soliman, S. M., Salem, H. M., Ahmed, A. I., Mahmood, M., et al. (2022). Plant growth-promoting microorganisms as biocontrol agents of plant diseases: mechanisms, challenges and future perspectives. *Front. Plant Sci.* 13:923880. doi: 10.3389/fpls.2022.923880
- Ezebo, R. O., and Okigbo, R. N. (2024). Biological control of post-harvest rot in water yam (*Dioscorea alata* L.) using antagonistic fungi. *Int. J. Pathog. Res.* 13, 101–116. doi: 10.9734/ijpr/2024/v13i6325
- FAO (2025). *FAOSTAT Statistical Database*. Rome: Food and Agriculture Organization of the United Nations. Available online at: <https://www.fao.org/faostat/> (accessed August 18, 2025).
- Galván, G. A., Wietsma, W. A., Putrasamedja, S., Permadi, A. H., and Kik, C. (1997). Screening for resistance to anthracnose (*Colletotrichum gloeosporioides* Penz) in *Allium cepa* and its wild relatives. *Euphytica* 95, 173–178. doi: 10.1023/A:1002914225154
- Ghanbarzadeh, B., Safaei, N., Mohammadi E. G., Younes R. D., and Fatemeh, K. (2016). Biological control of Fusarium basal rot of onion using *Trichoderma harzianum* and *Glomus mosseae*. *J. Crop Prot.* 5, 359–368. doi: 10.18869/modares.jcp.5.3.359
- Glickmann, E., and Dessaux, Y. (1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Appl. Environ. Microbiol.* 61, 793–796. doi: 10.1128/aem.61.2.793-796.1995
- Gopal (2015). Onion research in India: status and challenges. *Progr. Hortic.* 47, 1–19. doi: 10.5958/2249-5258.2015.00001.9
- Guarro, J. T., Svidinski, E., Zaror, L., Forjaz, M. H., Gene, J., Fischman, O. (1998). Subcutaneous hyalohyphomycosis caused by *Colletotrichum gloeosporioides*. *J. Clin. Microbiol.* 36, 3060–3065. doi: 10.1128/JCM.36.10.3060-3065.1998
- Guzman-Guzman, P., Porras-Troncoso, M. D., Olmedo-Monfil, V., and Herrera Estrella, A. (2019). *Trichoderma* species, versatile plant symbionts. *Phytopathology* 109:616. doi: 10.1094/PHYTO-07-18-0218-RVW
- Han, S., Chi, Y., Liu, L., and Yi, H. (2013). Biological characteristics of *Trichoderma* strain. *Chinese Agric. Sci. Bull.* 29, 37–41 (in Chinese). doi: 10.11924/j.issn.1000-6850.2013-1038
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. (2004). *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* 2, 43–56. doi: 10.1038/nrmicro797
- Harman, G. E., and Shores, M. (2007). “The mechanisms and applications of opportunistic plant symbionts,” in Vurro, M., Gressel, J., eds. *Novel Biotechnologies for Biocontrol Agent Enhancement and Management* (Amsterdam: Springer), 131–153.
- Helman, Z. S. D., Palacios-P. E., Rodríguez-H. A., Melchor, D., Rodríguez, M., and Mario J. G. (2019). *Trichoderma asperellum*, a potential biological control agent of *Stemphylium vesicarium*, on onion (*Allium cepa* L.). *Biol. Cont.* 140, 104–105. doi: 10.1016/j.bioccontrol.2019.104105
- Hernandez, R. A., Herrera-A. M., Zapata-S D. H., Becerra M. E., Rodríguez M. M., and Sepúlveda J. G. (2023). *Trichoderma asperellum* promotes the development and antioxidant activity of white onion (*Allium cepa* L.) plants. *Hortic. Environ. Biotechnol.* 64, 25–39. doi: 10.1007/s13580-022-00467-x
- Intiaj, A., and Lee, T. (2008). Antagonistic effect of three *Trichoderma* species on the *Alternaria porri* pathogen of onion Blotch. *World J. Agri.Sci.* 4, 13–17.
- Jaiswal, A. K., Mengiste, T. D., Myers, J. R., Egel D. S., and Hoag, L. A. (2020). Tomato domestication attenuated responsiveness to a beneficial soil microbe for plant growth promotion and induction of systemic resistance to foliar pathogens. *Front. Microbiol.* 11:604566. doi: 10.3389/fmicb.2020.604566
- Jukes, T. H., and Cantor, C. R. (1969). *Evolution of Protein Molecules*. New York, NY: Academic Press, 21–132.
- Kale, R. B., Gavhane, A. D., Thorat, V. S., Gadge, S. S., Wayal S. M., Gaikwad S. Y., et al. (2024). Efficiency dynamics among onion growers in Maharashtra: a comparative analysis of drip irrigation adopters and non-adopters. *BMC Plant Biol.* 24:237. doi: 10.1186/s12870-024-04875-2
- Kalyani, G., Balsubramanian, S., and Chandra, P. (2014). Plant based butters: review. *J. Food Sci. Tech.* 52, 3965–3976. doi: 10.1007/s13197-014-1572-7
- Keswani, C., Mishra, S., Sarma B. K., Singh S. P., and Singh H. B. (2014). Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. *App. Microb. Biotechnol.* 98, 533–544. doi: 10.1007/s00253-013-5344-5
- Khan, R. A. A., Najeeb, S., Chen, J., Wang, R., Zhang, J., Hou, J., et al. (2023). Insights into the molecular mechanism of *Trichoderma* stimulating plant growth and immunity against phytopathogens. *Physiol. Plant.* 175:e14133. doi: 10.1111/ppl.14133
- Kim, Y. S., Balaraju, K., and Jeon, Y. H. (2016). Biological control of apple anthracnose by *Paenibacillus polymyxa* APEC128, an antagonistic rhizobacterium. *Plant Path.* 32, 251–259. doi: 10.5423/PPJ.OA.01.2016.0015
- Kumar, K., Amaresan, N., Bhagat, S., Madhuri, K., and Srivastava, R. C. (2012). Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. *Indian J. Microbiol.* 52:3. doi: 10.1007/s12088-011-0205-3
- Kumar, M. A., and Sharma, P. (2016). Morphological characterization of biocontrol isolates of *Trichoderma* to study the correlation between morphological characters and biocontrol efficacy. *Int. Lett. Natural Sci.* 55, 57–67. doi: 10.56431/p-9s33dk
- Kumar, S., Raj, S., Maurya, D. K., Kashyap, P. L., Srivastava, A. K., and Anandaraj, M. (2013). Cross-species transferability of microsatellite markers from *Fusarium oxysporum* for the assessment of genetic diversity in *Fusarium udum*. *Phytoparasitica* 41, 615–622. doi: 10.1007/s12600-013-0324-y
- Li, X., Liao, Q., Zeng, S., Wang, Y., and Liu, J. (2025). The use of *Trichoderma* species for the biocontrol of postharvest fungal decay in fruits and vegetables: challenges and opportunities. *Postharvest Biol. Technol.* 219:113236. doi: 10.1016/j.postharvbio.2024.113236
- Liu, T. X. (2004). Seasonal population dynamics, life stage, composition of T tabaci (*Thysanoptera: Thripidae*) and predacious natural enemies on onions in south Texas. *Southwest Entomol.* 29, 127–135.
- Liu, Z., Xu, N., Pang, Q., Khan, R. A. A., Xu, Q., Wu, C., et al. (2023). A salt-tolerant strain of *Trichoderma longibrachiatum* HL167 is effective in alleviating salt stress, promoting plant growth, and managing Fusarium wilt disease in cowpea. *J. Fungi* 9:304. doi: 10.3390/jof9030304
- Lorini, I., Torres, L., and Guimaraes, D. R. (1986). Study about population fluctuations of *T. tabaci* in an onion crop. *Int. J. Entomol.* 62, 1–9.
- Louden, B. C., Haarmann, D., and Lynne, A. M. (2011). Use of blue agar CAS assay for siderophore detection. *J. Microb. Bio. Edu.* 12, 51–53. doi: 10.1128/jmbe.v12i1.249
- Machuca, A., and Milagres, A. M. F. (2003). Use of CAS-agar plate modified to study the effect of different variables on the siderophore production by *Aspergillus*. *Lett. App. Microbiol.* 36, 177–181. doi: 10.1046/j.1472-765X.2003.01290.x
- Manthesha, H. D., Mallikarjun, K., Yenjerappa, S. T., Aswathanarayana, D. S., and Vikas, K. (2022). Management of onion twister disease under field condition. *The Pharma Inn. J.* 1, 551–555.
- Manzar, N., Kashyap, A. S., Goutam, R. S., Rajawat, M. V. S., Sharma, P. K., Sharma, S. K., et al. (2022). *Trichoderma*: advent of versatile biocontrol agent, its secrets and insights into mechanism of biocontrol potential. *Front. Sust.* 14:12786. doi: 10.3390/su141912786
- McKenzie, C. L., Cartwright, B., Miller, M. E., and Edelson, J. V. (1993). Injury to onions by *Thrips tabaci* (Thysanoptera: Thripidae) and its role in the development of purple blotch. *Environ. Entomol.* 22, 1266–1277. doi: 10.1093/ee/22.6.1266
- Meera, T., and Balabaskar, P. (2012). Isolation and characterization of *Pseudomonas fluorescens* from rice fields. *Int. J. Food Agri. Vet. Sci.* 2, 113–120.
- Melo, I. G., and Costa, C. P. (1983). Estimativas de parametros geneticos em cebola (*Allium cepa* L.) populacoes Pira Rosa e Pira Tropical, para o car'ater resistencia a *Colletotrichum gloeosporioides* Penz. *Summa Phytopath.* 9, 207–213.
- Mergawy, M. M., Metwaly, H. A., and Shoeip A. M. (2022). Evaluation of the efficacy of some bioagents accompanied with bio-and mineral fertilizers in controlling early blight of tomato and improvement yield. *Egypt. J. Phytopath.* 50, 31–50. doi: 10.21608/ejp.2022.133129.1059
- Metwally, R. A., and Al-Amri, S. M. (2020). Individual and interactive role of *Trichoderma viride* and arbuscular mycorrhizal fungi on growth and pigment content of onion plants. *Lett. App. Microbiol.* 70, 79–86. doi: 10.1111/lam.13246
- Mishra, R. K., and Gupta, R. P. (2012). *In vitro* evaluation of plant extracts, bio-agents and fungicides against purple blotch and *Stemphylium* blight of onion. *J. Med. Plant Res.* 6, 5840–5843. doi: 10.5897/JMPRI0-246
- Musheer, N., and Ashraf, S. (2017). Effect of *Trichoderma* spp. on *Colletotrichum gloeosporioides* Penz and Sacc. causal organism of turmeric leaf spot disease. *Trends Biosci.* 10, 9605–9608.
- Paibomesai, M., Celetti, M., and Tesfaendrias, M. (2012). Update on *Stemphylium* leaf blight of onions in Ontario. *Hort. Matt.* 12, 11–12.
- Pawar, S. B., Bhosale, S. B., and Chavan, A. M. (2020). Antagonistic activity of *Trichoderma harzianum* on fungi of onion (*Allium cepa* L.). *Purakala* 31, 710–714.
- Pedrero-Mendez, A., Insuasti, H. C., Neagu, T., Illescas M., Rubio M. B., and Monte, E. (2021). Why is the correct selection of *Trichoderma* strains important? The case of wheat endophytic strains of *T. harzianum* and *T. simmonsii*. *J. Fungi.* 7:1087. doi: 10.3390/jof7121087
- Poppeliers, S. W., Sánchez-Gil, J. J., and de Jonge, R. (2023). Microbes to support plant health: understanding bioinoculant success in complex conditions. *Curr. Opin. Microbiol.* 73:102286. doi: 10.1016/j.mib.2023.102286
- Prakasam, V., and Sharma, P. (2012). *Trichoderma harzianum* (Th-3) a potential strain to manage the purple blotch of onion (*Allium cepa* L.) caused by *Alternaria porri* under North Indian Plains. *J. Agric. Sci.* 4, 266–272. doi: 10.5539/jas.v4n10p266
- Prasad, R. D., and Anwar, M. (2020). Economic analysis of biocontrol-based integrated management of root rot in chickpea using *Trichoderma* spp. *J. Biol. Control.* 34, 129–134.
- Priya, R. U., Sataraddi, A., and Darshan, S. (2015). Efficacy of non-systemic and systemic fungicides against purple blotch of onion (*Allium cepa* L.) caused by *Alternaria porri* (Ellis) Cif. *Int. J. Recent Sci. Res.* 6, 6519–6521.

- Promwee, A., Issarakraisila, M., Intana, W., Chamswang, C., and Yenjit, P. (2014). Phosphate solubilization and growth promotion of rubber tree (*Hevea brasiliensis* Muell. Arg.) by *Trichoderma* strains. *J. Agric. Sci.* 6:8. doi: 10.5539/jas.v6n9p8
- Rai, S., Kashyap, P. L., Kumar, S., Srivastava, A. K., and Ramteke, P. W. (2016). Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. *Springerplus* 5:1939. doi: 10.1186/s40064-016-3657-4
- Rajput, L. S., Kumar, S., Natara, J. V., Shivakumar, M., Maheshwari, H. S., and Ghodki B S (2022). Evaluation of novel fungicide for the management of soybean anthracnose disease and yield loss estimation. *Legume Res.* 10, 1–8. doi: 10.18805/LR-4783
- Ramzan, U., Abid, K., Zafar, M. A., Anwar, A. M., Nadeem, M., Tanveer, U., et al. (2023). *Trichoderma*: multitasking biocontrol agent. *Int. J. Agric. Biosci.* 12, 77–82. doi: 10.47278/journal.ijab/2023.047
- Rivera-Méndez, W., Obregón, M., MoránDiez, M. E., Hermosa, R., and Monte, E. (2020). *Trichoderma asperellum* biocontrol activity and induction of systemic defenses against *Sclerotium cepivorum* in onion plants under tropical climate conditions. *Biol. Cont.* 141:104145. doi: 10.1016/j.biocontrol.2019.104145
- Saini, S., Raj, K., Saini, A. K., Kumar, R., Saini, A., Khan, A., et al. (2024). Unravelling the synergistic interaction of *Thrips tabaci* and newly recorded, *Thrips parvispinus* with *Alternaria porri* (Ellis.) Cif, inciting onion purple blotch. *Front. Microbiol.* 15:1321921. doi: 10.3389/fmicb.2024.1321921
- Salunkhe, V. N., Gedam, P., Pradhan, A., Gaikwad, B., Kale, R., and Gawande, S. (2022). Concurrent waterlogging and anthracnose-twister disease in rainy-season onions (*Allium cepa*): Impact and management. *Front. Microbiol.* 8:1063472. doi: 10.3389/fmicb.2022.1063472
- Samuels, G. J., Ismael, A., and Mulaw, T. B. (2012). The *longibrachiatum* clade of *Trichoderma*: a revision with new species. *Fungal Divers.* 55, 77–108. doi: 10.1007/s13225-012-0152-2
- Sathish, L., Pavithra, N., and Ananda, K. (2012). Antimicrobial activity and biodegrading enzymes of endophytic fungi from eucalyptus. *Int. J. pharmacol. Sci. Res.* 3, 2574–2583.
- Schwartz, H. F., du Toit, L. J., and Coutinho, T. (2015). *Diseases of Onion and Garlic (Allium cepa L. and A. sativum L., Respectively)*. St Paul, MN: The American Phytopathological Society.
- Schwartz, H. F., and Mohan, S. K. (2007). *Compendium of Onion and Garlic Diseases and Pests*. 2nd ed. St. Paul, MN: The American Phytopathological Society, 136.
- Schwyn, B., and Neillands, J. B. (1987). Universal chemical assay for the detection and determination of siderophore. *Anal. Biochem.* 160, 47–56. doi: 10.1016/0003-2697(87)90612-9
- Sharma, D., and Chauhan, A. (2024). Kharif onion production in India-present status and future potential: a review. *Agric. Rev.* 45, 653–660. doi: 10.18805/ag.R-2455
- Sharma, P., Sharma, N., and Sindhan, G. S. (2011). Economic viability of *Trichoderma harzianum* in management of soil-borne diseases in vegetables. *Indian Phytopath.* 64, 281–284.
- Sharma, S. K., Sharma, M. P., Ramesh, A., and Joshi, O. P. (2012). Characterization of zinc solubilizing *Bacillus* isolates and their potential to influence zinc assimilation in soybean seeds. *J. Microbiol. Biotechnol.* 22, 352–359. doi: 10.4014/jmb.1106.05063
- Siddiqui, S., Sultan, N., Khatoon, A., and Kamal, A. (2025). Exploring the multifaceted roles of *Trichoderma* in agriculture: from growth stimulation to pollution remediation. *Biochem. Cell. Arch.* 25:49. doi: 10.51470/BCA.2025.25.1.49
- Simkin, A. J., Kapoor, L., Doss, C. G. P., Hofmann T, A., Lawson, T., and Ramamoorthy, S. (2022). The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield in planta. *Photosynthesis Res.* 152, 23–42. doi: 10.1007/s11120-021-00892-6
- Singh, A., Jain, A., Sarma, B. K., Upadhyay, R. S., and Singh, H. B. (2013). Rhizosphere microbes facilitate redox homeostasis in *Cicer arietinum* against biotic stress. *Ann. Appl. Biol.* 163, 33–46. doi: 10.1111/aab.12030
- Singh, A., Pandey, S., Srivastava, M., Shahid, M., Kumar, V., Trivadi, S., et al. (2015). Physiological and biochemical analysis of *Trichoderma* species isolated from different location U. P. *Afr. J. Agri. Res.* 10, 4837–4843. doi: 10.5897/AJAR2015.10374
- Singh, V., Keswani, C., Ray, S., Upadhyay, R. S., Dhananjaya, P., Singh, P. R., et al. (2019). Isolation and screening of high salinity tolerant *Trichoderma* spp. with plant growth property and antagonistic activity against various soilborne phytopathogens. *Arch. Phytopathol. Plant. Protec.* 52, 667–680. doi: 10.1080/03235408.2019.1648917
- Singh, V., Ray, S., Bisen, K., Keswani, C., Upadhyay R., S., Sarma, B. K., et al. (2017). Unravelling the dual applications of *Trichoderma* spp. as biopesticide and biofertilizer. *Adv. PGPR Res.* 364–374. doi: 10.1079/9781786390325.0364
- Solanki, M. K., Singh, N., Singh, R. K., Singh, P., Srivastava, A. K., Kumar, S., et al. (2011). Plant defense activation and management of tomato root rot by a chitin-fortified *Trichoderma/Hypocrea* formulation. *Phytoparasitica* 39, 471–481. doi: 10.1007/s12600-011-0188-y
- Srinivas, P. S., and Lawande, K. E. (2007). Seedling root dip method for protecting onion plants from thrips. *Indian J. Plant Protect.* 35, 206–209.
- Suhardi, H. A. (1993). Anthracnose on shallot (*Allium cepa* group aggregatum) in Java. *Onion Newslitt. Trop.* 5, 48–50.
- Sultana, N., Hossain, I., and Ahmed, M. U. (2021). Economic evaluation of *Trichoderma*-based management for damping-off in vegetables. *Bang. J. Agric. Res.* 46, 243–253.
- Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis. *Mol. Biol. Evol.* 38, 3022–3027. doi: 10.1093/molbev/msab120
- Tanya, M., Trina, S., Sunita, M., and Srikanth, D. (2020). Measuring and assessing the yield loss and yield loss model of green gram due to Anthracnose of green gram (*Vigna radiata*). *Legume Res.* 43, 678–682.
- Vincent, J. M. (1927). Distribution of fungal hyphae in the presence of certain inhibitors. *Nature* 159, 239–241.
- Vojnović, D., Maksimović, I., Horecki, A., T., Žunić, D., Adamović, B., and Milić, A. (2023). Biostimulants affect differently biomass and antioxidant status of onion (*Allium cepa*) depending on production method. *Horticulturae* 9:1345. doi: 10.3390/horticulturae9121345
- Wang, X., Chen, W., Zhang, J., Hu, W., Li, M., Zhao, P., et al. (2025). Effector Cpe1 secreted by *Trichoderma longibrachiatum* induces plant disease resistance. *Biol. Control* 202:105726. doi: 10.1016/j.biocontrol.2025.105726
- Wang, X., Zhao, Z., Li, H., Wei, Y., Hu, J., Yang, H., et al. (2024). The growth-promoting and disease-suppressing mechanisms of *Trichoderma* inoculation on peanut seedlings. *Front. Plant Sci.* 15:1414193. doi: 10.3389/fpls.2024.1414193
- Wheeler, B. E. J. (1969). *An Introduction to Plant Diseases*. London: John Wiley and Sons Limited, 301.
- Zhang, L., Niaz, S. I., Khan, D., Wang, Z., Zhu, Y., and Zhou, H. (2017). Induction of diverse bioactive secondary metabolites from the mangrove endophytic fungus *Trichoderma* sp. (strain 307) by co-cultivation with *Acinetobacter johnsonii* (strain B2). *Mar Drugs* 15:35. doi: 10.3390/md15020035
- Zhou, X. F., Zhang, X. Y., Cai, W. Y., Yan, T., Jing, W. X., and Xu, H. W. (2020). The effect of *Trichoderma harzianum* on the growth-promoting effect of cucumber seedlings. *J. Jilin Norm. Univ.* 41, 93–99.