Journal of Applied Biology & Biotechnology Vol. 14(4), pp. 108-114, July-August, 2026
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2026.292854

Screening of *Trichoderma* isolates from Punjab and Himalayan region for hydrolytic enzyme production

Ranjna Kumari[1], Mayukh Sarkar[2], T. S. Archana[2], Vipul Kumar[2]*, Swapna Rekha Panda[3], Amol Kantilal Wagh[3]

[1]Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India.
[2]Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India.
[3]Department of Chemical Engineering, UPL University of Sustainable Technology, Vataria, Gujarat, India.

ARTICLE INFO

Article history:
Received on: 27/10/2025
Accepted on: 17/01/2026
Available online: 25/05/2026

Key words:
Biocontrol,
Bioformulation,
Cellulase,
Enzyme index,
Protease,
Rhizosphere colonization,
Trichoderma.

ABSTRACT

Sustainable agriculture increasingly relies on eco-friendly and biocontrol strategies, and this study aimed to screen *Trichoderma* isolates for cellulase and protease activity associated with plant growth promotion and disease suppression. The present study was conducted to evaluate the enzymatic potential of several *Trichoderma* isolates collected from distinct agroclimatic zones of India. The results revealed substantial inter-isolate variation in cellulase and protease activity. Isolate PBT (Punjab Trichoderma) 13 recorded higher protease activity and cellulase activity, whereas isolates such as PBT1 and PBT21 exhibited relatively lower enzymatic activity. This study provides region-specific evidence on the qualitative variability of cellulase and protease activity among *Trichoderma* isolates, highlighting enzyme-based screening as a practical preliminary approach for biocontrol selection. The observed inter-isolate differences in cellulase and protease activity reflect ecological adaptation and highlight the importance of enzymatic screening for the preliminary selection of promising *Trichoderma* isolates with potential application in sustainable plant disease management.

1. INTRODUCTION

Global agriculture is facing dual challenges of meeting rising food demand while minimizing environmental degradation caused by the intense use of chemical pesticides and fertilizers [1]. This has driven a paradigm shift toward sustainable, biologically based plant protection strategies [2], among which various *Trichoderma* isolates have gained prominence for enhancing crop productivity and soil health [3]. While *Trichoderma* isolates are widely recognized for their antagonistic activity against phytopathogens, not all isolates exhibit comparable biocontrol efficacy [4]. The variability in performance highlights the importance of understanding the functional traits that underpin effective biocontrol. The effectiveness of a strain largely depends on its metabolic potential, particularly the production of hydrolytic enzymes such as cellulase and protease [5]. These enzymes directly contribute to biocontrol by facilitating pathogen cell wall degradation, mycoparasitism, and rhizosphere colonization [6,7]. Accordingly, enzyme activity is widely regarded as a crucial marker for initial screening and characterization of biocontrol-effective *Trichoderma* strains [8,9].

From a functional perspective, cellulase contributes to cellulose degradation and nutrient mobilization in soil [10], whereas protease breaks down structural proteins in the cell wall, weakening phytopathogen defense [11]. Together, these enzymes support pathogen suppression and indirectly promote plant growth and soil health [12]. However, the expression of these enzymatic traits is not uniform across the *Trichoderma* population, as enzyme activity is influenced by genetic diversity, ecological origin, and environmental adaptation [13]. This emphasizes the significance of region-specific screening, since isolates adapted to local agroclimatic conditions may exhibit enhanced functional efficacy [14].

Despite this understanding, previous studies have mainly focused on chitinase and β-1,3-glucanase activities [15], while the contribution of cellulase and protease to the enzymatic biocontrol potential of regional *Trichoderma* isolates has not been sufficiently explored. To address this gap, the present study was undertaken to qualitatively screening cellulase and protease activity in 21 *Trichoderma* isolates collected from agroecological zones of Punjab, Katra (Jammu and Kashmir) and Mandi (Himachal Pradesh) [Figure 1], with the objective of identifying isolates with enzymatic traits relevant to biocontrol-based disease management, where cellulase and protease contribute by facilitating pathogen cell wall degradation during mycoparasitism.

2. MATERIALS AND METHODS

2.1. Collection of Soil Samples

A total of 21 rhizosphere soil samples were collected during 2023–2024 from uncultivated fields across Himalayan (Jammu and Kashmir

---

*Corresponding Author:
Vipul Kumar,
Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India.
E-mail: vipul.19845 @ lpu.co.in*

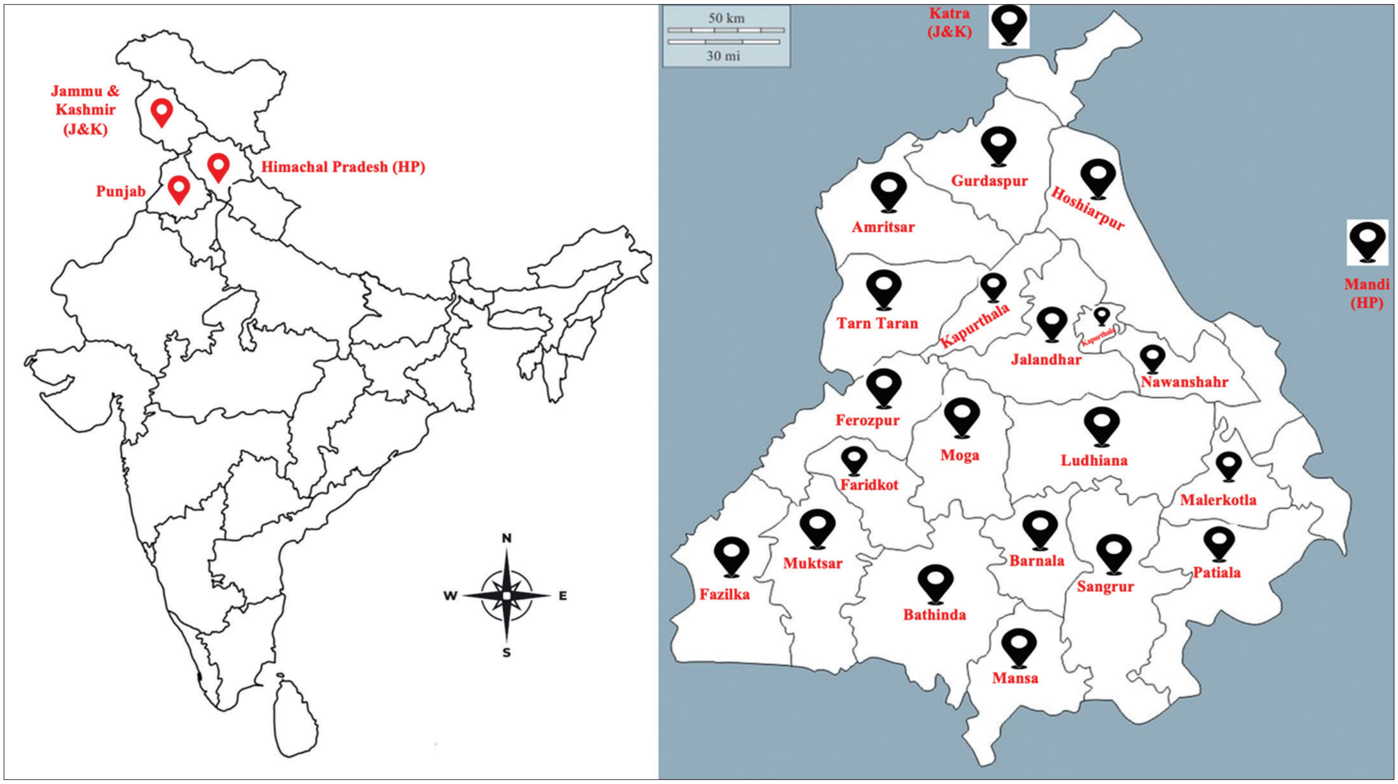

Figure 1: Distribution of soil sample collection sites used for the study.

and Himachal Pradesh) and Punjab, India. To avoid the agrochemical impact, rhizosphere soil from naturally growing vegetation in uncultivated fields was collected, thereby allowing the isolation of *Trichoderma* with inherent biocontrol potential [27]. Soil samples were collected using an auger from the top 0 to 15 cm, because this zone has the highest microbial population, organic matter concentration, and root-microbe interaction, making it the most biologically active soil horizon [28]. To enhance representativeness and tackle spatial heterogeneity approximately 500 g of soil per sampling point was taken from five subsamples within 25 m^2 area to form a composite sample. The samples were then placed in sterile polybags, kept cool during transportation, and processed within 24 h. Global positioning system coordinates of each site were recorded [Table 1].

2.2. Isolation of the *Trichoderma* strains

For isolation, 1 g of soil was suspended in 90 mL of sterile distilled water in 100 mL conical flask and homogenized on a vortex at 200 rpm for 15 min, followed by serial dilution up to 10^{-6} [16]. Aliquot (100 µL) from each dilution was disseminated on Rose Bengal Agar plates, which served as a selective medium for *Trichoderma* due to its inhibitory effect on rapidly growing fungal pathogenic molds and bacterial contaminants and incubated at 25°C for 3 days [29]. Distinct colonies were transferred to potato dextrose agar (PDA) plates and purified by single spore isolation before qualitative enzyme assay [17]. Isolates were identified based on pigmentation, conidiophore structure, and conidial morphology [30]. For further studies, *Trichoderma* cultures were maintained on PDA slants at 4°C [15].

2.3. Background on biocontrol screening of *Trichoderma* isolates

The biocontrol efficacy of *Trichoderma* isolates against *Fusarium oxysporum* and *Sclerotium rolfsii* was previously evaluated using dual culture technique under a completely randomized design with three replications and has been reported in detail [15].

2.4. Molecular characterization of *Trichoderma* isolates

Isolates with maximum pathogen growth inhibition were subjected to molecular identification using the cetyltrimethylammonium bromide DNA extraction method and internal transcribed spacer (ITS) region sequencing with universal primers ITS1 and ITS4 under standardized polymerase chain reaction conditions. The amplified ITS products were sequenced by a commercial sequencing service (Mr. Biologist India Pvt. Ltd., Pune, India) using Sanger sequencing technology. Sequence similarity searches were conducted using the BLASTn tool of the NCBI GenBank database, and species identification was confirmed based on the highest sequence identity [31].

2.5. Media preparation for the enzymatic assay

Casein Agar Medium (CAM) and Czapek Mineral Salt Agar (CMSA) were prepared for the detection of protease and cellulase activity, respectively. CAM was composed of yeast extract (10 g/L), glucose (0.5 g/L), K_2HPO_4 (0.5 g/L), $MgSO_4.7H_2O$ (0.2 g/L), agar (20 g/L) in 1000 mL distilled water and was supplemented with casein (10 g/L) as a substrate for proteolytic enzyme activity, while CMSA consisted of $NaNO_3$ (2 g/L), K_2HPO_4 (1 g/L), $MgSO_4.7H_2O$ (0.5 g/L), KCl (0.5 g/L), $FeSO_4.7H_2O$ (0.01 g/L), sucrose (30 g/L), and agar (20 g/L) in 1000 mL distilled water. The CMSA medium was supplemented with carboxymethyl cellulose (CMC; 10 g/L) as a carbon source to facilitate cellulase induction. All chemicals were procured from HiMedia Laboratories Pvt. Ltd., India. The pH of CAM was adjusted to 7 using 1N NaOH and pH of CMSA was adjusted to 5 using 1N HCl before sterilization. Media components, including CMC, were autoclaved at 121.7°C for 20 min under 15 psi pressure.

Table 1. Geographic coordinates of soil sampling sites used for *Trichoderma* isolation and enzyme assay.

Isolates	Soil sampling sites	Global positioning system location
PBT1	Faridkot	30.6774° N, 74.7539° E
PBT2	Barnala	30.3745° N, 75.5461° E
PBT3	Bathinda	30.2110° N, 74.9455° E
PBT4	Amritsar	31.6340° N, 74.8723° E
PBT5	Fatehpur Sahib	30.6435° N, 76.3970° E
PBT6	Fazilka	30.4036° N, 74.0280° E
PBT7	Ferozpur	30.9331° N, 74.6225° E
PBT8	Gurdaspur	32.0414° N, 75.4031° E
PBT9	Malerkotla	30.5246° N, 75.8783° E
PBT10	Katra (J&K)	32.99059° N, 74.93717° E
PBT11	Jalandhar	31.3260° N, 75.5762° E
PBT12	Kapurthala	31.3723° N, 75.4018° E
PBT13	Ludhiana	30.9010° N, 75.8573° E
PBT14	Mandi (HP)	32.1024° N, 77.5619° E
PBT15	Mansa	29.9995° N, 75.3937° E
PBT16	Moga	30.8230° N, 75.1734° E
PBT17	Muktsar	30.4762° N, 74.5122° E
PBT18	Nawanshahr	31.1256° N, 76.1186° E
PBT19	Patiala	30.3398° N, 76.3869° E
PBT20	Sangrur	30.2451° N, 75.8449° E
PBT21	Tarn Taran	31.4539° N, 74.9268° E

2.6. Dye preparation for enzymatic assay

For protease detection, a Bromocresol Green (BCG) solution (0.03% w/v in sterilized distilled water) was prepared freshly in sterile distilled water and adjusted to maintain a pH of 8, which enabled the visualization of proteolytic activity as a transparent zone against a greenish-blue background [32]. For cellulase detection, a Congo red solution 0.1% (w/v in sterilized distilled water) was prepared and used as a primary stain, followed by treatment with 1M NaCl to destain and enhance zone clarity [33]. Plates were stained with respective dyes, kept undisturbed for 15 min to ensure uniform color development, and subsequently examined for clear halo zone formation.

2.7. Enzymatic assay

Enzymatic assays were carried out to evaluate the functional efficiency of *Trichoderma* isolates. Each plate of 9 cm diameter contained 20 mL of medium approximately centrally incubated with 0.5 cm diameter mycelial disc from an actively growing culture. Plates were incubated at 28 ± 2°C under 12 h light/12 h dark photoperiod and 82% relative humidity. After 7 days, the assay was performed under controlled incubation conditions, and zone formation around the colonies was measured in centimeters along two perpendicular axes, and the mean value was used for analysis. The enzymatic activity was expressed as the Enzyme Index (EI), calculated using the following formula:

$$EI=\frac{\text{Diameter of hydrolysis zone}}{\text{Diameter of fungal colony}}$$

All measurements were recorded as the mean of three independent replicates to minimize measurement bias.

2.7.1. Protease assay

Protease activity of *Trichoderma* isolates was determined using CAM following the standard plate assay method [18]. A 5-day-old 0.5 cm mycelial disc of *Trichoderma* isolates was placed at the center of each plate, as actively growing mycelium at this stage ensures uniform metabolic activity and reliable extracellular enzyme secretion [34]. The plates were incubated for 7 days and then treated with BCG Dye (pH 8 ± 0.2) which enables clear visualization of proteolytic activity. Protease activity was demonstrated by the development of a transparent halo zone surrounding the fungal colony, which was contrasted with a greenish-blue background, resulting from casein hydrolysis. The diameter of the colony and clear zone was measured.

2.7.2. Cellulase assay

The cellulase activity of *Trichoderma* isolates was tested using CMSA Medium for cellulase activity. Media was sterilized, poured into separate Petri plates, and a 0.5 cm mycelial disc of *Trichoderma* was placed at the center of each plate. The inoculated plates were kept in Biochemical Oxygen Demand incubator set at 28 ± 2°C for 7 days. After incubation, the petri-dishes were uniformly stained with 0.1% aqueous Congo red dye for 15 min, a duration optimized to ensure adequate binding of the dye to non-hydrolyzed cellulase without overstaining. Plates were then washed with sterilized water and flooded with sodium chloride (1 M NaCl) for 90 s, to enhance contrast between hydrolyzed and non-hydrolyzed regions [33]. The presence of a yellowish opaque zone around the colonies indicated production of cellulase enzyme. Both the colony diameter and the diameter of the clear zone were measured along two perpendicular axes, and mean values were calculated from three independent replicates. Measurement was performed manually; no digital imaging software was used [18].

2.8. Data Analysis

Data obtained from the enzymatic assay were subjected to one-way analysis of variance (ANOVA) to determine differences among *Trichoderma* isolates. Each treatment consisted of three independent replicates (n = 3). Before analysis, the Shapiro–Wilk test for normality and Levene's test for homogeneity of variances were used to verify that the data met the assumptions of the ANOVA. In addition, residual plots were visually inspected to confirm that ANOVA assumptions were adequately met. The balanced design (n = 3) guarantees the robustness of ANOVA to such deviations, even though there were slight differences from normality. Variances were homogeneous ($P > 0.05$) at the 95% confidence level [24,25]. Following the ANOVA, Duncan's Multiple Range Test (DMRT) was used to separate means at $P \leq 0.05$, as DMRT is well suited for biological screening studies where the objective is to identify the difference among multiple treatments with greater sensitivity compared to more conservative tests such as Tukey's Honestly Significant Difference [26]. The packages, ggplot2 for graphical visualization, dplyr for data handling, and agricolae for DMRT were used in all statistical analysis, which were carried out in RStudio (version 2024.12.1).

3. RESULTS

3.1. Identified Species

ITS sequence analysis identified the potent isolates as *Trichoderma lixii* (PBT9; accession no. MK288146.1), *Trichoderma harzianum* (PBT13; accession no. MF87546.1), *Trichoderma virens* (PBT3;

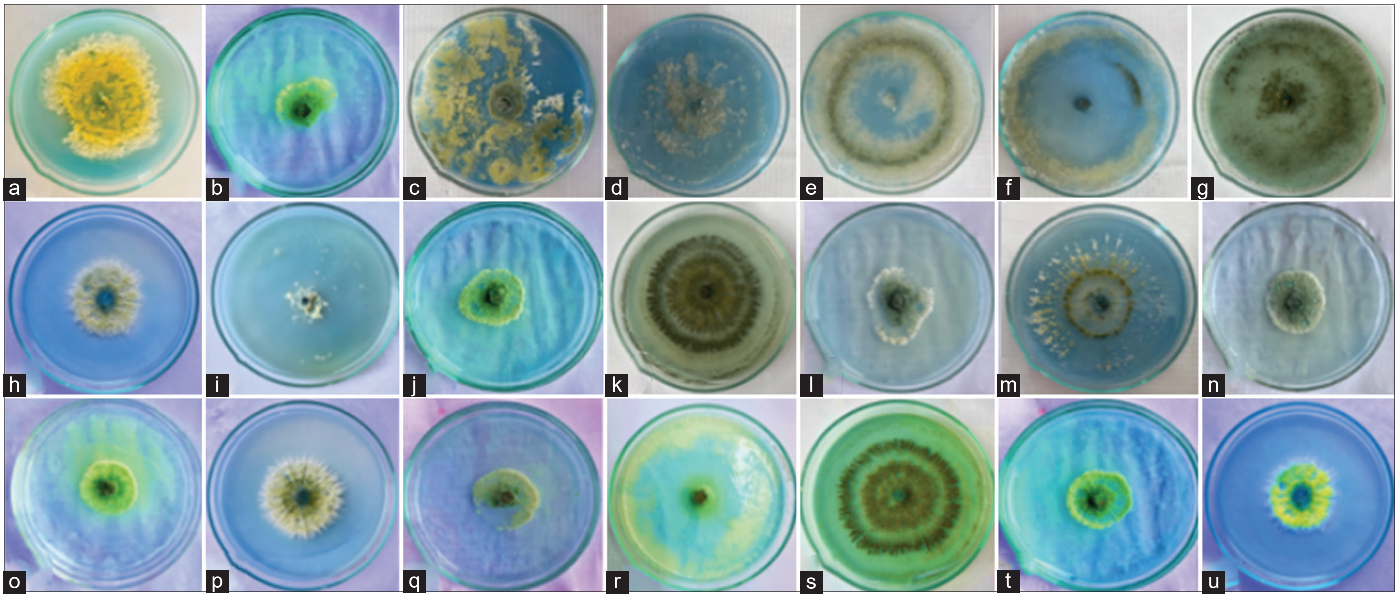

Figure 2: (a-u) Plate-based protease activity zones of *Trichoderma* isolates showing a halo zone formed on casein agar after incubation which indicates qualitative protease activity.

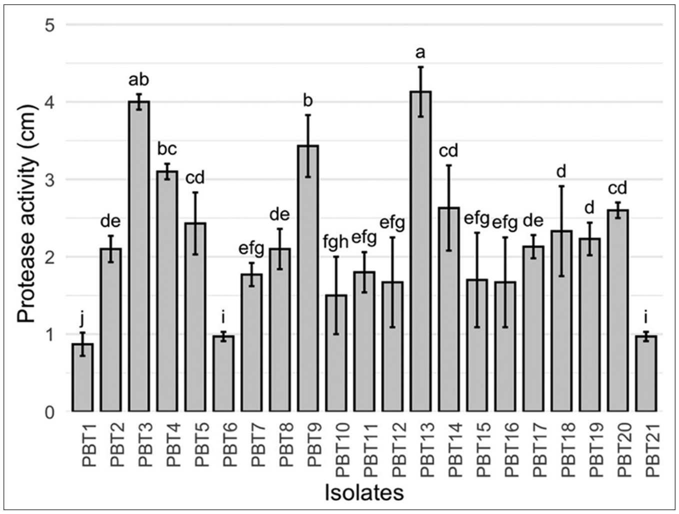

Figure 3: Qualitative comparison of protease activity of *Trichoderma* isolates. Different letters above bars indicate significant difference according to Duncan's multiple range test at $P \leq 0.05$.

accession no. MN452840.1), *Trichoderma asperellum* (PBT 4; accession no. MN046976.1) [15].

3.2. Protease Activity

The protease activity of *Trichoderma* isolates differed significantly across regions [ANOVA, $P \leq 0.05$; Table 2]. The highest protease activity was observed in the isolate PBT13, which formed a distinct statistical group (DMRT, group "a"), indicating significantly superior enzyme production. This was followed by PBT3 and PBT9, which also showed significantly higher activity than most other isolates (group "ab" and "b"). Moderate protease activity was recorded in isolates PBT4 and PBT14, while the lowest protease activity was found in PBT6, PBT21, and PBT1 [Figures 2 and 3, Table 2].

Table 2: Quantitative measurement of cellulase and protease activity in *Trichoderma* isolates.

Isolates	Location	Cellulase activity (cm)	Protease activity (cm)
PBT1	Faridkot	$1.17^{ef}\pm0.15$	$0.87^{j}\pm0.15$
PBT2	Barnala	$1.13^{ef}\pm0.06$	$2.10^{de}\pm0.17$
PBT3 (*Trichoderma virens*)	Bathinda	$2.10^{a}\pm0.10$	$4.00^{a}\pm0.10$
PBT4 (*Trichoderma asperellum*)	Amritsar	$1.80^{b}\pm0.10$	$3.10^{bc}\pm0.10$
PBT5	Fatehpur Sahib	$1.20^{ef}\pm0.10$	$2.43^{cd}\pm0.40$
PBT6	Fazilka	$1.63^{cd}\pm0.21$	$0.97^{i}\pm0.06$
PBT7	Ferozpur	$1.20^{ef}\pm0.10$	$1.77^{efg}\pm0.15$
PBT8	Gurdaspur	$1.23^{ef}\pm0.15$	$2.10^{de}\pm0.26$
PBT9 (*Trichoderma lixii*)	Malerkotla	$1.90^{b}\pm0.62$	$3.43^{b}\pm0.40$
PBT10	Katra (J&K)	$1.47^{de}\pm0.06$	$1.50^{fgh}\pm0.50$
PBT11	Jalandhar	$1.70^{bc}\pm0.20$	$1.80^{efg}\pm0.26$
PBT12	Kapurthala	$1.10^{ef}\pm0.10$	$1.67^{efg}\pm0.58$
PBT13 (*Trichoderma harzianum*)	Ludhiana	$2.13^{a}\pm0.15$	$4.13^{a}\pm0.32$
PBT14	Mandi (HP)	$1.30^{de}\pm0.20$	$2.63^{cd}\pm0.55$
PBT15	Mansa	$1.70^{bc}\pm0.10$	$1.70^{efg}\pm0.61$
PBT16	Moga	$1.33^{de}\pm0.21$	$1.67^{efg}\pm0.58$
PBT17	Muktsar	$2.03^{ab}\pm0.35$	$2.13^{de}\pm0.15$
PBT18	Nawanshahr	$0.97^{f}\pm0.06$	$2.33^{d}\pm0.58$
PBT19	Patiala	$1.07^{f}\pm0.12$	$2.23^{d}\pm0.21$
PBT20	Sangrur	$1.13^{ef}\pm0.06$	$2.60^{cd}\pm0.10$
PBT21	Tarn Taran	$1.03^{f}\pm0.06$	$0.97^{i}\pm0.06$

Values represent mean±standard deviation (n=3). Mean followed by the same letter (s) within a column are not significantly different according to Duncan's Multiple Range Test at $P<0.05$. One-way analysis of variance indicated significant variation among isolates for both enzyme activities (protease: F=19.12, $P=4.69\times10^{-15}$; cellulase: F=11.12, $P=5.45\times10^{-11}$).

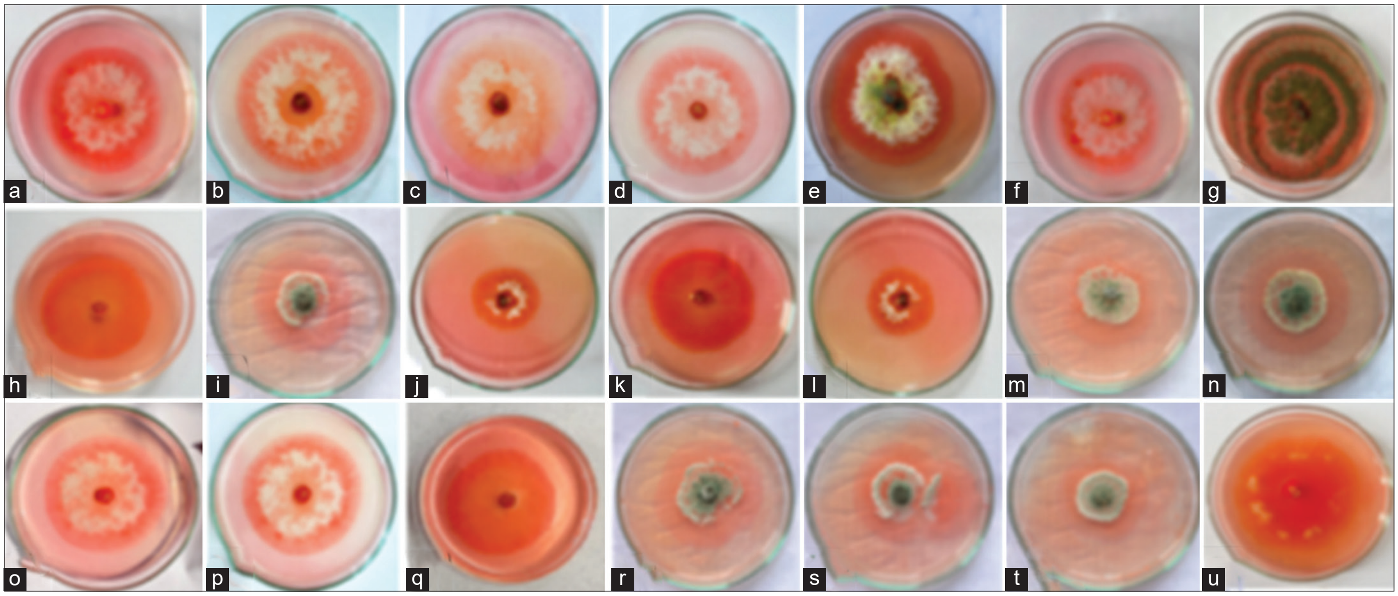

Figure 4: (a-u) Plate-based cellulase activity of *Trichoderma* isolates showing a halo zone formed on carboxymethyl cellulose agar after incubation which indicates qualitative cellulase activity.

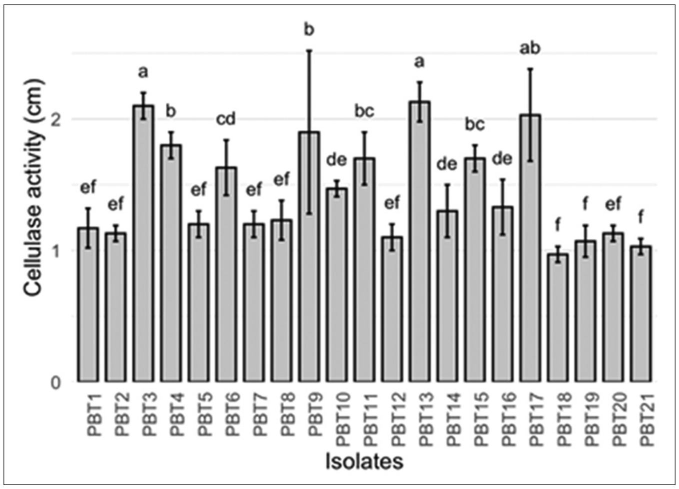

Figure 5: Qualitative comparison of cellulase activity among *Trichoderma* isolates. Different letters above bars indicate significant differences according to Duncan's multiple range test at $P \leq 0.05$.

3.3. Cellulase Activity

Plate assay revealed substantial variation in cellulase activity among *Trichoderma* isolates, where clearance zone diameter was used as an indicator of cellulase production [Table 2]. PBT13 and PBT3 showed the highest protease activity and were statistically comparable and PBT9, which were grouped among the top-performing isolates [Figures 4 and 5, Table 2].

4. DISCUSSION

Hydrolytic enzyme production is an important mechanism in *Trichoderma*-mediated antagonism, as these enzymes facilitate pathogen cell wall degradation and mycoparasitism. Previous studies have demonstrated that *Trichoderma* isolates from diverse environments consistently produce extracellular cellulase and protease, reflecting their metabolic adaptability and functional plasticity [36]. In particular, significant amounts of cellulolytic enzymes secreted by *Trichoderma* play a key role in suppressing phytopathogens by breaching host cell walls during penetration [19,22]. Consistent with this mechanism, Mishra (2010) reported that higher cellulase activity contributed to the suppression of mycelial growth of *Pythium aphanidermatum* [23].

Protease activity in *Trichoderma* has been directly linked to reduced disease severity and pathogen fitness in several *Trichoderma*-pathogen interaction studies, establishing a functional link between protease production and disease suppression [37]. At the mechanistic level, fungal proteases contribute to cell wall lysis through cleavage of peptide bonds in structural proteins [20], and their role in reducing disease severity and pathogen sporulation has been demonstrated in several host-pathogen systems [21].

Beyond protease and cellulase, multiple hydrolytic enzymes collectively contribute to *Trichoderma*-mediated biocontrol, including chitinase and β-1,3-glucanase involved in mycoparasitism and pathogen cell wall degradation, reinforcing the multifactorial enzymatic basis of antagonism [15]. The production of these enzymes is regulated by both genetic factors and environmental factors, such as nutrient availability, carbon source, pH, and the presence of pathogen-derived signals, which influence enzyme induction and secretion in *Trichoderma* [38]. In this context, the present study complements the earlier findings and further supports the enzymatic basis of their antagonistic potential.

The observed diversity in enzyme production among isolates highlights the heterogeneous nature of *Trichoderma* population, which may arise from genetic variation and adaptation to local environmental conditions. Such functional diversity emphasizes the importance of region-specific screening when identifying *Trichoderma* isolates for biocontrol-related applications [35].

The present study was limited to qualitative plate assays and evaluating cellulase and protease activity under *in vitro* conditions. Other biocontrol-related enzymes were not assessed, and field-level validation of biocontrol efficacy was not included.

5. CONCLUSION

The comparative evaluation of *Trichoderma* isolates revealed variability in cellulase and protease activities among isolates collected from different agroecological regions. Some isolates exhibited higher enzyme activity, indicating their relevance for further investigation in relation to crop productivity and suppression of phytopathogens. The outcome of this study lies in establishing a region-specific, enzyme-based qualitative screening approach to identify *Trichoderma* isolates with biocontrol-relevant enzyme traits. These findings support the value of enzyme screening as an integral part of *Trichoderma* characterization for sustainable plant disease management. Future research should focus on quantitative enzyme profiling, multi-enzyme assays, formulation development, improving the enzyme production ability of potential isolates, and field validation to translate these laboratory observations into practical biocontrol applications.

6. AUTHOR'S CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. ACKNOWLEDGMENT

This study was carried out as a part of the first author's Ph.D. at the Lovely Professional University. The authors gratefully acknowledge the training, technical, and institutional support provided by the Lovely Professional University, Punjab, and UPL University of Sustainable Technology, Gujarat, which contributed significantly to the success of this research work.

8. FUNDING

There is no funding to report.

9. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

The article does not contain any experiments on animal or human subjects.

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. PUBLISHER'S NOTE

13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Yasir M, Hossain A, Pratap-Singh A. Pesticide degradation: Impacts on soil fertility and nutrient cycling. Environments. 2025;12:272. https://doi.org/10.3390/environments12080272
2. Gómez-Lama Cabanás C, Mercado-Blanco J. Groundbreaking technologies and the biocontrol of fungal vascular plant pathogens. J Fungi (Basel). 2025;11:77. https://doi.org/10.3390/jof11010077
3. Abdul-Halim AM, Shivanand P, Krishnamoorthy S, Taha H. A review on the biological properties of *Trichoderma* spp. as a prospective biocontrol agent and biofertilizer. J Appl Biol Biotechnol. 2023;11:34-46. https://doi.org/10.7324/JABB.2023.11504
4. Cyriac A, Sible GV, Johnson JM, Radhika NS, Krishnan AG. Antagonistic efficacy of *Trichoderma* isolates against soil-borne plant pathogens *Pythium aphanidermatum* and *Rhizoctonia solani*. J Biol Control. 2021;35:48-56. https://doi.org/10.18311/jbc/2021/26283
5. Algifari MH, Prismantoro D, Hafsari AR, Miranti M, Wan-Mohtar WAAQI, Ozturk AB, *et al*. Performance of hydrolytic enzymes produced by Trichoderma in sustainable crop production: Current insights and future perspectives. Sustain Environ. 2025;11(1):2593746. https://doi.org/10.1080/27658511.2025.2593746
6. Patel TK. Fungal biocontrol agents for disease management in crops. In: Microbial Approaches for Sustainable Green Technologies. Boca Raton: CRC Press; 2024. p. 176-95. https://doi.org/10.1201/9781003407683
7. Narayanasamy P. Mechanisms of action of fungal biological control agents. In: Biological Management of Diseases of Crops. Vol. 1. Dordrecht: Springer; 2013. p. 99-200. https://doi.org/10.1007/978-94-007-6380-7_3
8. Khatri DK, Tiwari DN, Bariya HS. Chitinolytic efficacy and secretion of cell wall-degrading enzymes from *Trichoderma* spp. in response to phytopathological fungi. J Appl Biol Biotechnol. 2017;5(6):1-8. https://doi.org/10.7324/JABB.2017.50601
9. Paveen P, Kumar V, Gupta RK, Sadawarti RK, Alamri S, Siddiqui MH, *et al*. Integrated use of biochar, *Cassia fistula*, and *Trichoderma* for sustainable management of *Sclerotium rolfsii* in chickpea. BMC Plant Biol. 2025;25:895. https://doi.org/10.1186/s12870-025-06848-5
10. Dehghani F, Wagner RC, Blagodatskaya E, Schlüter S, Reitz T. Microbial decomposition of cellulose in soil: Roles of resource stoichiometry and water content. Eur J Soil Sci. 2025;76:e70184. https://doi.org/10.1111/ejss.70184
11. Paiva-Silva C, Proença Pereira J, Marcolino F, Figueiredo A, Santos RB. Silenced cutters: Mechanisms and effects of protease inhibition in plant-pathogen interactions. J Exp Bot. 2025;76:4248-61. https://doi.org/10.1093/jxb/eraf156
12. Mishu NJ, Hasan MR, Islam SMN, Nayeema J, Hossain MM. Additive effects of *Trichoderma* isolates in enhancing growth and suppressing southern blight in tomato. PLoS One. 2025;20:e0329368. https://doi.org/10.1371/journal.pone.0329368
13. El-Sobky MA, Eissa RA, Abdel-Lateif KS, Fahmi AI, El-Zanaty AM, Hassan MM, *et al*. Genetic diversity and cellulolytic activity of *Trichoderma* spp. from Egyptian soils. Egypt J Biol Pest Control. 2024;34:24. https://doi.org/10.1186/s41938-024-00784-6
14. Garcha S, Katyal P, Sharma V. Microbial diversity in soil under different land-use systems of sub-mountainous Punjab. J Indian Soc Soil Sci. 2016;64:271-5. https://doi.org/10.5958/0974-0228.2016.00038.4

15. Kumari R, Kumar V, Arukha AP, Rabbee MF, Ameen F, Koul B. Screening of the biocontrol efficacy of potent *Trichoderma* strains against *Fusarium oxysporum* f.sp. ciceri and *Scelrotium rolfsii* causing wilt and collar rot in Chickpea. Microorganisms. 2024;12:1280. https://doi.org/10.3390/microorganisms12071280
16. Akhtar R, Showkat S, Saini N. Isolation and identification of soil-borne fungi from agricultural fields of Dehradun, India. Int J Environ Sci. 2022;4:60-5.
17. Xue M, Wang R, Zhang C, Wang W, Zhang F, Chen D, *et al*. Screening and identification of Trichoderma strains isolated from natural habitats in China with potential agricultural applications. Biomed Res Int. 2021;2021:7913950. https://doi.org/10.1155/2021/7913950
18. Cherkupally R, Amballa H, Reddy BN. *In vitro* antagonistic activity of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *Melongenae*. Int J Appl Agric Res. 2017;12:87-95.
19. Kapat A, Zimand G, Elad Y. Effect of *Trichoderma harzianum* isolates on hydrolytic enzymes of *Botrytis cinerea*. Physiol Mol Plant Pathol. 1998;52:127-37. https://doi.org/10.1006/pmpp.1997.0140
20. Yike I. Fungal proteases and their pathophysiological effects. Mycopathologia. 2011;171:299-323. https://doi.org/10.1007/s11046-010-9386-2
21. Haggag WM, Kansoh AL, Aly AM. Proteases from *Talaromyces flavus* and *Trichoderma harzianum* and their antifungal activity. Plant Pathol Bull. 2006;15:231-9. https://doi.org/10.6649/PPB.200612_15(4).0003
22. Gruber S, Seidl-Seiboth V. Self-versus non-self: Fungal cell wall degradation in *Trichoderma*. Microbiology. 2012;158:26-34. https://doi.org/10.1099/mic.0.052613-0
23. Mishra VK. *In vitro* antagonism of *Trichoderma* spp. against *Pythium aphanidermatum*. J Phytol. 2010;2:9.
24. Glass GV, Peckham PD, Sanders JR. Consequences of failure to meet ANOVA assumptions. Rev Educ Res. 1972;42:237-88. https://doi.org/10.3102/00346543042003237
25. Lix LM, Keselman JC, Keselman HJ. Alternatives to the one-way ANOVA F test. Rev Educ Res. 1996;66:579-619. https://doi.org/10.3102/00346543066004579
26. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. 2nd ed. New York: John Wiley & Sons; 1984.
27. Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Ściseł J. *Trichoderma* in agriculture: Biocontrol and plant growth stimulation. Int J Mol Sci. 2022;23:2329. https://doi.org/10.3390/ijms23042329
28. Shen Y, Xiong S, You C, Zhang L, Li Y, Hong Z, *et al*. Soil microbial biomass across a chronosequence. Forests. 2023;14:470. https://doi.org/10.3390/f14030470
29. Atlas RM. Handbook of Microbiological Media. 4th ed. London: CRC Press; 2010. https://doi.org/10.1201/9781420039726
30. Gams W, Bissett J. Morphology and identification of Trichoderma. In: *Trichoderma and Gliocladium*. Vol. 1. London: Taylor & Francis; 2002.
31. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, *et al*. Gapped BLAST and PSI-BLAST. Nucleic Acids Res. 1997;25:3389-402. https://doi.org/10.1093/nar/25.17.3389
32. Vijayaraghavan P, Vincent SGP. Detection of protease activity using bromocresol green dye. J Biochem Technol. 2013;4:628-30.
33. Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A. Detection of microbial cellulases on agar plates. Curr Microbiol. 2008;57:503-7. https://doi.org/10.1007/s00284-008-9276-8
34. Rajani P, Rajasekaran C, Vasanthakumari MM, Olsson SB, Ravikanth G, Shaanker RU. Inhibition of plant pathogens by endophytic *Trichoderma*. Microbiol Res. 2021;242:126595. https://doi.org/10.1016/j.micres.2020.126595
35. Rojas-Villa N, Ormeño-Vásquez P, Pedrozo P, Oré-Asto B, Moriano-Camposano J, Álvarez LA. Functional diversity in *Trichoderma* from low-anthropogenic Peruvian soils reveals distinct antagonistic strategies enhancing the biocontrol of Botrytis cinerea. Agriculture. 2026;16(1):112. https://doi.org/10.3390/agriculture16010112
36. Antal Z, Kredics L, Manczinger L, Ferenczy L. Extracellular enzyme profiles of mycoparasitic Trichoderma strains. IOBC/WPRS Bull. 2001;24(3):337-40.
37. Kredics L, Antal Z, Szekeres A, Hatvani L, Manczinger L, Vágvölgyi CS, *et al*. Extracellular proteases of *Trichoderma* species. Acta Microbiol Immunol Hung. 2005;52:169-84. https://doi.org/10.1556/AMicr.52.2005.2.3
38. Yan S, Xu Y, Yu XW. From induction to secretion: A complicated route for cellulase production in *Trichoderma reesei*. Bioresour Bioprocess. 2021;8:107. https://doi.org/10.1186/s40643-021-00461-8

How to cite this article:
Kumari R, Sarkar M, Archana TS, Kumar V, Panda SR, Wagh AK. Screening of *Trichoderma* isolates from Punjab and Himalayan region for hydrolytic enzyme production. J Appl Biol Biotech 2026;14(4):108-114. DOI: 10.7324/JABB.2026.292854