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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Trichoderma* SPECIES PREVAILING IN THE MAJOR PULSE GROWING AREAS OF INDIA

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ABSTRACT

The present study was aimed to characterize the *Trichoderma* species prevailing in the major pulse growing areas of India. The *Trichoderma* isolates (54) were collected from the rhizospheric soils of chickpea, pigeonpea and rice-pulse cropping system in Madhya Pradesh, Uttar Pradesh and Maharashtra states of India. The isolates were characterized using their culture-morphological and molecular characteristics. Compact mycelial growth of *Trichoderma* spp. was seen in PDA medium, less dense growth in malt extract agar and powdery growth in oatmeal agar. Colony colour in media was generally green to dark green and occasionally yellowish green to dark green with white tinge; and the pigmentation in the reverse side varied from white, yellowish or faint yellow. Phialides were ampulliform to sub-globose or lageniform divergent or crowded whorls of 2-5, the middle of phialides were markedly swollen, conidia were sub-globose to ovoid, the conidiophores highly branched having intercalary or terminal chlamydospores of sub-globose to ellipsoid or pyriform shape. Based on PCR amplification of ITS region sequences 44 isolates were characterized at molecular level. The partial ITS sequences were analyzed using MegaBLAST with NCBI GenBank database to confirm their identity. The study revealed the presence of 5 species of *Trichoderma* viz., *T. viride, T. asperelloides*, *T. koningiopsis, T. asperellum* and *T. harzianum*.

Keywords: Characterization, chickpea, culture, identification, ITS region, morphology, *Trichoderma*

INTRODUCTION

Trichoderma species are widely used bio-pesticide and commercialized in agriculture. They are used as bio-protectants, bio-stimulants and biofertilizers in many crops (Saba *et al.*, 2012; Zhang *et al.*, 2014). *Trichoderma* strains are strong opportunistic invaders, fast growing, prolific producers of powerful antibiotics and most resistant to insecticides and fungicides (Druzhinina *et al.*, 2011; Zhang *et al.*, 2014; Guo *et al.*, 2019). They have developed an astounding ability to interact, both parasitically and symbiotically, with different substrates and living organisms and are highly effective against several root, soil-borne, and foliar pathogens (Sain and Sathyanarayana, 2016; Chagas *et al.*, 2017; Halifu *et al.*, 2019). Therefore, the choice of compatible effective *Trichoderma* strains is

important in designing safe biocontrol strategies in crop-specific areas (Rajasekhar *et al*., 2016; Zin and Badaluddin, 2020).

Identification is the first and most crucial step in strain selection for biocontrol purposes. Morphological characterization is often adequate up to genus or species level identification of *Trichoderma* and remains the primary tool to identify the species of *Trichoderma* prior to identity confirmation (Sharma and Singh, 2014). Morphological characteristics of conidia and phialides help in differentiation of these species from each other. Molecular characterization of effective suitable strains of *Trichoderma* is helpful in understanding the mechanism of mode of action and in differentiation of strains from one other (Benitez *et al*., 2004). The identification of location-specific strains through molecular marker assisted studies are of immense significance in developing tools to monitor the genetic and environmental fate of these bioagents. The present study was aimed to characterize and identify *Trichoderma* species prevailing in the rhizospheric soils of some pulse (chickpea and pigeon pea) and rice growing areas of Central India through cultural and phenotypic characterization as well as by molecular methods.

MATERIALS AND METHODS

Isolation of Trichoderma by serial dilution technique

The rhizospheric soil samples were collected from different parts of Uttar Pradesh, Madhya Pradesh and Maharashtra states of India. The fungi were isolated by using serial dilution technique (Tuite, 1969; Dhingra and Sinclair, 1995), and plated on Trichoderma specific medium (TSM) (Elad and Chet, 1983) modified by Saha and Pan (1997). On TSM plates, 1 g soil sample was added to 100 mL sterilized distilled water and homogenized for 1 min, and a series of dilutions were prepared in sterile water. Each dilution (100 µL) was inoculated onto the surface of plates containing modified TSM, spread evenly and incubated in dark for 5-7 days at 25±1°C. The colonies were isolated, stained with methylene blue and identified microscopically. The selected colonies of *Trichoderma* were transferred to PDA plate. The pure cultures were obtained and maintained on PDA slants at 25±1°C.

Cultural characterization of Trichoderma

The cultural characteristics of *Trichoderma* isolates were studied in three growth media, *viz*., PDA, oat meal agar (OMA) and malt extract agar (MEA). Mycelial discs (6 mm) of young growing culture of respective isolates of *Trichoderma* were inoculated in the centre of Petriplates containing either of the solidified PDA or OMA or MEA medium and incubated at 28±1°C for 7 days. The growth characteristics of isolates were monitored daily and all the distinguishing characters recorded. The experiments were replicated five times. The characteristic features recorded were colony, growth rate, presence or absence of pigments, hyphae/mycelial form, sporulation, conidiation and presence of any distinguishing odour.

Anamorphic characterization of Trichoderma isolates

Morphological characterization of *Trichoderma* isolates was done by growing them in PDA medium in order to unequivocally verify their genus and species. Morphological characteristics *viz*., conidiophores length, width and branching pattern, phialides width, length and shape, phialospores shape, length and width, chlamydospores shape, length and width, presence of intercalary phialides and number of phialides in whorl. For estimation of above parameters, slide culture technique (Zoberi, 1967) using half strength PDA was used and incubated at 25±1°C. All conidiophores, phialides, conidia and chlamydospores were measured following the methods of Lieckfeldt *et al.* (2001). At least 50 observations were randomly recorded while measuring all the morphological characteristics of *Trichoderma* spp. The isolates were identified upto species level based on the taxonomic keys and monograph of Rifai (1969) and Bissett (1991 a-c).

Molecular characterization of Trichoderma isolates

DNA extraction: DNA extraction was done by the modified method of Raeder and Broda (1985). Mycelial mats of 4 days old culture of *Trichoderma* were harvested, dried, weighed (1 g) and grounded in pre-chilled pestle and mortar with liquid nitrogen. Powdered mycelium was transferred into centrifuge tubes carrying 25 mL pre-heated (65±1^{o}C) lysis buffer. The tubes were incubated at 65±1^{o}C for an hour with occasional stirring by vortexing every 15 min, followed by addition of equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). The samples were centrifuged at 15000 rpm for 10 min at room temperature. Upper aqueous phase was precipitated with 2 volumes of ice-cold ethanol and 0.1 volume of 3 M sodium acetate (pH, 5.20) and incubate for 1 h at 4^{o}C. Then the material was spinned at 15000 rpm for 15 min at room temperature and the pellets were washed with 70% ethanol and again spinned at 10000 rpm for 10 min. Nucleic acid was eluted by elution buffer (10 mM tris HCl and 1 mM sodium EDTA; pH, 8). The DNA was dissolved and treated with 10 µL RNase and incubated for 1 h at 37±1^{o}C. After incubation 500 µL of water saturated phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and centrifuged at 10000 rpm for 20 min. The upper aqueous phase was collected and 50 µL of 3 M sodium acetate and 1 mL ethanol added and incubated at -80±1^{o}C for 1 h. The samples were centrifuged at 10000 rpm for 10 min and the supernatant was discarded. The pellets were washed with 70% ethanol, air-dried and 100 µL of 1 X TE buffer added to it to dissolve the pellet and used as template for PCR reactions.

PCR amplification of ITS region: Partial 18 S ribosomal RNA gene, ITS 1, 5.8 S rRNA gene and ITS 2 complete sequence and partial 28 S ribosomal RNA gene were amplified using primer pair ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') obtained from Sigma Aldrich (White *et al*., 1990), with slight modifications (Hermosa *et al*., 2000). PCR amplification was performed using thermo cycler (Bioer, LifePro). The ITS region was amplified with an initial denaturation for 5 min at 95±1°C, followed by 35 cycles of denaturation at 94±1°C for 1 min, annealing of primers at 60±1°C for 30 sec, and extension at 72±1°C for 30 sec. The amplification was completed with one cycle of final extension at 72±1°C for 5 min. PCR reaction mixture consisted of DNA. The PCR mixture (25 µL) contained 10X PCR buffer, 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 0.5 µM of each of forward and reverse primer, 1 µL Thermo Taq DNA polymerase (3U μL^{-1}) and 20 ng DNA template. The amplification products were run in 1.0% agarose gel and visualized under UV-transilluminator. Gel image was taken using gel documentation system.

The partial sequencing of purified PCR product was undertaken at Xcelris Labs, Gujarat (India) using the aforementioned forward primer. The partial ITS sequence was analyzed using MegaBLAST (Basic Local Alignment Search Tool) (Morgulis *et al.*, 2008) with NCBI GenBank database (*http://www.ncbi.nlm.nih.gov*) to assess their identity. The analysis was performed on Phylogeny.fr platform (Dereeper *et al*., 2008). The sequences were aligned with MUSCLE (v3.8.31) and configured for highest accuracy (Edgar, 2004). After alignment, ambiguous regions (i.e. containing gaps and/or poorly aligned) were removed with Gblocks (v0.91b) (Castresana, 2000). The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.1/3.0 aLRT) (Guindon and Gascuel, 2003). The HKY85 substitution model was selected assuming an estimated proportion of invariant sites (of 0.573) and 4 gamma-distributed rate categories to account for rate heterogeneity across sites. The gamma shape parameter was estimated directly from the data (gamma = 97.586). Reliability for internal branch was assessed using the aLRT test (SH-Like) (Anisimova and Gascuel, 2006). Graphical representation and edition of the phylogenetic tree were performed with TreeDyn (v198.3) (Chevenet, 2006).

RESULTS AND DISCUSSION

A total of 54 isolates were obtained from Madhya Pradesh (16 isolates), Uttar Pradesh (17 isolates) and Maharashtra (21 isolates) and coded accordingly with the initials as state. Table 1 shows the

Table 1: Details of location, pH, isolation source (rhizospheric soil) of the samples from where *Trichoderma* spp. were isolated from pulse growing areas of India

Isolate name	Rhizo-spheric soil	Isolates collected from			Site details			
		Village	District	State	Soil pH	Latitude	Longitude	Altitude (m asl)
UP:Bam001	Rice	Bambawad	Gautam Budhnagar	Uttar Pradesh	7.2	28.55 N	77.59 E	210
UP:Bam002	Rice	Bambawad	Gautam Budhnagar	Uttar Pradesh	6.9	28.55 N	77.59 E	210
UP:Bam003	Rice	Bambawad	Gautam Budhnagar	Uttar Pradesh	6.8	28.55 N	77.59 E	210
MP:Umr004	Chickpea	Umreth	Chindwara	Madhya Pradesh	7.4	22.19 N	78.76 E	47
MP:Dol005	Chickpea	Dolapanjra	Chwarpatha	Madhya Pradesh	7.5	22.93N	79.04 E	365
MP:Kod006	Chickpea	Kodia	Chwarpatha	Madhya Pradesh	7.2	22.93 N	79.04 E	361
UP:Sad007	Chickpea	Sadasani	Kamasin	Uttar Pradesh	6.8	25.49 N	80.88 E	141
UP:Kus008	Chickpea	Kusmara	Kurara	Uttar Pradesh	6.7	25.97 N	80.07 E	351
UP:Gur009	Chickpea	Gurdaha	Maudaha	Uttar Pradesh	6.5	25.60N	80.15E	80
UP:Gaj010	Chickpea	Gajaura	Ganjdundwara	Uttar Pradesh	6.9	25.58 N	80.12 E	257
UP:Kak011	Chickpea	Kakrau	Jalaun	Uttar Pradesh	6.4	26.12 N	79.71 E	123
UP:Pah012	Chickpea	Pahari	Mirzapur	Uttar Pradesh	7.1	25.10 N	82.37 E	87
MS:Sat013	Chickpea	Sategaon	Palam	Maharashtra	5.4	21.11 N	77.24 E	347
MS:Mar014	Chickpea	Mardasgaon	Parbhani	Maharashtra	5.6	18.99 N	76.74 E	392
MS:Gop015	Chickpea	Gopa	Parbhani	Maharashtra	5.9	18.99 N	76.74 E	392
MS:Mar016	Chickpea	Mardasgaon	Parbhani	Maharashtra	7.1	18.99 N	76.74 E	392
MS:Aar017	Chickpea	Aarni	Parbhani	Maharashtra	7.2	18.39 N	76.49 E	347
MS:Tak018	Chickpea	Takwiki	Osmanabad	Maharashtra	6.9	18.39 N	76.49 E	679
MS:Mal019	Chickpea	Malumbara	Gangakhed	Maharashtra	7.2	19.50 N	76.75 E	679
MP:Khe020	Chickpea	Kherua	Narsingpur	Madhya Pradesh	7.1	22.93 N	79.06 E	500
MP:Gau021	Chickpea	Gaunee	Chwarpatha	Madhya Pradesh	6.8	22.93 N	79.04 E	356
MP:Deo022	Chickpea	Deori	Chwarpatha	Madhya Pradesh	6.8	21.06 N	80.36 E	340
MP:Mau023	Chickpea	Mauja Attas	Chwarpatha	Madhya Pradesh	6.9	22.93N	79.04 E	495
MP:Sim024	Chickpea	Simariya	Chwarpatha	Madhya Pradesh	7.2	24.48 N	84.92 E	381
MS:Jal025	Chickpea	Kategaon	Jalna	Maharashtra	6.2	19.83 N	75.88 E	502
MS:Bil026	Chickpea	Bilhera	Chwarpatha	Madhya Pradesh	6.6	22.93 N	79.04 E	356
MS:Puy027	Chickpea	Puyani	Nanded	Maharashtra	6.3	19.01 N	76.94 E	366
MP:Bhe028	Chickpea	Bhesa Khedi	Devas	Madhya Pradesh	6.5	22.93 N	79.04 E	526
MS:Sun029	Chickpea	Sunegaon	Nanded	Maharashtra	6.1	19.01 N	76.94 E	383
MP:Kha030	Chickpea	Khairi	Narsingpur	Madhya Pradesh	6.9	22.93 N	79.06 E	414
MP:Gop031	Chickpea	Gopa	Parbhani	Madhya Pradesh	7.2	18.99 N	76.74 E	392
MS:Tor032	Chickpea	Toradi	Raigad	Maharashtra	6.8	18.04 N	73.16 E	312
MP:Kha033	Chickpea	Khamarpani	Chhindwara	Madhya Pradesh	7.1	22.63 N	79.21 E	593
MS:Mas034	Chickpea	Masgaon	Gondia	Maharashtra	6.8	21.45 N	80.20 E	300
UP:Gah035	Chickpea	Gahrauli	Pratapgarh	Uttar Pradesh	6.4	29.57 N	77.58 E	403
UP:Lah036	Chickpea	Lahori	Farukkabad	Uttar Pradesh	6.2	27.38 N	79.58 E	153
MP:Lal037	Chickpea	Lalgaon	Rewa	Madhya Pradesh	7.1	22.93 N	79.04 E	393
UP:Pac038	Chickpea	Pachoha	Kamasin	Uttar Pradesh	6.2	25.51 N	80.90 E	141
UP:Kar039	Chickpea	Kariya	Unnao	Uttar Pradesh	6.2	26.53 N	80.48 E	129
UP:Pat040	Chickpea	Patanpur	Hamirpur	Uttar Pradesh	6.8	25.76 N	80.04 E	500
MS:Chi041	Chickpea	Chinchtakli	Nanded	Maharashtra	6.9	18.98 N	72.83 E	362
UP:Tik042	Chickpea	Tikri	Allahabad	Uttar Pradesh	6.4	28.83 N	78.77 E	99
MS:Puy043	Chickpea	Puyani	Nanded	Maharashtra	6.9	19.01 N	76.94 E	366
MS:Cha044	Chickpea	Chandsuraj	Gondia	Maharashtra	6.9	21.23 N	80.61 E	587
MP:Kod045	Chickpea	Kodia	Narsingpur	Madhya Pradesh	7.0	22.94 N	79.19 E	361
MS:Sat046	Chickpea	Sategaon	Parbhani	Maharashtra	6.4	21.11 N	77.24 E	347
MS:Kol047	Chickpea	Kolewadi	Satara	Maharashtra	6.1	17.68 N	74.01 E	592
UP:Chi048	Chickpea	Chitauli	Mirzapur	Uttar Pradesh	6.2	25.13 N	82.56 E	80
MP:Gai049	Chickpea	Gai gohan	Parasia	Madhya Pradesh	6.7	22.49 N	78.40 E	47
MP:Bic050	Chickpea	Bichiyaghati	Chwarpatha	Madhya Pradesh	7.1	22.93 N	79.04 E	775
MS:Sun051	Chickpea	Sunegaon	Latur	Maharashtra	6.8	18.40 N	76.56 E	515
MP:Pad052	Chickpea	Padariya	Chhatarpur	Madhya Pradesh	7.1	22.93 N	79.04 E	226
UP:Kha053	Chickpea	Khamarkha	Banda	Uttar Pradesh	6.3	25.51 N	80.90 E	141
MS:Kaj054	Chickpea	Kajala	Badnapur	Maharashtra	6.8	20.33 N	75.71 E	523

details of location, pH and isolation source (rhizospheric soil) samples from where *Trichoderma* spp. were isolated from pulse growing areas of India. Five species of *Trichoderma viz*., *T. viride*, *T. asperelloides*, *T. koningiopsis*, *T. asperellum* and *T. harzianum* isolated from the rhizospheric soils of chickpea, pigeonpea and rice from above mentioned states were morphologically characterized on the basis of their sporophore structure and the taxonomic keys (Rifai, 1969; Bissett, 1991a-c).

Cultural characteristics of Trichoderma

There was strong variation in the growth pattern of *Trichoderma* species and other cultural characters of antagonists when inoculated on the three test-media. *T. viride* was fast growing, dark green, dense mycelia growth, more powdery pustules, compactly tufted on PDA whereas on OMA compactly tufted, effuse, pustules powdery, arachnoid and on MEA, compactly tufted, aerial mycelium cottony, pustules powdery were seen. Colony colour was snow white to dark, dirty green and whitish to yellowish green on PDA; whereas on OMA it was whitish green to dark green and on MEA it was whitish mycelium and colony colour was dark green in most isolates (Fig. 1). *T. viride* forms ring like zone in PDA medium (Shahid *et al*., 2013; Waghunde *et al*., 2016).

T. asperellum culture was light to dark green, floccose to arachnoid, loose to compactly tufted on PDA, fluffy, cottony and raised on OMA; while on MEA some cultures of *T. asperellum* showed compact and effuse conidiation. Cultures of *T. asperelloides* showed cushion-like, loosely tufted to compactly tufted conidiation on PDA. On OMA aerial mycelium fluffy, cottony, pustules powdery, floccose to arachnoid were seen while it was compactly tufted and floccose to arachnoid on MEA. In most of the isolates, colony colour was light yellow to dark green on PDA, whitish to dark green on OMA and whitish yellow/ bright green to dark green on MEA. Sporulation was high on PDA and MEA, and moderate to high on OMA (Fig. 2). The isolates identified as *T. harzianum* cultures showed floccose, powdery owing to dense conidiation on PDA and flat submerged in some isolates. On OMA mycelium was compactly tufted and effuse while on MEA aerial mycelium was fluffy, raised, profuse with powdery pustules. Colony colour varied from yellowish white to dirty dark green in PDA, dark whitish to dull and dark green in OMA and whitish yellow to dark green in MEA. Sporulation was high in in all the tested media, except for one isolate (UP:Lah036) (Fig. 3).

T. asperelloides culture was colorless on PDA, whitish to dark green on OMA and dark green on MEA. Colony was loosely tufted, fluffy on both PDA and OMA but compactly tufted on MEA. The pustules were powdery on both OMA and MEA. Mycelium form was arachnoid, sporulation high and odour indistinct in all the media (Fig. 4). *T. koningiopsis* culture was compactly tufted and pustules were powdery in all the test media. Sporulation was high in all the media. Coconut like odor was found on OMA and indistinct on PDA and MEA. Mycelium form was arachnoid on PDA and MEA but floccose on OMA (Fig. 5). Similar observations were made by several workers who reported that *T. harzianum* and *T. viride* were fast growing green coloured mycoparasitic fungi with distinct coconut or faintly earthy aroma (Rifai, 1969; Samuels, 1996; Lieckfeldt *et al*., 2001; Pan and Bhagat, 2008; Sharma and Sharma, 2014).

Identification based on anamorphic characters

Anamorphic characteristics of *Trichoderma* isolates are presented in Table 2 which revealed that the phialide size of *T. viride* varied from 3.42-14.66 x 1.15-3.5 µm to 2.27-18.31 x 0.85-2.67 µm; whereas chlamydospore size varied from 5.37-20.99 x 5.32-13.95 µm to 7.46-13.48 x 12.45-14.31 µm and conidial size from 1.3 x 1.8 µm to 1.5-1.8 µm. The phialides of *T. asperellum* were lageniform to bottle shaped with divergent whorls of 2-4 and sometimes 3-4. The shape of chlamydospores varied from ellipsoid to oval, pyriform or globose, born intercalary and terminal. The size of phialides of *T. asperellum* isolates varied from 3.57-14.74 x 1.03-2.86 µm to 3.74-13.29 x 1.35-3.62 µm; while the size of phialospores varied from 1.5-4.13 x 1.5-4.2 µm to 1.25-3.67 x 1.03-4.23 µm. The chlamydospores sizes varied from 11.28-21.78 x 6.86-12.34 µm to 9.23-23.24 x 7.89-14.62 µm.

The size of phialides in *T. asperelloides* isolates varied from 4.32-17.8 x 1.14-3.47 µm to 3.76-16.89 x 1.42-3.93 µm. The conidial size varied from 1.36-3.39 x 1.1-2.99 µm to 1.35-4.06 x 1.35-

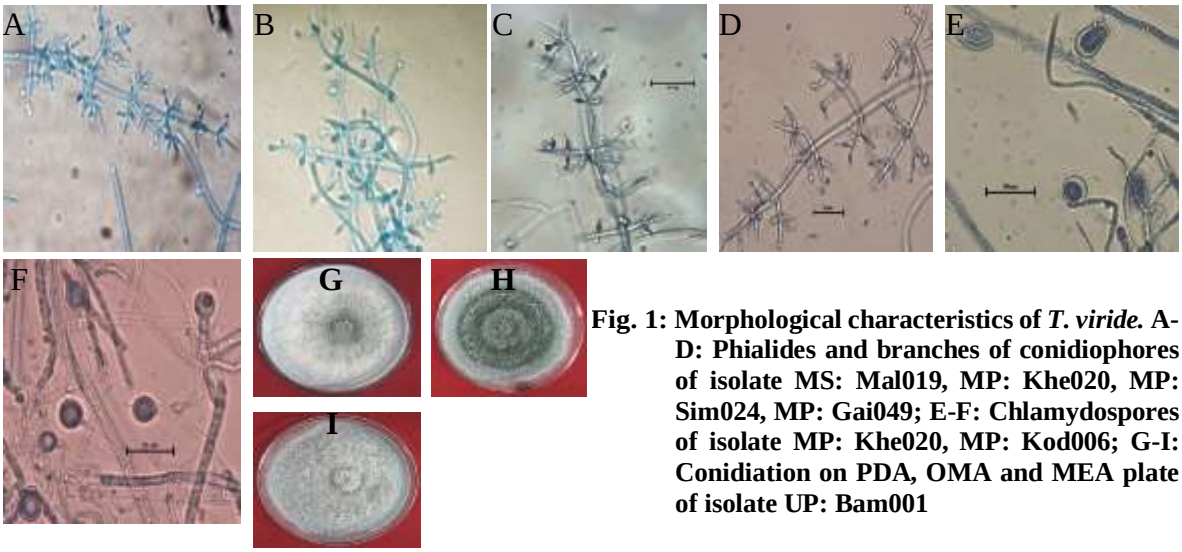


Fig. 1: Morphological characteristics of *T. viride.* A-D: Phialides and branches of conidiophores of isolate MS: Mal019, MP: Khe020, MP: Sim024, MP: Gai049; E-F: Chlamydospores of isolate MP: Khe020, MP: Kod006; G-I: Conidiation on PDA, OMA and MEA plate of isolate UP: Bam001

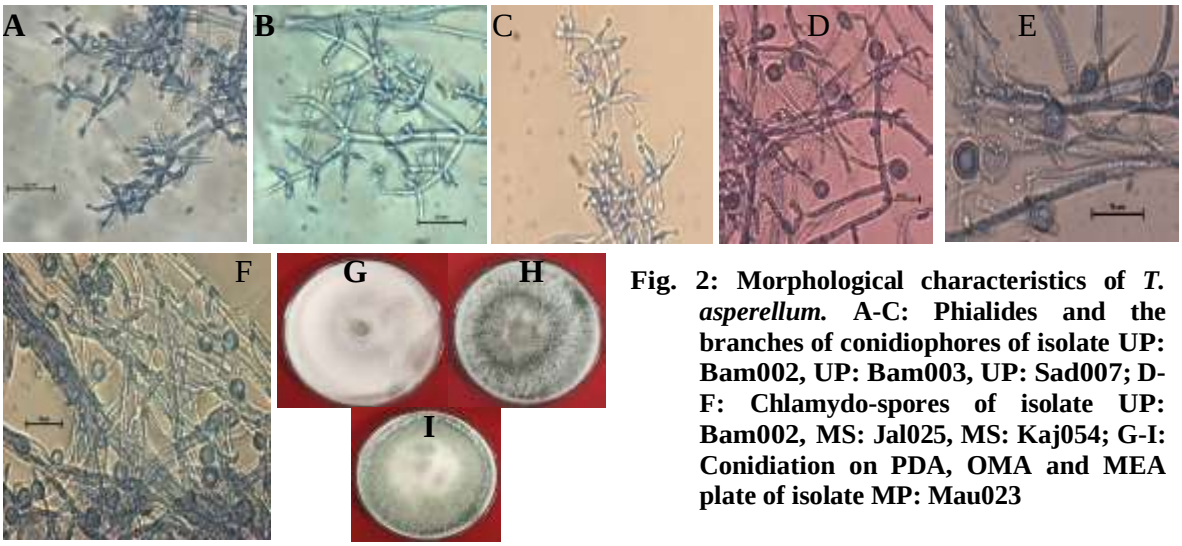


Fig. 2: Morphological characteristics of *T. asperellum.* A-C: Phialides and the branches of conidiophores of isolate UP: Bam002, UP: Bam003, UP: Sad007; D-F: Chlamydo-spores of isolate UP: Bam002, MS: Jal025, MS: Kaj054; G-I: Conidiation on PDA, OMA and MEA plate of isolate MP: Mau023

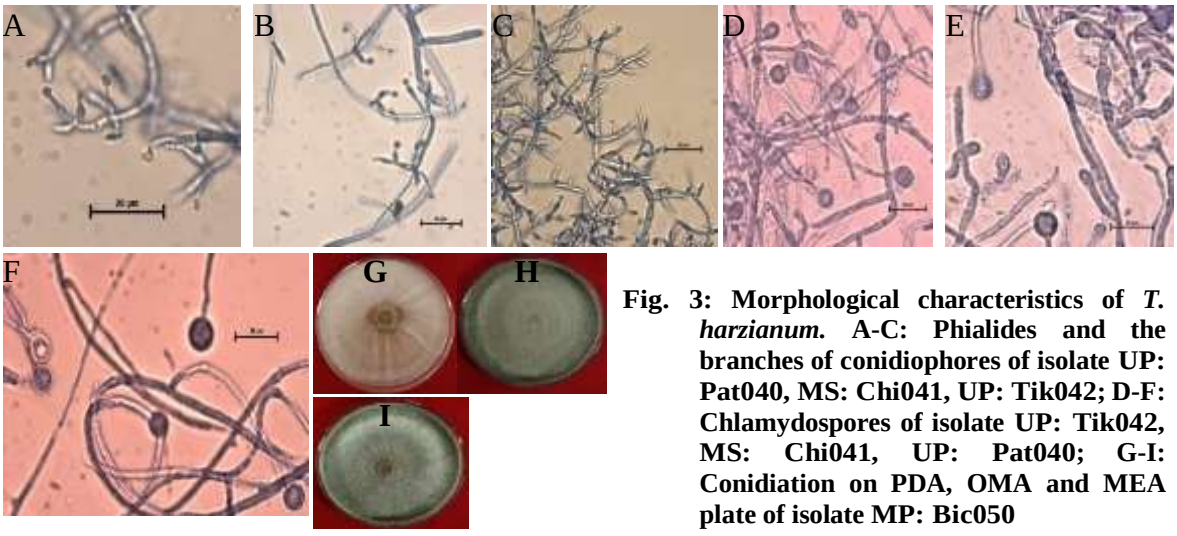


Fig. 3: Morphological characteristics of *T. harzianum.* A-C: Phialides and the branches of conidiophores of isolate UP: Pat040, MS: Chi041, UP: Tik042; D-F: Chlamydospores of isolate UP: Tik042, MS: Chi041, UP: Pat040; G-I: Conidiation on PDA, OMA and MEA plate of isolate MP: Bic050

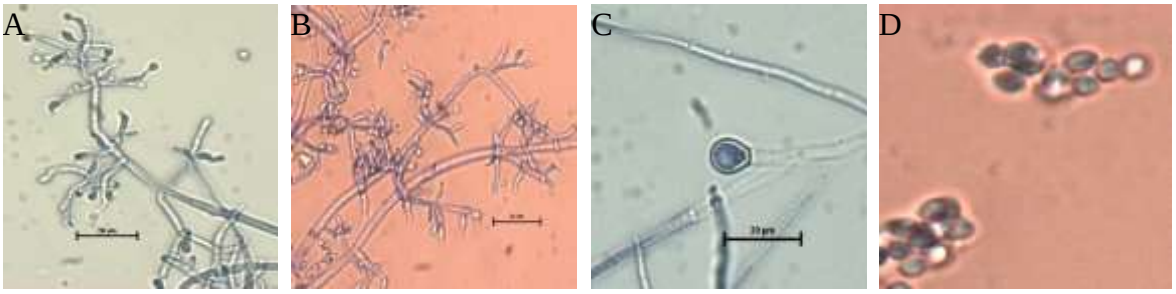


Fig. 4: Morphological characteristics of *T. asperelloides.* A-B: Phialides and branches of conidiophores of isolate UP: Pac038, MS: Mar014, C: Chlamydospore of MS: Mar014; D: Conidia of MS: Mar014, E-G: Conidiation of *T. asperellum* on PDA, OMA and MEA plate of isolate MS: Mar014

Fig. 5: Morphological characteristics of *T. koningiopsis.* A-B: Phialides and the branches of conidiophores of isolate UP: Pac038, MP: Kha030; C-E: Conidiation of *T. koningiopsis* on PDA, OMA and MEA plate of isolate MS:Mar014

Table 2: Anamorphic characteristics of *Trichoderma* isolates

Isolates	Conidial size (µm)	Conidial shape	PW	Phialides	IP	Chlamydospore	Identified as
UP:Bam001	2.69-2.99 x 1.56-3.49	Ovoidal to globose	2-3	Phialides ampulliform to subglobose, more swollen in middle than base or tip,3.42-14.66 x 1.15-3.5 µm	+	Not seen	*T. viride*
UP:Bam002	1.34-4.28 x 0.69-2.88	Globose to sub-globose	2-3	Phialides ampulliform constricted at the base, strongly swollen in the middle 3.57-14.74 x 1.03-2.86 µm	+	Size 11.28-21.78 x 6.86-12.34 µm, Ovoid, ellipsoid, both intercalary & terminal	*T. asperellum*
UP:Bam003	1.5-4.13 x 1.5-4.2	Subglobose to globose	3-4	Phialides ampulliform to subglobose or lageniform, 3.77-13.77 x 1.18-4.13 µm	+	Not seen	*T. asperellum*
MP:Umr004	1.03-3.5 x 1.64-3.37	Round to slightly oval	2-3	Phialides lageniform, convergent phialide, 2.27-18.31 x 0.85-2.67 µm	-	Not seen	*T. viride*
MP:Dol005	1.36-3.56 x 1.87-3.45	Oval to ellipsoidal	3-4	Regularly paired phialides, slender irregularly bent, 2.28-18.1 x 1.06-2.28 µm	+	Not seen	*T. viride*
MP:Kod006	0.85-3.51 x 1.5-7.6	Small round to oval	2-3	Phialides verticillate, slender with open fingers, abruptly tapered towards the apices, 3.8-23.2 x 0.9-2.9 µm	-	Size 0.22-20.99 x 8.43-13.95 µm, Ovoid, ellipsoid, both intercalary & terminal	*T. viride*
UP:Sad007	1.2-2.5 x 1.79-3.51	Round oval to short ellipsoidal	3-4	Phialides lageniform to bottle shaped with divergent phialides, 5.26-11.9 x 1.04-2.28	+	Not seen	*T. asperellum*
UP:Kus008	1.19-3.31 x 1.38-5.34	Globose	2-3	Phialides verticillate, slender, abruptly tapered towards the apices, 4.95-12.33 x 1.09-3.24 µm	-	Not seen	*T. viride*
UP:Gur009	0.92-3.63 x 1.4-3.48	Globose to subglobose	2-3	Phialides ampulliform, narrow at the base and sharp pointed neck, 3.64-15.63 x 0.74-2.53 µm	-	Size 5.37-12.34 x 5.32-12.86 µm, terminal	*T. viride*
UP:Gaj010	1.32-2.75 x 1.43-3.27	Ovoidal to Globose	3-4	Phialides verticillate to sub-globose, more swollen in middle than base or tip, 4.55-15.29 x 1.15-3.48 µm	+	Not seen	*T. asperellum*
UP:Kak011	1.76-3.32 x 1.23-2.77	Ovoidal to subglobose	2-3	Phialides long like bottle shaped narrow at tip, 3.42-14.66 x 1.33-2.75 µm	-	Not seen	*T. viride*

UP:Pah012	1.14-3.11 x 1.58-3.38	Globose to subglobose	3-4	Phialides verticillate, slender, abruptly tapered towards the apices 4.41-16.08 x 0.99-2.38 µm	+	Size 7.46-13.48 x 12.45-14.31 µm, Ovoid and globose	*T. viride*
MS:Sat013	0.96-3.47 x 1.23-3.53	Round oval to ellipsoidal	3-4	Phialides ampulliform to subglobose, more swollen in middle than base or tip,3.74-15.22 x 0.97-2.34 µm	+	Not seen	*T. asperellum*
MS:Mar014	1.36-3.39 x 1.1-2.99	Globose to oval	2-3	Phialides ampulliform, narrow at the base but attenuate abruptly and sharp pointed neck, 4.32-15.8 x 1.14-2.87 µm	-	5.84-14.23 x 13.64-15.85, Ovoid and globose, both intercalary and terminal	*T. asperelloides*
MS:Gop015	1.56-2.83 x 0.99-3.04	Globose	3-4	Phialides ampulliform to subglobose, more swollen in middle than base or tip, 4.81-12.55 x 1.17-3.7 µm	+	Size 8.33-12.12 x 4.66-13.94 µm, Terminal, subglobose to ovoid	*T. viride*
MS:Mar016	1.36-4.09 x 0.92-3.24	Round oval to ellipsoidal	2-3	Phialides verticillate, slender with open fingers, abruptly tapered towards the apices, 3.48-14.73 x 1.14-3.44	-	Not seen	*T. viride*
MS:Aar017	1.54-3.58 x 1.47-3.69	Round oval to ellipsoidal	3-4	Phialides ampulliform to subglobose, more swollen in middle than base or tip, diver-gent whorls 4.68-16.69 x 1.83-2.63 µm	+	Not seen	*T. viride*
MS:Tak018	1.42-4.99 x 1.8-3.37	Round to ovoidal	3-4	Phialides ampulliform to subglobose, more swollen in middle than base or tip, flexous, divergent whorls branch at right angle, size 4.63-11.85 x 1.72-2.32 µm	+	Not seen	*T. asperellum*
MS:Mal019	1.66-3.32 x 1.16-3.01	Globose	4-5	Phialides ampulliform to subglobose, more swollen in middle than base or tip, 2.34-12.57 x 1.23-3.45 µm	+	Not seen	*T. viride*
MP:Khe020	1.71-3.51 x 1.57-3.32	Oval to short ellipsoidal	2-3	Phialides verticillate, swollen in middle, abruptly tapered towards the apices,3.84-10.85 x 1.19-4.01 µm	-	Size 10.32-14.2 x 7.45-12.78 µm, oblong, terminal and intercalary	*T. viride*
MP:Gau021	1.52-3.32 x 1.57-3.38	Oval to globose	2-3	Phialides ampulliform to subglobose, more swollen in middle than base or tip, 3.74-13.29 x 1.35-3.62 µm	-	Not seen	*T. asperellum*
MP:Deo022	2.22-4.41 x 1.64-3.69	Globose, ovoidal to tapered at apices	3-5	Phialides ampulliform, lageniform, convergent whorls, swollen at middle & tapered at neck or tip, 3.7-14.69 x 1.97-2.92 µm	-	Not seen	*T. asperellum*
MP:Mau023	1.31-3.67 x 1.32-3.38	Round oval to ellipsoidal	3-4	Phialides lageniform or ampulliform and strongly swollen in the middle, µm 4.62-10.58 x 1.65-4.27 µm	+	Not seen	*T. asperellum*
MP:Sim024	1.56-4.21 x 1.79-3.9	Oblong to narrowly ellipsoidal	3-4	Phialides lageniform, convergent phialide, 3.02-15.89 x 1.17-4.56 µm	+	Not seen	*T. viride*
MS:Jal025	1.25-4.13 x 1.45-3.72	Round oval to ellipsoidal	3-4	Phialides ampulliform to subglobose, more swollen in middle than base or tip, 5.35-14.8 x 1.57-3.94 µm	+	9.23-23.24 x 7.89-14.62 µm, ellipsoid, intercalary and terminal	*T. asperellum*
MS:Bil026	2.28-4.01 x 1.06-3.19	Oblong to narrowly ellipsoidal	2-3	Phialides lageniform or ampulliform and strongly swollen in the middle, 4.55-14.04 x 1.56-3.35 µm	-	Not seen	*T. viride*
MS:Puy027	1.52-4.44 x 1.12-3.86	Oblong, narrowly ellipsoidal	3-4	Phialides ampulliform or lageniform, more swollen in middle, abruptly tapered at tip, convergent whorl, pyramidally branched, 4.87-10.5 x 2.10-3.29 µm	+	Not seen	*T. asperellum*
MP:Bhe028	1.56-4.05 x 1.5-3.41	Ovoidal, round, somewhat triangular	2-3	Phialides ampulliform to lageniform, swollen at base and middle and tapered at apices, convergent whorl, 4.35-16.81 x 2.32-2.89 µm	-	Not seen	*T. asperellum*
MS:Sun029	2.18-4.64 x	Oblong, round, ovoidal	2-5	Phialides long bottle shaped, swollen in middle and tapered at both ends, conidiophore flexous, convergent phialides,5.36-13.57 x 2.25-3.05 µm	+	Not seen	*T. viride*
MP:Kha030	2.18-3.59 x 1.82-3.44	Round oval to ellipsoidal	2-3	Phialides ampulliform to subglobose, more swollen in middle than base or tip, 4.09-14.72 x 1.36-3.34 µm	-	Not seen	*T. koningiopsis*
MP:Gop031	1.25-3.67 x 1.03-4.23	Ovoidal to globose	3-4	Phialides ampulliform or lageniform, more swollen in middle and abruptly tapered towards the tip, 5.02-13.69 x 1.25-3.75 µm	+	Not seen	*T. asperellum*

MS:Tor032	1.35-4.49 x 1.7-4.16	Subglobose to ovoidal, round	2-3	Phialides long, convergent whorl, branching flexous, 6.12-19.6 x 1.96-2.45 µm	-t	Not seen	*T. asperellum*
MS:Kha033	1.65-4.16 x .02-4.1	Globose to ovoidal	3-4	Phialides ampulliform to lageniform, abruptly tapered & bent at tip, pyramidally branched, flexous, 5.81-15.3 x 2.14-2.51 µm	+	4.18-12.22 x 4.9-10.52 µm, spherical, globose both intercalary and terminal	*T. asperellum*
MS:Mas034	0.03-4.48 x 1.7-3.85	Ovoidal to globose	3-4	Phialides long ampulliform or lageniform, swollen in middle and tapered at both ends, divergent whorls, 5.87-15.89 x 1.98-2.82 µm	+	9.86-10.03 x 9.77-10.78 µm, ovoidal to globose, terminally seen	*T. viride*
UP:Gah035	1.7-4.25 x 1.49-3.81	Globose to oval	2-3	Phialides lageniform or ampulliform, branching at nearly right angle, flexous, convergent, 7.17-12.73 x 1.99-2.70 µm	-	11.47-14.69 x 9.77-16.91 µm, Ovoidal to globose, both intercalary & terminal	*T. asperellum*
UP:Lah036	1.9-3.59 x 1.1-3.38	Globose to ovoidal	3-4	Phialide lageniform, ampulliform, strongly swollen at middle, less at base and tapered at apices, convergent whorls, 5.4-12.54 x 2.13-6.22 µm	-	9.17-14.62 x 9.23-23.24 µm, both intercalary and terminal, ovoidal, globose, oblong or pyriform	*T. harzianum*
MP:Lal037	1.7-3.34 x 1.14-4.43	Globose to Subglobose, ellipsoidal	2-3	Phialide long, ampulliform, convergent, hyaline hyphae, smooth walled, 5.49-15.86 x 1.94-2.61 µm	+	Not seen	*T. asperellum*
UP:Pac038	1.35-3.81 x 1.7-3.81	Oval, globose, ellipsoidal	2-3	Phialide lageniform or ampulliform, abruptly bent at tip, divergent whorls, 3.76-16.89 x 1.85-3.54 µm	-t	8.82-12.96 x 10.84-13.62 µm, both terminal & inter-calary, globose and ovoidal	*T. asperelloides*
UP:Kar039	1.35-4.06 x 1.35-3.93	Oblong to narrowly ellipsoidal	3-4	Phialide verticillate, slender irregularly bent, 3.76-16.89 x 1.42-3.93 µm	+	8.82-12.96 x 10.84-13.62 µm, both intercalary and terminal	*T. asperellum*
UP:Pat040	2.17-4.45 x 1.9-4.31	Globose to subglobose	2-3	Phialide lageniform to bottle shaped with divergent phialides,4.08-20.02 x 1.76-3.13 µm	-	8.22-12.18 x 8.11-13.42 µm, globose to subglobose, pyriform	*T. harzianum*
MS:Chi041	2.17-4.45 x 1.9-4.31	Ovoidal to Globose	2-3	Phialide subglobose to ellipsoidal or ampulliform, constricted at the base, narrowing abruptly at the apex to a very short conidia, 4.08-20.02 x 1.06-3.54 µm	-	8.22-12.18 x 8.11-13.42 µm, both intercalary and terminal	*T. harzianum*
UP:Tik042	1.1-4.65 x 1.54-4.48	Subglobose to globose, ovoidal, ellipsoidal	3-4	Phialide lageniform, strongly swollen in middle, abruptly tapered and bent near tip, convergent, conidiophore irregularly branched, 4.27-10.4 x 1.67-3.79 µm	+	7.61-10.73 x 8.16-12.67 µm, abundant, ovoidal, oblong, pyriform, tapered at both ends	*T. harzianum*
MS:Puy043	1.91-3.81 x 1.99-4.16	Subglobose to globose	2-3	Phialide long cylindrical narrow at tip, some are swollen in middle than on base, divergent whorl, 6.48-12.43 x 1.7-2.81 µm	-	7.17-11.96 x 7.32-13.06 µm, both present intercalary and terminal	*T. viride*
MS:Cha044	1.82-4.91 x 1.23-5.08	Globose to subglobose	2-3	Phialide ampulliform or lageniform, swollen at base and middle, convergent whorl, 4.25-11.37 x 2.14-3.22 µm	+	8.46-9.96 x 11.2-14.43 µm, both intercalary and terminal, ovoidal	*T. asperellum*
MP:Kod045	1.8-3.58 x 1.8-4.74	Globose to subglobose, ovoidal	2-3	Phialide lageniform or ampulliform, irregularly bent, strongly swollen in middle, 4.51-12.86 x 2.17-3.15 µm	+	Not seen	*T. viride*
MS:Sat046	1.61-4.97 x 1.91-4.28	Subglobose to globose, ovoidal	2-3	Phialide ampulliform, regularly paired, divergent whorls, conidiophores straight and flexous, 5.67-16.72 x 2.17-3.42 µm	+	Not seen	*T. viride*
MS:Kol047	1.61-5.48 x 1.91-4.28	Globose to subglobose	2-3	Phialide lageniform sharply tapered towards the tip of phialide, 5.67-16.72 x 1.14-4.1 µm	-	Not seen	*T. harzianum*
UP:Chi048	2.12-4.35 x 1.82-4.48	Globose	3-4	Phialide long cylindrical, narrow at apices, convergent whorl, 3.46-13.75 x 2.41-3.31 µm	+	10.01-16.58 x 7.94 -12.51 µm,both intercalary and terminal, pyriform	*T. viride*
MP:Gai049	1.27-4.82 x 1.52-4.19	Globose to subglobose	3-4	Phialide lageniform, abruptly bent, convergent whorl, straight branching, primary branch arises at right angle, 5.87-13.88 x 2.24-3.53 µm	+	Not seen	*T. viride*
MP:Bic050	1.27-4.82 x 1.52-4.19	Ovoidal to globose	3-4	Phialide bottle shaped, slender, 5.87-13.88 x 1.69-4.19 µm	+	Not seen	*T. harzianum*
MS:Sun051	2.08-4.64 x 1.35-4.01	Round oval to short ellipsoidal	3-5	Phialide lageniform, sharply tapered towards the tip of phialide, 5.61-17.86 x 1.35-4.44 µm	+	Not seen	*T. viride*

MP:Pad052	1.69-4.69 x 1.49-4.06	Globose to subglobose	3-5	Phialide lageniform, swollen mainly at base, tapered towards apices, 5.68-9.55 x 2.0-3.37 µm	+	11.26-12.46 x 12.5-13.41 µm, both terminal and intercalary, globose	*T. asperellum*
UP:Kha053	1.54-5.02 x 1.65-4.56	Globose to subglobose, ovoidal	2-3	Phialide lageniform, long slender, primary branches arising at right angle, onidiophore straight, 5.22-14.98 x 2.22-2.92 µm	+	7.22-14.86 x 5.01-13.88 µm, both intercalary and terminal, globose to sub-globose, pyriform, oblong	*T. viride*
MS:Kaj054	1.74-6.01 x 1.27-4.06	Globose	2-3	Phialide long slender, flexous, uniform thickness, filamentous, 5.32-13.25 x 2.39-3.24 µm	+	7.84-14.09 x 7.65-13.69 µm, numerous, mainly intercalary, globose to subglobose	*T. asperellum*

PW = Phialide in whorls (No.); IP = Intercalary phialide, + present, - absent

3.93 µm. The conidia were globose to oval or oblong to narrowly ellipsoidal. The size of phialides of *T. harzianum* isolates varied from 5.61-17.86 x 1.35-4.44 µm to 4.08-20.02 x 1.06-3.54 µm. The phialide shape of *T. harzianum* varied from subglobose to ellipsoidal or ampulliform, constricted at base, narrowing abruptly at apex or lageniform sharply tapered towards the tip. In case of *T. koningiopsis*, phialide shape ranged from ampulliform to subglobose, more swollen in middle than tip or base (4.09-14.72 x 1.36-3.34 µm (MP:Kha030) under the fluorescence microscope (Nikon Eclipse, 80i). Similar findings were reported by Samuels *et al*. (1999) for *T. asperellum.* Zhu and Zhuang (2015) reported that conidiophores were repeatedly branched, irregularly arranged in whorls, appeared as clusters of divergent, usually asymmetrical bent, flask-shaped/cylindrical to nearly subglobose. Micromorphology of *Trichoderma* spp. have been studied to observe the branching pattern which produces cellulolytic enzymes (Novy *et al*., 2016).

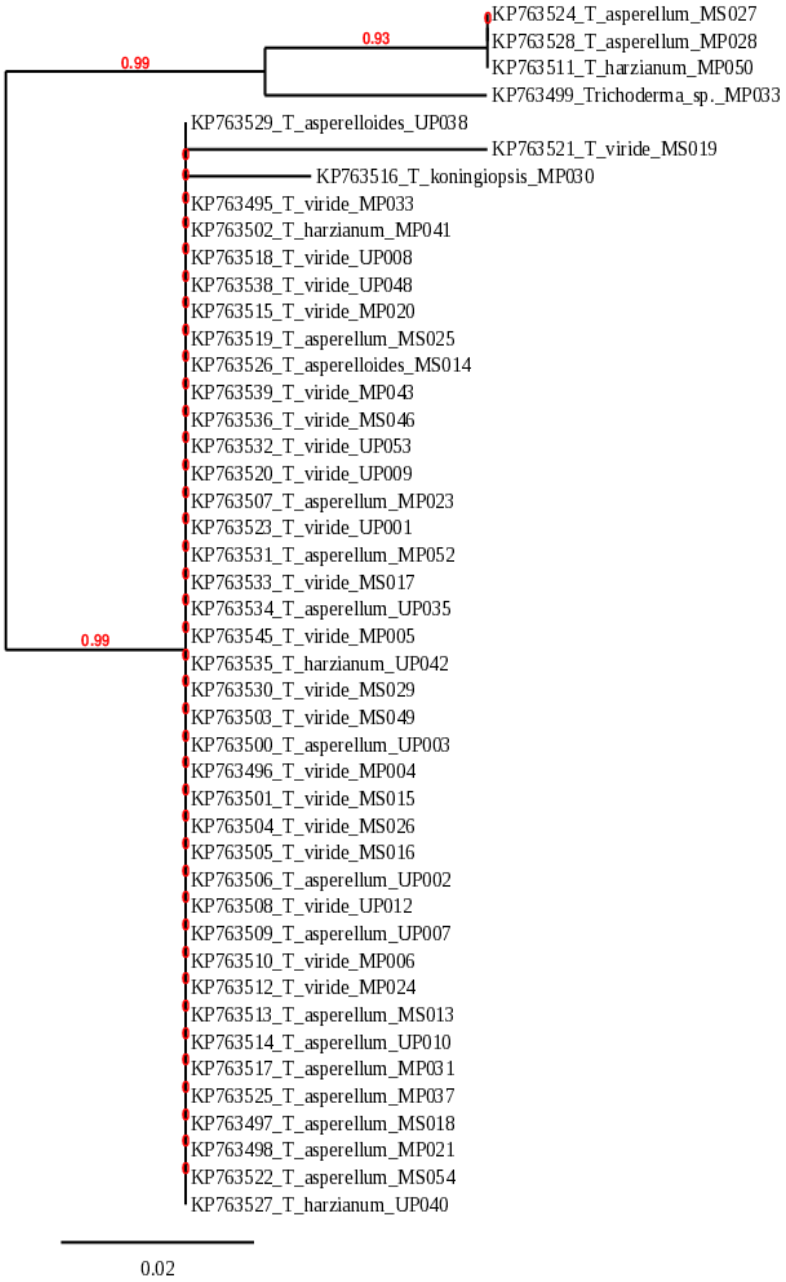
The cultural and anamorphic characteristics are the major determinants for morphological identification of *Trichoderma* spp. at species level. *T. harzianum* and *T. viride* are fast growing green-coloured fungi. Pan and Bhagat (2008) suggested that the conidiophore branching was at nearly right angle or less for *T. harzianum*, and at nearly acute angles in case of *T. viride*. Our findings are agreement with earlier findings (Domsch *et al.*, 1980; Bissett, 1991a-c).

Molecular characterization of Trichoderma isolates

Out of the 54 *Trichoderma* isolates, 44 isolates were molecularly identified grounded on their higher antagonistic potential *in vitro*. Based on ITS region (partial 18S rRNA gene, complete sequence internal transcribed spacer 1, 5.8S rRNA gene, and internal transcribed spacer 2, and partial 28S rRNA gene sequence) sequencing, phylogenetic tree was constructed. Based on ITS sequences and BLAST search, the isolates UP:Bam001, MP:Umr004, MP:Dol005, MP:Kod006, UP:Kus008, UP:Gur009, UP:Kak011, UP:Pah012, MS:Gop015, MS:Mar016, MS:Aar017, MS:Mal019, MP:Khe020, MP:Sim024, MS:Bil026, MS:Sun029, MS:Puy043, MS:Sat046, UP:Chi048, MP:Gai049 and UP:Kha053 identified as *T. viride*, isolate UP:Bam002, UP:Bam003, UP:Sad007, UP:Gaj010, MS:Sat013, MS:Tak018, MP:Gau021, MP:Mau023, MS:Jal025, MS:Puy027, MP:Bhe028, MP:Gop031, UP:Gah035, MP:Lal037, MP:Pad052 and MS:Kaj054 were identified as *T. asperellum*, isolate MS:Mar014 and UP:Pac038 were identified as *T. asperelloides*, isolate MP:Kha030 identified as *T. koningiopsis*, isolate MP:Kha033 identified as *Trichoderma* sp., isolate UP:Pat040, MS:Chi041, UP:Tik042, MP:Bic050 identified as *T. harzianum* (Fig. 1).

Multiple sequence alignment was performed by MUSCLE program. After alignment, ambiguous regions were removed with Gblocks program. Minimum length of a block after gap cleaning was 10. No gap positions were allowed in the final alignment. All segments with contiguous non-conserved positions bigger than 8 were rejected. The minimum number of sequences for a flank position was 85%. Molecular phylogeny was depicted using PhyML and aLRT program (*http://www.phylogeny.fr/index.cgi*). PhyML is phylogeny depiction software based on the maximum-likelihood principle. The minimum number of sequences for a conserved position was 23. There were 44 nucleotide sequences analyzed. There were 235 new positions in final data set (38% of the original 607 positions). The minimum number of sequences for a flanking position was 38. No gap positions were allowed. Phylogenetic analysis of the strains based on maximum likelihood

Fig. 1: Phylogenetic tree based on ITS sequences of *Trichoderma* spp. reconstructed by using maximum likelihood method implemented in PhyML program [Data set with 1000 bootstrap replications; branch lengths are represented with genetic distances [The scale at the bottom represents genetic distances in nucleotide substitutions per site]



method resulted into two major clusters with one subcluster formed from first cluster. Subcluster of cluster I formed with two *T. asperellum* and one *Trichoderma harzianum* and second subcluster formed with *Trichoderma* sp. In second cluster, *T. viride* (KP763521), *T. koningiopsis* (KP763516) and *T. asperelloides* (KP763529) were separated from other *Trichoderma* isolates. Other isolates in second cluster comprised of twenty *T. viride*, three *T. harzianum*, fourteen isolates of *T. asperellum* and one isolate of *T. asperelloides*. The higher number of gaps were found in *T. koningiopsis* (MP:Kha030) accession No. KP763516 and *T. viride* (MS:Mal019) accession No. KP763521. The highest number of insertions and deletions (InDels) were found in *Trichoderma harzianum* (MP:Bic050) accession number- KP763511. Table 3 showed the nucleotide sequences submitted in NCBI with their GenBank accession numbers.

The identification of *Trichoderma* species is extremely difficult. DNA based characters have assumed a crucial role, so this method should be used for reliable, dependable and genuine identification of all *Trichoderma* spp. Molecular identification of *Trichoderma* isolates are necessary so they can further be formulated for use as plant growth promoters and to combat various diseases and it will also minimize the use of agrochemicals in pulse growing areas of India.

Table 3: Nucleotide sequences submitted NCBI with their GenBank accession numbers and Sequence Id with best match after BLAST search

Isolate name	PCR product size (bp)	Morphological identification	Sequence based identity (partial ITS sequence)	Sequence Id with best match	Identity (%)	NCBI GenBank Accession numbers
UP:Bam001	535	*T. viride*	*T. viride*	KM078038	100	KP763523
UP:Bam002	547	*T. asperellum*	*T. asperellum*	KM066549	100	KP763506
UP:Bam003	539	*T. asperellum*	*T. asperellum*	KM875466	99	KP763500
MP:Umr004	531	*T. viride*	*T. viride*	KM078038	100	KP763496
MP:Dol005	525	*T. viride*	*T. viride*	HQ259989	100	KP763545
MP:Kod006	547	*T. viride*	*T. viride*	HM438946	99	KP763510
UP:Sad007	546	*T. asperellum*	*T. asperellum*	KC859432	100	KP763509
UP:Kus008	546	*T. viride*	*T. viride*	GQ221864	99	KP763518
UP:Gur009	536	*T. viride*	*T. viride*	JF304319	99	KP763520
UP:Gaj010	546	*T. asperellum*	*T. asperellum*	JQ387727	100	KP763514
UP:Pah012	546	*T. viride*	*T. viride*	KM078038	99	KP763508
MS:Sat013	547	*T. asperellum*	*T. asperellum*	KM527926	100	KP763513
MS:Mar014	536	*T. asperelloides*	*T. asperelloides*	KJ174189	100	KP763526
MS:Gop015	547	*T. viride*	*T. viride*	HM438946	99	KP763501
MS:Mar016	547	*T. viride*	*T. viride*	GQ221864	99	KP763505
MS:Aar017	533	*T. viride*	*T. viride*	KP763523	100	KP763533
MS:Tak018	540	*T. asperellum*	*T. asperellum*	MH013951	100	KP763497
MS:Mal019	545	*T. viride*	*T. viride*	KM078038	98	KP763521
MP:Khe020	423	*T. viride*	*T. viride*	KM820885	100	KP763515
MP:Gau021	537	*T. asperellum*	*T. asperellum*	GU589845	99	KP763498
MP:Mau023	535	*T. asperellum*	*T. asperellum*	KC113288	100	KP763507
MP:Sim024	547	*T. viride*	*T. viride*	HM438946	100	KP763512
MS:Jal025	537	*T. asperellum*	*T. asperellum*	JQ387727	100	KP763519
MS:Bil026	547	*T. viride*	*T. viride*	KM820885	100	KP763504
MS:Puy027	553	*T. asperellum*	*T. asperellum*	MK871318	100	KP763524
MP:Bhe028	554	*T. asperellum*	*T. asperellum*	MK871318	100	KP763528
MS:Sun029	538	*T. viride*	*T. viride*	MN025411	99.81	KP763530
MP:Kha030	278	*T. koningiopsis*	*T. koningiopsis*	KP316429	99	KP763516
MP:Gop031	547	*T. asperellum*	*T. asperellum*	KC859432	100	KP763517
MP:Kha033	573	*Trichoderma* sp.	*Trichoderma* sp.	MK871250	100	KP763499
UP:Gah035	533	*T. asperellum*	*T. asperellum*	GU589845	99.81	KP763534
MP:Lal037	539	*T. asperellum*	*T. asperellum*	MT529837	100	KP763525
UP:Pac038	538	*T. asperelloides*	*T. asperelloides*	KJ174189	100	KP763529
UP:Pat040	538	*T. harzianum*	*T. harzianum*	KC576716	99	KP763527
MS:Chi041	546	*T. harzianum*	*T. harzianum*	MK871221	99.27	KP763502
UP:Tik042	540	*T. harzianum*	*T. harzianum*	MT150599	99.81	KP763535
MS:Puy043	537	*T. viride*	*T. viride*	KP763530	100	KP763539
MS:Sat046	532	*T. viride*	*T. viride*	JF304319	99	KP763536
UP:Chi048	537	*T. viride*	*T. viride*	MN025411	100	KP763538
MP:Gai049	531	*T. viride*	*T. viride*	KM078038	100	KP763503
MP:Bic050	563	*T. harzianum*	*T. harzianum*	KM491893	100	KP763511
MP:Pad052	537	*T. asperellum*	*T. asperellum*	GU589845	99.81	KP763531
UP:Kha053	532	*T. viride*	*T. viride*	MT007532	99.81	KP763532
MS:Kaj054	539	*T. asperellum*	*T. asperellum*	KY381942	99.81	KP763522

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