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Molecular Identification, Antagonistic Assay and Enzyme Profiling of Selected *Trichoderma* Isolates

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Abstract

The aim of this study was to find and characterize *Trichoderma* isolates by antagonistic and enzymatic activity to evaluate their potential as biocontrol agents against Dry root rot (DRR). *Trichoderma* isolates were isolated from thepulses rhizosphere of different districts of Uttar Pradesh. Twenty one *Trichoderma* isolates were identified using ribosomal DNA internal transcribed spacer (ITS) regions and translation elongation factor 1-alpha (tef1). In addition, enzymatic profiling of *Trichoderma* isolates was done indicated strong cell wall degrading enzyme activities and plant growth promoting traits of *Trichoderma* isolates. Overall, our results suggested that the isolated *Trichoderma* spp. have prodigious potential for plant growth promotion and can be used as biocontrol agents against dry root rot.

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Biocontrol; Chickpea; Growth Promotion; Pulses; *Trichoderma*.

Introduction

Pulses are the second most important group of crops after cereals. These are rich source of protein in vegetarian diet which contains around 20-30% protein.[1] Chickpea (*Cicer arietinum* L.) has occupied a prominent position among major pulses. Mainly soil-borne pathogens cause major losses in chickpea yield. Dry root rot caused by *Rhizoctonia bataticola*, collar rot by Sclerotium rolfsii and wilt by *Fusarium oxysporum* f. sp. ciceris, wet root rot caused by *Rhizoctonia solani*, are the major diseases of chickpea caused by soil borne pathogens. According to some reports suggested that dry root rot (DRR) is emerging as a potential threat to chickpea production.[2,3] The disease commonly appears all over the podding and flowering stage. Most noticeable symptom of dry root rot is impulsive drying of the entire plant disseminated around the field. Yellowing and drooping of petioles and leaves on the tip only occurs at later stage. Shows sign of rotting and the tap root turns black and later lacking of lateral and finer roots. The dead roots were fragile and show mincing of bark and crosswise roots. Earlier, dry root rot was not that significant in chickpea. Though, in recent years due to changing weather conditions, extended drought hexes, it

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has become a major menace to production of chickpea. As a cost effective option bio controls are ecologically environment friendly. So, the present research has been commenced to assess the effects of *Trichoderma* sp. keeping in view the severity and losses caused by dry root rot disease.

Trichoderma species are widely distributed in nature, extending from tundra to humid ecosystems. Their capability to flourish in varied regions can be ascribed to their diverse metabolic competences and natural competitive bellicosity. *Trichoderma* spp. possesses a extensive range of survival and proliferation mechanisms, including physical attack and degradation of other fungi, as well as the consumption of complex carbohydrates. Attributable to these characteristics, *Trichoderma* spp. Embraces substantial economic importance and is exploited in numerous marketable applications for instance industrial enzyme production, antibiotic production, heterologous protein expression, and biocontrol of plant pathogenic fungi.[4] The biocontrol potential of *Trichoderma* was first acknowledged in the initial 1930s. Subsequently then, widespread exploration has been steered on this genus as an antagonist against soil-borne plant pathogens, including *Rhizoctonia bataticola*, the causative agent of Dry root rot of chickpea.

This biological control operates through various mechanisms, including the secretion of hydrolytic enzymes.[4] Extensive research has been conducted on the mechanism of mycoparasitism. Several genomic and proteomic studies have been done to determinenovel hydrolytic enzymes.[5,6] These studies have also aimed to understand the synergistic effects between dissimilar hydrolytic enzymes and antibiotics,[7] in addition to examine cell signalling throughout the formation of cell wall degrading enzymes (CWDEs).[8] Regardless of the application of different methodologies to reconnoitre the multiplicity of *Trichoderma* species and their biocontrol potential, there have been limited publications on the molecular and physiological studies of CWDEs.

Therefore, present research work aimed to characterize CWDE production and to find out the potential isolates of *Trichoderma* isolated from pulses rhizospheric areas of Uttar Pradesh with high biocontrol potential, plant growth promotion ability by improving the soil quality and restricting Dry root rot of chickpea.

Materials & Methods

Collection of Soil Samples from Pulses Rhizosphere

Wide-ranging collection of 76 soil samples was done from diverse rhizospheric areas of Uttar Pradesh following the methods by[9] during 2021-2022. (Table1.)

Isolation of *Trichoderma* from rhizospheric soil

Isolation was done using serial dilution technique suggested by.[10] On *Trichoderma* Selective media.[11,12] Each soil samples were prepared and 100µl of dilution sample was spread on TSM culture media (MgSO4 0.2g, K2HPO4 0.9g, KCl 0.15g, NH4NO3 3g, Glucose/ Dextrose 3g, Chloramphenicol 0.25g, Fenaminosulf 0.305g, PCNB 0.20g, Rose Bengal 0.15g, Agar 18g, distilled water 1000ml, pH 7).[13] Plates were incubated at 28˚± 2 for 3 days and then observed for the growth of morphologically different colonies appearing on the plate. The selected colonies were further purified on the PDA plates and preserved at 4˚C.

Purification of *Trichoderma* isolates

Trichoderma isolates were extracted and purified by single spore culture. The spores of the *Trichoderma* isolates were inoculated on petriplates with Potato dextrose agar (PDA) medium.[14]

Morphological Characterization of *Trichoderma* isolates

Mycelial growth of *Trichoderma* isolates were studied by the method given by.15 Each isolates were grown on potato dextrose agar (PDA) for 4-5 days. After 48 hours of inoculation morphological parameters weremeasured and observed. The type of shape, growth habit, kind of colour and firmness for each *Trichoderma* isolates were examined day by day.[14]

Measurement of Spore Density

Ten days old conidial suspension of *Trichoderma* isolates were taken in a beaker and stirred in shaker to determine spore density. Volume of Beaker with conidial suspension of *Trichoderma*isolates was maintained 500 ml with sterile water and 1 drop Tween-20 (act as spreader) was added to it and stirred well to dissolve. To count spore desnsity,1

drop of conidial suspension was taken on the center of hem cytometer and a cover slip was placed on it. Under 40X microscopic lens,spores were counted. Average number of spore per unit cell used in the following formula, the number of spore per 1 ml was determined.[16]

Number of spores per cubic mm sporulation = (Number of sporulation x Dilution/number of smaller square counted) x 4000

Extraction of DNA and PCR amplification sequencing and DNA analysis of *Trichoderma* strains

Liquid culture of *Trichoderma* strains were grown in potato dextrose broth (Himedia) and maintained on a rotary shaker at 25-28°C for 5 days. After one week mycelial mat was filtered to use for CTAB method for the DNA isolation. Purity and quantity of DNA of each isolates were checked by gel electrophoresis, determined with the nano drop spectrophotometer and the DNA concentrationwas observed 10ngul-1. PCR and sequencing was used to amplify ITS region 1 and 4 of the rRNA gene cluster, and the translation elongation factor 1-alpha (tef1), TEF 728R (CAT CGA GAA GTT CGA GAA GG) & 986F (TAC TTG AAG GAA CCC TTA CC) The ITS region was amplified using the following programme; 3 min at 94°C followed by 35 cycles each of 30sec at 94°C, 30sec at 55°C, 1 min at 72°C and, finally, 10 min at 72°C. For tef amplification following programme; 5 min at 94°C followed by 30 cycles each of 1 min at 94°C, 1min at 56.4°C, 1 min at 72°C and finally, 5 min at 72°C. The amplified products were analyzed by electrophoresis in 2% agarose gel in 1 X TAE buffer at 60 volt, molecular marker used was of 1 kb. The raw sequence FASTA files of ITS1 and ITS4, tef were checked for quality, trimmed, amended and accumulated using CLC Genomics Workbench 7.5 (CLCBio, Aarhus, Denmark).[17]

The ITS and tef sequences of the *Trichoderma* isolates were aligned with the reference sequences of *Trichoderma* obtained from NCBI database using Clustal W software Ver. 2.0 and MEGA software for phylogenetic tree construction.

Identification of *Trichoderma* Isolates for their Antagonistic Activity

Evaluation of *Trichoderma* isolates were doneunder *in vitro* condition for their antagonistic effects against *Rhizoctonia bataticola* through dual culture technique.[18] The dominance effect of *Trichoderma* spp. was assessed by the percent mycelial inhibition of *R. bataticola* by the following formula.[19]

Per cent Inhibition of Radial Growth (PIRG) = R1-R2 x 100% /R1

R1 = Radial growth of *R. bataticola* without *Trichoderma* isolates in the particular plate (control) R2 = Radial growth of *R. bataticola* with *Trichoderma* isolates (treatment). Triple replication was used for each treatment.

Enzyme Production and Enzymatic Assays

Chitinolytic Enzyme Assay

Chitinolytic activity of *Trichoderma* strains were observed using chitinase detection medium.[20] The fresh culture of *Trichoderma* were inoculated into the decontaminated plates having chitinase detection medium and incubated at 28 ± 2°C for 2–3 days and witnessed violet coloured zone creation. The violet coloured zone was measured in mm indicating lower to higher Chitinolytic activity, + (1-20mm), ++ (21-39mm), +++ (40-44 mm), ++++ (>40 mm) and – (no zone).[21]

Estimation of Siderophore

The capability of *Trichoderma* spp. to produce Siderophore was identified in Chrome Azurol S (C.A.S) assay.[22] 5 mm discs of seven days old culture of *Trichoderma* strains were inoculated in culture medium. The plates were incubated at 28 ± 2°C for 6-7 days. Colour changed to magenta in the C.A.S. blue agar. The uninoculated control plates were incubated under the same conditions. Each treatment was replicated three times. Three replications for each treatment were designed.[23]

Phosphate solubilizing *Trichoderma* strains

Twenty one strains exerted ability for phosphate Solubilization on Pikovskaya medium with different efficacy. All the isolates of *Trichoderma* spp. were evaluated for phosphate solubilization on modified Pikovskaya's agar supplemented with bromocresol purple (100.0 mg/L). 5 mm mycelial disc of each *Trichoderma*isolates was placed on the center of agar plate and incubated at room temperature for 7 days. The activity of phosphate solubilizing *Trichoderma* strains observed as the agar plate yellow from purple zones of acidification.[24]

Screening for Cellulolytic Activity

Trichoderma strains were grown on the Czapek's-Mineral Salt Agar Medium suggested by.25 5-day old fungal culture of each strain separatedthen inoculated on the medium and incubated at 25 ± 2°C for 5-7 days. The aqueous Congo red (2% w/v) solution was flooded on the inoculated plates for 15min. Then, after washing with distilled water, plates were flooded with NaCl (1 M) for 1.5 min. Formation of Whitish-yellow area around the colonies was observed by the production of cellulase. Clear zone and diameter of the colony were measured.

Screening of Amylase producing *Trichoderma* using Starch Agar Plate

Screening of amylase producing *Trichoderma* was done by using starch agar (containing 1% starch and 2% agar) plate method.[26] The sterilized medium was poured to Petri dishes and inoculated with 5-day old culture of each strain. The plates kept for incubation for 48 hrs at 28°C. The plates were flooded with 1% of iodine solution for 5 min after incubation. Then, they were washed with distilled water to remove the excess iodine solution. Highest zone of clearance was observed and selected as potential strain.

Results

Collection of Isolation of *Trichoderma* isolates form rhizospheric areas of Uttar Pradesh

A total number of 21 *Trichoderma* isolates were successfully isolated. 13 isolates namely TR9, TR10, TR11, TR12, TR13, TR14, TR15, TR16, TR17, TR18, TR19, TR20 and TR21 were from Kanpur, UP while TR1, TR2 and TR6 from Chitrakoot, MP, while TR7 and TR8 were from Orcha, Jhansi and TR3 Badausa, UP, TR4 Fatehpur, UP and TR5 from Unnao, UP. (Table 1)

Table 1: Details of isolated *Trichoderma* isolates with respective code, isolation district, block, crop field.

S.No	Isolate Code	Area/ District	Rhizospheric soil	LAT	LONG
1	TR1	RAJOULA CHITRAKOOT, MP	CHICKPEA	25.12.930	80.51.004
2	TR2	RAJOULA CHITRAKOOT, MP	CHICKPEA	25.12.930	80.51.004
3	TR3	BADAUSA, UP	CHICKPEA	25.12.877	80.46.882
4	TR4	FATEHPUR ROSHAMI, UP	PIGEONPEA	26.40.819	80.11.353
5	TR5	MANGWADA, UNNAO	CHICKPEA		
6	TR6	CHITRAKOOT	CHICKPEA	25.12.930	80.51.004
7	TR7	FOOTERA(ORCCHA)JHANSI, UP	PIGEONPEA	25.35N	78.65E
8	TR8	FOOTERA(JHANSI)	CHICKPEA	25.35N	78.65E
9	TR9	NARAMAU, KANPUR, UP	PIGEONPEA	26.50N	80.25E
10	TR10	IIPR, KANPUR, UP	CHICKPEA	26.49N	80.28E
11	TR11	IIPR, KANPUR, UP	CHICKPEA	26.49N	80.28E
12	TR12	IIPR, KANPUR, UP	CHICKPEA	26.49N	80.28E
13	TR13	KANPUR	CHICKPEA	26.49N	80.28E
14	TR14	KANPUR	CHICKPEA	26.49N	80.28E
15	TR15	KANPUR	CHICKPEA	26.49N	80.28E
16	TR16	KANPUR	CHICKPEA	26.49N	80.28E
17	TR17	KANPUR	CHICKPEA	26.49N	80.28E
18	TR18	KANPUR	CHICKPEA	26.49N	80.28E
19	TR19	KANPUR	CHICKPEA	26.49N	80.28E
20	TR20	KANPUR	CHICKPEA	26.49N	80.28E
21	TR21	KANPUR	CHICKPEA	26.49N	80.28E

Morphological Characterization of *Trichoderma* spp.

As earlier studies say that many *Trichoderma* spp. are distinctly diverged on their cultural and morphological characters. Linear growth of mycelia ranged from 29.91 mm to 90.00 mmat 28±1ºC. Based on morphology, isolates were divided into four groups. (Table2).[27] conducted a similar study in Hebron University, Palestine.

Table 2: Morphological features of *Trichoderma* isolates used during the study

S.no.	*Trichoderma* strains	Shape	Colour	Growth Pattern	Colony evenness
1	TR1	Regular	Dark Green	Medium	compact
2	TR2	Regular	Dark Green	Fast	compact
3	TR3	Regular	Green	Fast	Very compact
4	TR4	Regular	Dark Green	Medium	Compact
5	TR5	Regular	Light Green	Fast	compact
6	TR6	Regular	Whitish Green	Slow	Loose
7	TR7	Regular	Light Green	Fast	compact
8	TR8	Regular	Whitish Green	Medium	compact
9	TR9	Regular	Green	Fast	Very compact
10	TR10	Regular	Yellowish Green	Fast	Very compact
11	TR11	Regular	Dark green	Medium	compact
12	TR12	Regular	Dark Green	Medium	Loose
13	TR13	Regular	Dark Green	Fast	Scattered
14	TR14	Regular	Light Green	Fast	Scattered
15	TR15	Regular	Dark Green	Medium	Compact
16	TR16	Regular	Light Green	Fast	Loose
17	TR17	Regular	Whitish yellow	Slow	Loose
18	TR18	Regular	Dark Green	Medium	Very compact
19	TR19	Regular	Whitish Green	Medium	Loose
20	TR20	Regular	Light Green	Medium	Compact
21	TR21	Regular	Dark Green	Fast	Very compact

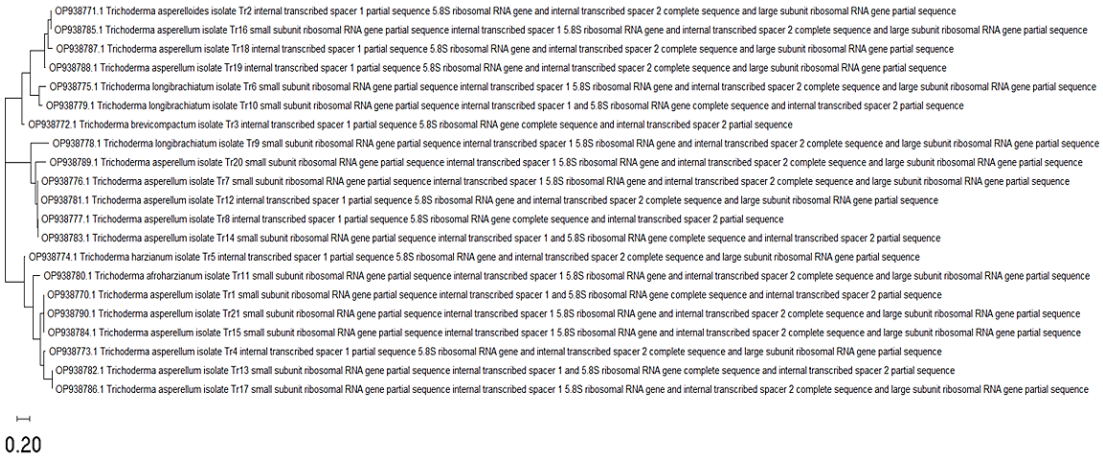

Fig. 1: Phylogenetic tree constructed by the neighbour-joining method derived from analysis of the ITS gene sequence of *Trichoderma* isolates and related sequences obtained from NCBI

Table 3: *Trichoderma* identified and submitted to NCBI having Accession number

S.No	*Trichoderma* identified	Isolate	ITS Gene	TEF Gene
1.	*Trichoderma asperellum*	TR1	OP938770	OP948258
2.	*Trichoderma* asperelloides	TR2	OP938771	OP948259
3.	*Trichoderma* brevicompactum	TR3	OP938772	OP948260
4.	*Trichoderma asperellum*	TR4	OP938773	OP948261
5.	*Trichoderma harzianum*	TR5	OP938774	OP948262
6.	*Trichoderma longibrachiatum*	TR6	OP938775	OP948263
7.	*Trichoderma asperellum*	TR7	OP938776	OP948264
8.	*Trichoderma asperellum*	TR8	OP938777	OP948265
9.	*Trichoderma longibrachiatum*	TR9	OP938778	OP948266
10.	*Trichoderma longibrachiatum*	TR10	OP938779	OP948267
11.	*Trichoderma afroharzianum*	TR11	OP938780	OP948268
12.	*Trichoderma asperellum*	TR12	OP938781	OP948269
13.	*Trichoderma asperellum*	TR13	OP938782	OP948270
14.	*Trichoderma asperellum*	TR14	OP938783	OP948271
15.	*Trichoderma asperellum*	TR15	OP938784	OP948272
16.	*Trichoderma asperellum*	TR16	OP938785	OP948273
17.	*Trichoderma asperellum*	TR17	OP938786	OP948274
18.	*Trichoderma asperellum*	TR18	OP938787	OP948275
19.	*Trichoderma asperellum*	TR19	OP938788	OP948276
20.	*Trichoderma asperellum*	TR20	OP938789	OP948277
21.	*Trichoderma asperellum*	TR21	OP938790	OP948278

Fig 2: Phylogenetic tree constructed by the neighbour-joining method derived from analysis of the TEF gene sequence of Trichoderma isolates and related sequences obtained from NCBI

Molecular Characterization of *Trichoderma* isolates
Molecular identification based on ITS and Tef genes confirmed that the isolates belong to six genera *Trichoderma harzianum*, *Trichoderma longibrachiatum*, *Trichoderma asperellum*, *Trichoderma* asperelloides, *Trichoderma brevicompactum* and *Trichoderma afroharzianum*. The results of the phylogenetic analysis based on the ITS and Tef gene sequences are given in the figure-1.

Table 4: Antagonistic potential of Trichoderma isolates against Rhizoctonia bataticola

Trichoderma Isolates	Inhibition % of *Rhizoctonia bataticola*
TR1	65.26g
TR2	65.33g
TR3	60.3h
TR4	65.48g
TR5	70.04de
TR6	70.41de
TR7	56.11i
TR8	70.7d
TR9	71.81cd
TR10	74.56abc
TR11	72.78bcd
TR12	66.93efg
TR13	75.85ab
TR14	65.74fg
TR15	66.78efg
TR16	72.78bcd
TR17	76.48a
TR18	59.04hi
TR19	69.26def
TR20	74.63abc
T21	70.96cd
Control	0j

* Values are mean of three replications

Identification of *Trichoderma* Isolates for their Antagonistic Potential
Evaluation of antagonistic effect of *Trichoderma* isolates against *Rhizoctonia bataticola* using dual culture tests showed that 11 isolates Tr5, Tr6, Tr8, Tr9, Tr10, Tr11, Tr13, Tr16, Tr17, Tr20 and Tr21 reduced the mycelial growth of *R. bataticola* more than 70% (Table 4). Maximum mycelial inhibition was 76.48% by isolate Tr17 and 75.85 % by Tr13. Ten isolates inhibited mycelial growth of *R. bataticola* more than 50% but less than 70%. The isolates overgrew on the *R. bataticola* colonies which had irregular morphology and were lysing indicating the incidence of strong mycoparasitism.

Chitinolytic Activity by *Trichoderma* Isolates
The maximum chitinase was produced by only five isolates Tr17, Tr13, Tr20, Tr10 and Tr11. These isolates measured violet colour zone more than 4.5 cm after 3 days of incubation. Seven isolates produced violet zone ranging from 4.0 to 4.4 cm; seven isolates produced violet zone ranging from 2.1-3.9 cm. Two isolates did not produce chitinase at all (Table 5).

Table 5: Screening of *Trichoderma* isolates for Chitinase production on solid medium supplemented with colloidal chitin

	Chitinolytic activity
Tr17	+++
Tr13	+++
Tr20	+++
Tr10	+++
Tr11	+++
Tr12	++
Tr19	++
Tr16	++
Tr15	++
Tr14	++
Tr3	++
Tr2	++
Tr1	+
Tr4	+
Tr21	+
T r6	+
Tr9	+
Tr8	+
Tr5	+
Tr7	-
Tr18	-

Siderophore Estimation
Results showed that *Trichoderma* isolates produced siderophores. However, the siderophore production varied in different isolates. Maximum production was observed in three isolates Tr10, Tr11 and Tr17 (change in colour zone more than 18 mm).

Four isolates did not produce the siderophore. The colour changed zone was measured more than 3.5 mm but less than 16.5 mm in 12 isolates. Colour change zone area less than 3.5 was produced by 2 isolates (Table 6)

Table 6: Siderophore activity by *Trichoderma* isolates

	Siderophore production activity
Tr10	++++
Tr11	++++
Tr17	++++
Tr20	+++
Tr13	+++
Tr12	+++
Tr3	+++
Tr16	+++
Tr9	+++
Tr14	++
Tr19	++
Tr4	++
Tr1	++
Tr2	++
Tr21	++
Tr8	+
Tr15	+
Tr6	-
Tr5	-
Tr7	-
Tr18	-

Cellulase Production

Based on the clear zone formation after flooding the culture plates withaqueous Congo red (2% w/v) followed by flooding with NaCl (1 M), only two isolates Tr10 and Tr17 produced cellulase activity. Rest all isolates did not produced cellulase.

Amylase Production

Production of amylase based on clear zone after flooding the plates with iodine was observed in 21 isolates. Eight isolates viz., Tr13, Tr10, Tr12, Tr17, Tr11, Tr16, Tr19 and Tr20 were identified which produced maximum amylase (>15 mm clear zone). Two isolates Tr14 and Tr15 clear zone from 9.1 to 15 mm. Other isolates produced clear zone less than 9 mm. (Table 7)

Table 7: Amylase production by *Trichoderma* isolates

	Amylase Production
Tr13	+++
Tr10	+++
Tr12	+++
Tr17	+++
Tr11	+++
Tr16	+++
Tr19	+++
Tr20	+++
Tr15	++
Tr14	++
Tr3	+
Tr4	+
Tr5	+
Tr2	+
Tr6	+
Tr21	+
Tr9	+
Tr18	+
Tr1	+
Tr7	+
Tr8	+

Phosphate Solubilization by *Trichoderma* isolates

Twenty one strains were found to solubilizing phosphate with varied efficiencies. All strains produced distinct halos around the colony on the plate indicating efficiency of the isolate to solubilizing phosphate. Halo of more than 25mm was form by eight isolates namely Tr10, Tr12, Tr20, Tr11, Tr17, Tr13, Tr19, Tr16 indicating more efficient in solubilizing phosphates. (Table 8)

Statistical Analysis

To analyse data of Antagonistic potential of *Trichoderma* isolates against *Rhizoctonia bataticola* Duncan Multiple Range Test has been done (Table 2). For phylogenetic analysis Clustal W software ver 2.0 was used. Values within a column followed by the same letter(s) are not significantly different at the P=0.05 level according to Duncan's multiple range test.

Table 8: Phosphate solubilizing activity by *Trichoderma* isolates

Phosphate Solubilizing *Trichoderma*	
Tr10	+++
Tr12	+++
Tr20	+++
Tr11	+++
Tr17	+++
Tr13	+++
Tr19	+++
Tr16	+++
Tr15	++
Tr1	++
Tr3	++
Tr2	++
Tr21	++
Tr4	++
Tr14	++
Tr6	++
Tr9	++
Tr8	++
Tr5	++
Tr7	+
Tr18	+

*Scale: zone measured in mm
Chitinase: (1-20= +), (21-39= ++), (40-44= +++), (45< = ++++), (No zone= -)
Siderophore: (1-3= +), (3-3.5= ++), (3.6-15= +++), (16< = ++++)(No zone= -)
Cellulase: (1-5= ++)(No zone= -)
Amylase: (1-5 =+),(5.1-9= ++), (9.1-15= +++), (15< = ++++)(No zone= -)
Phosphate: (1-15=+), (15.1-19= ++), (19.1-25= +++), (25< = ++++)(No zone= -)

The experiments were performed with three replicates. The analysis of variance (ANOVA) was executed using OPSTAT software. Mean values for treatments were equated by the least significant difference by critical difference at 95% level of confidence (p<0.05%). For descriptive statistical analysis, Microsoft Excel was used.[28]

Discussion

The antagonistic effect of selected *Trichoderma* strains, against *Rhizoctonia bataticola* was examined using the plate confrontation method in the present study. The results indicated that several strains unveiled the highest inhibition percentage compared to other strains. Additionally, noticeable morphological variations were observed after the confrontation assay. Previous research by[29] evaluated the five antagonists *Trichoderma* against dry root rot pathogen and observed that *T. viride* (96.40%) was best among others inhibiting the growth of *Rhizoctonia bataticola*. Furthermore, According to[30] *T. harzianum* found effective inhibiting the mycelial growth of *R. bataticola* causing dry root rot of chickpea. Microscopic investigationexposed that *Trichoderma* hyphae possibly will grow together with or penetrate and bind around *R. bataticola* hyphae, kerbing their expansion and in due course causing damage. These outcomes evidently point towards the antagonistic activity and biocontrol potential of *Trichoderma* strains against phytopathogenic fungi. The cell walls of pathogens serve as promising objectives for the development of antimicrobial agents. Most fungi have chitin-based cell walls, and disrupting these walls can significantly impact cell growth and morphology. Sequence analysis of twelve isolates was done to confirm species identity, which initially has been done based solely on morphological parameters. Comparison of oligonucleotide fragments of rDNA sequences, which included the 5.8S gene and the flanking ITS1 and ITS2 regions, with reference sequences from public databases, showed that they were very similar. In this research, we evaluated the efficacy of numerous cell wall degrading enzymes from *Trichoderma* spp. to identify that they could potentially be utilized as biocontrol agents capable of breaking down the cell walls of fungal, chitin degradation were observed in *Trichoderma* isolates. Some studies suggested that the physical interaction between mycoparasitic hypae and fungal pathogen is buoyed by the emission of a set of extracellular enzymes such as Chitinolytic enzyme[31,32,33] ß-glucanases[34,35] and proteinases[36] as well as secondary metabolites.[37,38] *Trichoderma* species like *T. asperellum, T. atroviride*, *T. harzianum* and *T. virens* are known to produce active cell wall degrading enzymes such as cellulase, chitinase, protease, and β-1,3-glucanases, which contest fungal pathogens. *Trichoderma* isolates were characterized by using the ITS & tef regions, metabolic profiling, and CWDE activities. Sequence analysis of ITS and tef led to the reclassification of the isolates. *Trichoderma* isolates produced common

metabolites with antifungal properties. Enzyme assays demonstrated robust CWDE activities. So, ourfindings suggest that the above *Trichoderma* isolates showed promising plant growth promoting and biocontrol activity against plant pathogens.

Conclusion

The study concludes that the collected soil showed of a large population of diverse fungi. Different species of *Trichoderma* were isolated from the soil. *T. harzianum*, *T. brevicompactum*, *T. afroharzianum*, *T. asperelloides, T. longibrachiatum* and *asperellum* showed antagonist ability against plant pathogenic fungi, *Rhizoctonia bataticola*. The isolated *Trichoderma* strains showed significant production of defence enzymes. These *Trichoderma* species can be explored further to be used as biocontrol agents.

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical Statement

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Authors' Contribution

Utkarsh Singh Rathore: All research work during Ph.D

Rudra Pratap Singh: Ph.D advisor, worked under him during Ph.D, helped in designing my research work

Sonika Pandey: helped in data collection & related work and paper writing.

R.K. Mishra: worked under him during my research work

Consent for Publication

All the authors have provided their consent for publication in this Journal. Attached

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