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Research Article

Exploration of native potential biological control agents from various agro-climatic regions of Arunachal Pradesh and its bio-efficacy against soil borne pathogens

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ABSTRACT: The biological control agent is one of the main components of eco-friendly management of insect pests and diseases for sustainable agricultural productivity and aids in reducing the excessive use of harmful pesticides. Hence, native *Trichoderma* spp. were isolated from various agro-climatic regions of Arunachal Pradesh covering ten districts. All the *Trichoderma* isolates were fast-growing spreading type covering the entire Petri-plates within 4-5 days, velutinous colonies with whitish colour, light green to dark green and yellowish colour floccose rising surface of mycelial growth with zero to four concentric ring-like zones of patterns. The microscopic study revealed that conidiophores are moderate to highly branched, light green to bluish colour, phialides of flask-shaped, present in whorls of 2-4, abruptly attenuated near the ends, and size ranging from 6.7 to 8.8 µm. Round to oval-shaped and light green to yellow colour conidia were arranged in clusters with size ranging from 3.2 to 5.3 µm. *Trichoderma* sp. (CHF.PP.23.05) showed the highest antagonistic properties with 77.7% and 83.3% inhibition against *Fusarium* sp. and *Rhizoctonia* sp. followed by CHF.PP.23.11 with 76.6% and 77.7%, respectively. NCBI blast of the resulting sequence of 552 bp DNA of *Trichoderma* sp. CHF.PP.23.05 (GenBank Acc. PQ056528) revealed high nucleotide homology of 99% (GenBankAccOR166806) similarity with *Trichoderma asperellum*. Hence, the native biocontrol agent, *T. asperellum* (CHF.PP.23.05) can be considered as potential biocontrol agent for the management of economically important agricultural and horticultural crops.

KEYWORDS: Native, molecular characterization, north-eastern region, soil-borne pathogen, *Trichoderma asperellum*

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INTRODUCTION

Arunachal Pradesh is situated among one of the world's top biodiversity hotspots, the eastern Himalaya biodiversity bordering Indo-Burma hotspot having a wide range of flora and fauna species and endemism diversity. However, the microbial diversity in this region is less explored as a result of lack of research activities. Study of microbial diversity in Arunachal Pradesh may reveal the beneficial soil microbes specially biocontrol agents. Effective alternative options are needed to tackle the environmental and health hazards arising from excessive use of chemical control. Among the eco-friendly control measures, biological control agents such as fungal antagonists, especially native *Trichoderma* strains, can be one of the effective and potential approaches for the management of various plant diseases. *Trichoderma* fungi are well known for their antagonism against several soil phytopathogens. It is free-living fungi that are found in association with soil and root ecosystems, thereby promoting plant growth.

Full exploitation of the BCA potential of *Trichoderma* strain could provide growth enhancement to domestic plants and crops. However, no remarkable work has been done to protect the crops from various soil-borne pathogens by native *Trichoderma* strains in the humid sub-tropical region of Arunachal Pradesh. Therefore, our research may offer an

opportunity to introduce and establish specific biocontrol agents in soils. Hence, with a view of its important fungicidal activities, the survey was conducted to explore the native *Trichoderma* species from the untouched nature, agro-climatic conditions of Arunachal Pradesh.

MATERIALS AND METHODS

Soil samples were collected from different bio-geographic regions of Arunachal Pradesh covering eighteen places from ten districts namely, Lower Subansiri, Changlang, Namsai, Lohit, Dibang Valley, Upper Siang, Longding, Lower Dibang Valley, Shiyomi and East Siang. The soil samples were air dried under shade and kept sealed in bag for further assay.

Isolation of native strain of *Trichoderma* from the collected soil samples was done as per procedure of Dhingra and Sinclair (1985).

The fungal cultural growths were purified by single spore isolation technique (Riker& Riker, 1936) for morphological characterization and further study. Mycelial discs of young growing culture of an isolate of *Trichoderma* were inoculate centrally into the petriplates containing solidified PDA and incubate at 27±2°C for one week. *Trichoderma* isolates were morphologically identified as per Rifai (1969) subsequently modified by Bissett (1991) considering texture of colony growth, colour, formation of zonation and pigmentation in reverse side of petriplate.

Antagonistic potential of the isolates of *Trichoderma* spp. were evaluated by dual culture technique and rated as per scale of Bell *et al.* (1982) and partial modification by Saha and Pan (1997). Percentage of radial growth inhibition (PRGI) calculated by using the formula (Skidmore & Dickinson, 1976): $[(R_1–R_2)/R1\times100]$, where R_1 = growth of the control and R_2 = growth in treatment.

For molecular characterisation *Trichoderma* isolate was grown in potato dextrose broth at 27± 2°C for 4-5 days and mycelial mat was harvested by filtration through Whatman No.1 filter paper and washed with sterile distilled water and dried for DNA extraction. Fungal genomic DNA was extracted using the method of Raeder and Broda (1985). DNA was resuspended in 50 µl of 10 mMTris–1 mM EDTA (pH 8) and quantified by use of ethidium bromide fluorescence (Sambrook *et al*., 1989).

PCR Amplification: The universal primers ITS1 F (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 R (5'-TCC TCC GCT TAT TGA TAT GC-3') were used for amplification (White*et al.,* 1990). Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyze. Consensus sequence of ITS rDNA gene was generated from forward and reverse sequence data using aligner software.

Phylogenetic Analysis: The ITS rDNA gene sequence was used to carry out BLAST with the database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA XI.

RESULTS AND DISCUSSION

Macroscopic identification: Eighteen *Trichoderma* spp. were isolated successfully from the different agro-climatic conditions of Arunachal Pradgrowth (%) of both *Fusarium* sp esh covering ten districts. *Trichoderma* isolates exemplify mostly fast growth and covered the entire petri-plates within 4-5 days, velutinous colonies with whitish colour, light green to dark green and yellowish colour floccose rising surface of mycelial growth, and thin sooty light green to dark green colonies with zero to four concentric ring-like zones of patterns were also observed (Figure 1). Matas *et al.*(2022) portray *Trichoderma* isolate as whitish mycelium of spongy consistency with two to three concentric rings like zones. Fast growing and light green to dark green colonies were also observed (Chaverri *et al.,* 2015; García-Núñez *et al.,* 2017). The characteristics cultural growth of different species of *Trichoderma* were found similar, consequently, it is difficult to identify the isolates solitary on macroscopic morphological study (Gupta *et al.,* 2013).

Microscopic identification:Slide culture was carried out for microscopic morphological study. Isolated *Trichoderma* illustrates septate hyphae, moderate to highly branched conidiophores, compact conidia and ampulliform types of phialides were present at the tip of the branches of *Trichoderma* conidiophores. Round to oval-shaped conidia with size ranges from 3.2 to 5.3 µm in diameter and light green to yellow colour were identified. Light green to bluish colourphialides of flask-shaped, present in whorls of 2-4, abruptly attenuated near the ends and ranges from 6.7 to 8.8 µm in size (Table1).Morphological study of conidia may be utilised for the identification of *Trichoderma* spp. Savitha and Sriram (2015) revealed that *T. harzianum* can be easily differentiated from other species as their size of the conidia are smaller and light green in colour when compare with *T. asperellum*and *T. virens*. Study of the pattern of conidiophores and shapes of phialides were played the important key role for identification of *Trichoderma* spp.(Naher *et al.,* 2019).Presence of ampulliform types of phialides, oval-round shaped conidia with greenish-yellow pigmentation

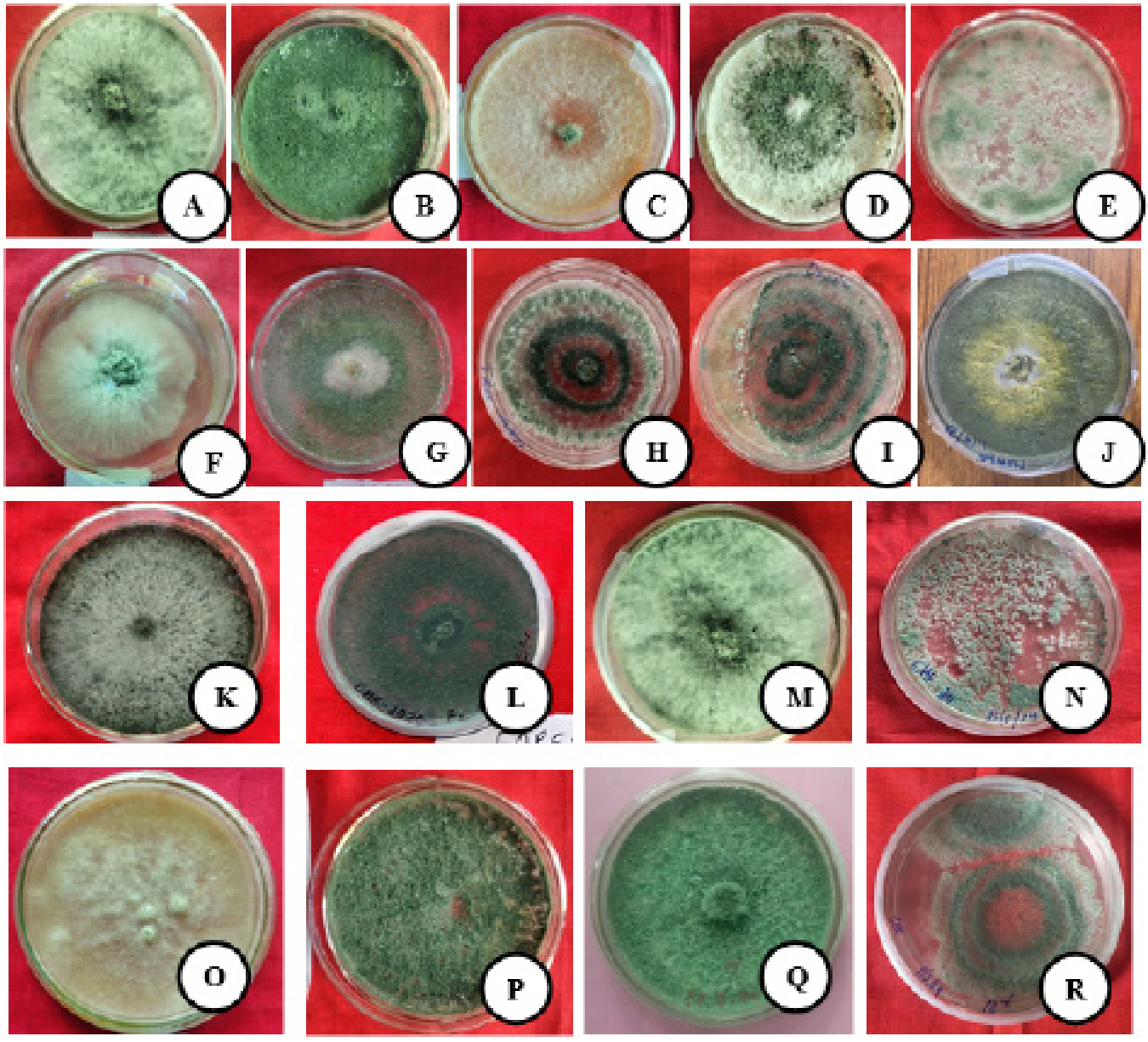


Figure 1. Morphological cultural growth of different *Trichoderma* isolates. A=CHF.PP.23.05; B=CHF.PP.23.06;C=CHF.PP.23.07;D=CHF.PP.23.08;E=CHF.PP.23.09;F=CHF.PP.23.10;G=CHF.PP.23.11;F=CHF.PP.23.12;I=CHF.PP.23.13;J=CHF.PP.23.14;K=CHF.PP.23.15;L=CHF.PP.23.16;M=CHF.PP.23.17;N=CHF.PP.23.18;O=CHF.PP.23.19;P=CHF.PP.23.20;Q=CHF.PP.23.21;R=CHF.PP.23.22.

Table 1. Morphological characterisation of *Trichoderma* isolates from ten different districts of Arunachal Pradesh

Place of collection	Isolate designation	Macroscopic observation	Microscopic observation	
		Colony morphology on PDA media	Conidia (µm)	Phialides(µm)
Ziro	CHF.PP.23.05	Thick light greenish rising floccose surface without concentric ring-like zone	4.7	6.8
Changlang	CHF.PP.23.06	Thin light green mycelia growth pattern without concentric ring-like zone	5.1	8.2
Namsai	CHF.PP.23.07	Thin whitish floccose surface without concentric ring-like zone	3.4	7.8
Tezu	CHF.PP.23.08	Thin whitish floccose surface at the base and dark green patches mainly at the centre without concentric ring-like zone	4.1	6.9

Ainini	CHF.PP.23.09	Whitish velutinous surface and scattered light green patches without concentric ring-like zone	4.6	8.1
Mariyang	CHF.PP.23.10	Thick greyish- green rising floccose surface with fine two concentric ring-like zones	4.2	7.4
Longding	CHF.PP.23.11	Thin greenish blue colony growth without concentric ring-like zone	3.0	7.6
Roing	CHF.PP.23.12	Light green rising floccose surface and dark green at the centre with fine three zonation	5.0	7.7
Hunli	CHF.PP.23.13	Dark green rising floccose surface with prominent four zonation	3.6	8.5
Desali	CHF.PP.23.14	Yellow-greenish thin floccose surface without any concentric ring-like zone	5.3	8.3
Monigong	CHF.PP.23.15	Thick whitish floccose surface and light green at the centre without concentric ring-likezone	3.4	7.2
Mechuka	CHF.PP.23.16	Thin dark green sooty surface with two zonation	4.8	8.8
Pasighat	CHF.PP.23.17	Thin light green mycelia growth pattern without concentric ring-like zone	3.5	7.4
Silli Village	CHF.PP.23.18	Thin light green and whitish patches without concentric ring-like zone	4.6	6.7
Bodak	CHF.PP.23.19	Thin whitish floccose surface with green pigmentation at the centre without concentric ring-like zone	3.2	6.8
Ranging	CHF.PP.23.20	Thin rising greenish sooty surface without concentric ring zone	4.4	6.8
Aing	CHF.PP.23.21	Thick whitish floccose surface and dark green patches at the centre without concentric ring-like zone	3.7	7.2
Ruksin.	CHF.PP.23.22	Light green rising floccose surface at the centre and whitish at the margin with fine three zonation	4.6	7.8

and branched conidiophores of *Trichoderma*species were also reported (Samuels *et al.,*1996). Various colony growth patterns of same species of *Trichoderma* is due to production of various types of enzymes such as cellulases and chitinases which provide to the colour changes of mycelia during its growth (Putty, 2010). Characterisation of morphology of *Trichoderma* strains, focuses on conidial pigmentation in white to bright green, mode of coniodiophore branching, facilitate their recognition at the genus level (Rifai, 1969). Therefore, both macroscopic and microscopic morphological studies collectively are required to bring the successful identification of *Trichoderma* species.

Results of antagonistic activity by dual culture technique of *Trichoderma* isolates and soil borne pathogen, *Fusarium* sp. (CHF.PP.24.07) and *Rhizoctonia* sp.(CHF. PP.24.01) isolated from research field trial at Department of Plant Protection, CHF, Pasighat, CAU(I), Arunachal Pradesh revealed the maximum PIRG (%) in CHF.PP.23.05 isolates from Ziro, Lower Subansiri district with 88.3% against *Rhizoctonia* and *Fusarium* (77.7%) which is followed by CHF.PP.23.11 with 76.6% and 77.7% inhibition against *Fusarium* and *Rhizoctonia* (Figures 2 and 3). PIRG of other remaining sixteen strains ranges from 54.4 % to 68.80% against *Fusarium* and in case of *Rhizoctonia* recorded 53.3% to 66.7%, respectively. Various study also showed *Trichoderma* spp as potential BCA exhibiting >50% growth inhibition of fungal pathogens such as *Sclerotium rolfsii, Ceratocystis paradox, Fusarium oxysporum, Colletotrichum capsicii and Rhizoctonia solani* (Waghunde *et al.,* 2016). Naher *et al.* (2019) showed that *T. parareesei* the best promising BCA species against *F. oxysporum* with 89% to 91% inhibition. In present study, all the tested *Trichoderma* isolates recorded >50% PIRG against soil borne pathogens. *Trichoderma harzianum* and *T. viride* have been widely considered as one of excellent and potential BCA to tone down *Fusarium* wilt and could replace hazardous chemical pesticides (Zandyavari *et al.,* 2024; Filizola *et al.*, 2019). Similarity to our finding Hamed *et al.* (2015) also reported that *T. asperellum* isolated from salinity soil also showed highly antagonistic effect against three important plant pathogens such as *Fusarium solani, Fusarium oxysporum,* and *Sclerotium* causing wilt disease and damping off causing disease, *Pythium myriotylum* (Mbarga *et al.,* 2012), it has been considered .as an successful BCA owing to its production of enzymes that hydrolyze cell walls of different microorganisms (Benítez *et al.,*2004). Hence, the strains CHF.PP.23.05 and CHF.PP.23.11 can be promoted as strong potential BCA for managing important soil-borne diseases after field trials.

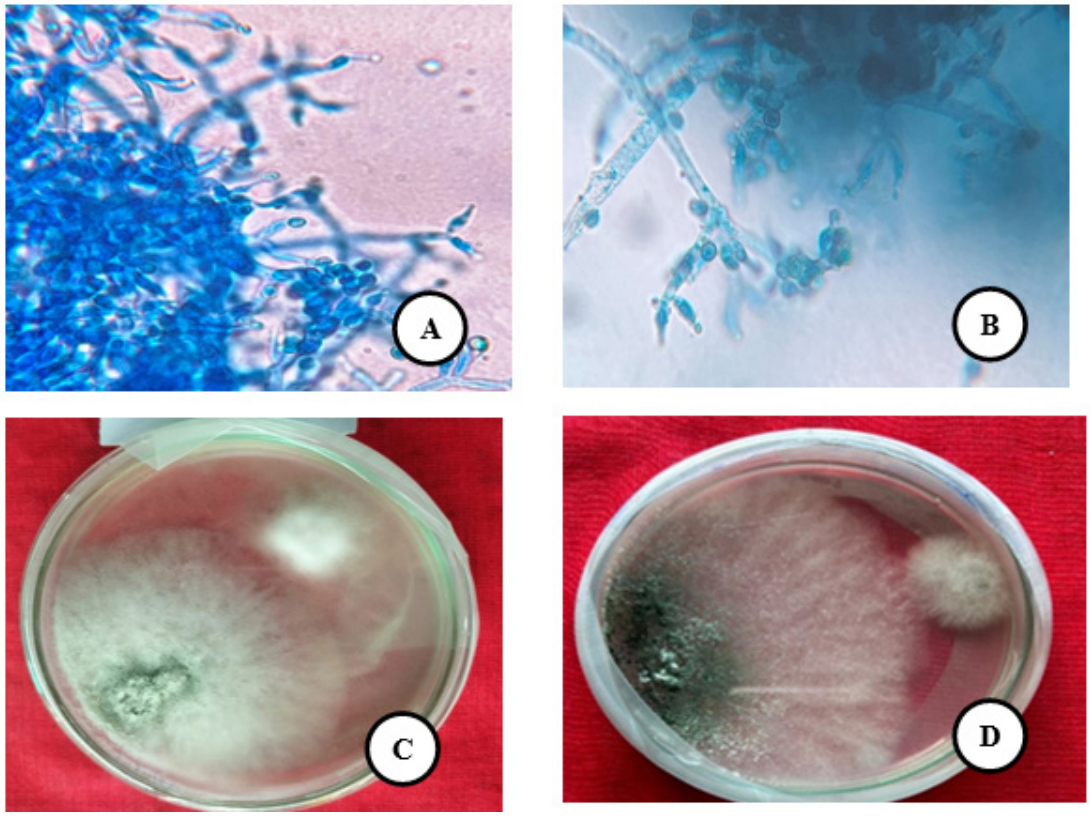
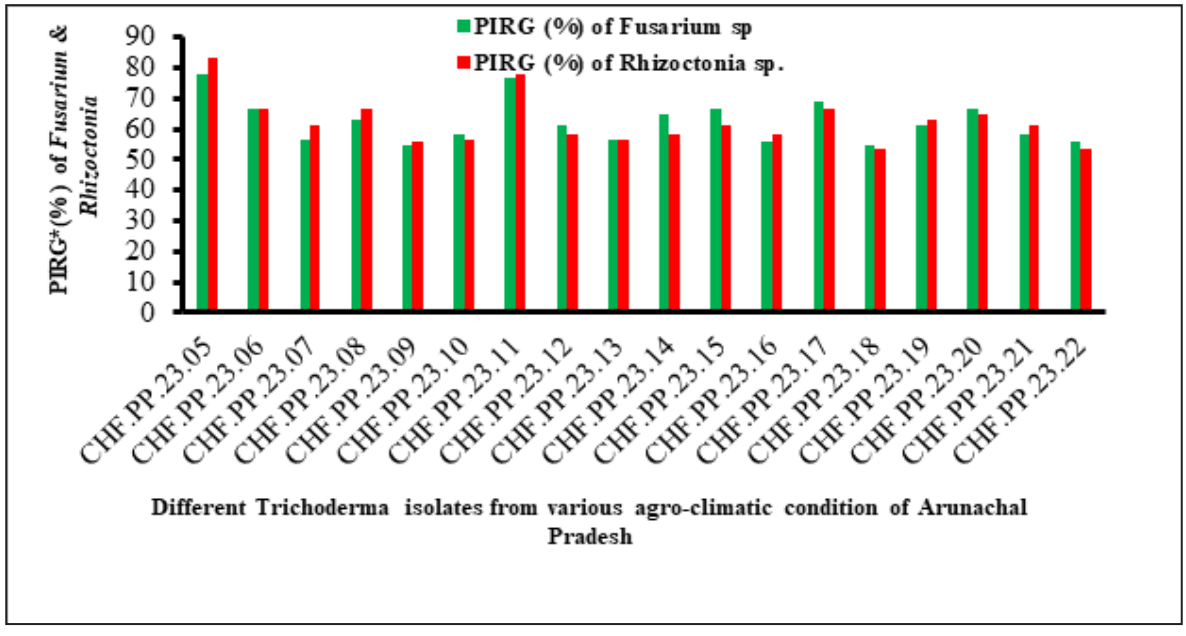


Figure 2. A, B: Microscopic morphological structure of strains CHF.PP.23.05 and CHF.PP.23.11; **C, D:** Antagonistic activity by dual culture technique of *T. asperellum* against *Fusarium* sp. and *Rhizoctonia* sp.



*PIRG= Percent Inhibition Radial Growth

Figure 3. Percentage inhibition radial growth (%) of both *Fusarium* sp. and *Rhizoctonia* sp. in the dual culture method with *Trichoderma* isolates.

Trichoderma isolate CHF.PP.23.05 from Ziro, Lower Subansiri district was selected for molecular identification as it showed the maximum PIRG against both target plant pathogens. Genomic DNA was isolated from BCA culture CHF.PP.23.05 and the fragment of the ITS rDNA gene was amplified using ITS1 and ITS4 primers. The ITS rDNA gene sequence was subjected to BLAST analysis in the NCBI gene bank. Based on the maximum identity score, seven sequences were selected and aligned using the multiple alignment software program Clustal W. A distance matrix was generated and the phylogenetic tree was constructed using MEGA version XI.

NCBI blast of the resulting sequence of 552 bp DNA of *Trichoderma* sp. CHF.PP.23.05 (GenBank Acc. PQ056528) revealed high nucleotide homology of 99 % (GenBank Acc. OR166806) similarity with *T. asperellum*. The study of molecular characterisation assists to portray the isolated cultures and confirm that the analysis used lead to a precise and reliable taxonomic identification (Matas-Baca *et al.,* 2022). Freeman *et al.* (2000) also have the same opinion that the primers ITS1 and ITS 4 are specific for fungi and amplified a DNA fragment of about 560 bp using *T. asperellum.*

CONCLUSION

The *Trichoderma asperellum* (CHF.PP.23.05) isolated from the agro-climatic region of Arunachal Pradesh serves as potential native strain from this region. It has highly effective antagonistic properties against soil-borne pathogens, *Fusarium* sp. and *Rhizoctonia* sp. with 77.7% and 83.3% inhibition under *in-vitro* conditions. This indicates the effectiveness of native strain of biocontrol agents for management of various plant pathogens. Therefore, native *T. asperellum* bearing Acc. PQ056528 can be used for developing potential bio-pesticide to manage soil borne diseases. However, there is a need for multi-location field trials in different economically important agricultural and horticultural crops of this region against the plant pathogens for its validation.

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