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CULTURAL AND ANAMORPHIC CHARACTERIZATION OF *TRICHODERMA* ISOLATES ISOLATED FROM RHIZOSPHERE OF FRENCH BEAN (*PHASEOLUS VULGARIS* L.) GROWING AREAS OF MANIPUR

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ABSTRACT
Trichoderma spp. were collected from the rhizosphere soil of french bean from different locations of Manipur and altogether three species of Trichoderma viz. *Trichoderma viride* (5 isolates), *Trichodera harzianum* (4 isolates) and *Trichoderma hamatum* (1no) were isolated. The growth patterns of antagonists were observed highest on Potato dextrose agar followed by two other growth media. Colours of the Trichoderma colony were showed white green to green and sometimes yellowish green to dark green. The growth of *T. viride* was light greenish with fluffy growth and concentric ring with dense growth at the margin of the colony except one isolate produced coconut like aroma in all the three media, growth of *T. harzianum* was fast to very fast with whitish greenish to green color, concentric ring, and hyaline hyphae without any significant aroma and in *T. hamatum,* it was fast with whitish green to greenish colour, highly fluffy and compact colony, hyaline hyphae without any significant aroma in all the three media. Anamorphic characters *viz*., size of phialides, phialospore and conidiophores of all *Trichoderma spp* were ranges from 3.7–8.4X1.2-3.7μm, 1.6-5.2X1.2-2.7μm and 4.2-38.8-2.1-5.8μm.

INTRODUCTION

Manipur is one of the states of North East India which is having a wide biodiversity resource. *Trichoderma* spp are most commonly use biocontrol agent against various plant diseases specially soil borne pathogens. It offers a chance to improve crop production in sustainable way and avoid the resistance of chemical pesticides to the target pathogens (Dekker, 1976). Trichoderma is completely safe and in 55 years of research there has been never recorded adverse reaction to humans and livestock's (Lorito 2005). Seed or seedling treatments of bioagents (*Trichoderma*) resulted in plant growth promotion, yield and also reduce disease severity (S. P. Singh *et al*., 2013; Jyotika Purohit *et al*., 2013). Identification of *Trichoderma* spp is a major constraint due to variability in nature. Therefore the present study was carried out to find the cultural and morphological variability of *Trichoderma* isolates from different french bean rhizosphere of Manipur.

MATERIALS AND METHODS

Collection of soil sample from rhizosphere of french bean

The soil samples were collected from the rhizosphere of french bean at different locations of Manipur for the presence of *Trichoderma* spp and isolated by soil dilution technique (Dhingra and Sinclair, 1995) using Trichoderma Specific Media (TSM) of modified methods of Saha and Pan (1997). GPRS (General Packet Radio Service) of the different places of collection were recorded by Garmin e Trex^R 10.

Cultural and anamorphic characteristics of *Trichoderma* spp.

The cultural and anamorphic characteristics of 10 isolates of *Trichoderma* spp were studied in three media, *viz*., potato dextrose agar (PDA), *Trichoderma* specific media (TSM) and oat meal agar (OMA) following the methods of Lieckfeidt *et al*. (2001) and Someshwar and Sitansu (2010).

RESULTS AND DISCUSSION

Altogether 30 soil samples were collected from a depth of 5-6 cm from various locations of valley areas of Manipur for the presence of *Trichoderma* spp. during 2011-12 and the details of GPRS location were presented at Table 1.

Identification of *Trichoderma* spp.

Altogether ten *Trichoderma* isolates were isolated and morphologically identified under microscope based on the taxonomic keys and monograph of Rifai (1969) and Bisett(1991a,b among these, five were *T.viride*, four were *T.harzianum* and one was *T.hamatumi*. This was confirmed by NCIPM (National Centre for Integrated Pest Management), New Delhi.

Growth characteristics of *Trichoderma* isolates

Growth of the 10 different isolates of *Trichoderma* on three different culture media *viz*., potato dextrose agar (PDA), *Trichoderma* specific media (TSM) and oat meal agar (OMA) were ranged from 6.93 cm to 9.00 cm (on PDA), 5.33 cm to

7.40cm(on TSM) and 6.93cm to 9.00 cm (on OMA) at 2 days after inoculation (Table 2). The mean radial growth on PDA was found to be the highest with 7.95 cm followed by OMA (6.98 cm) and TSM (6.20cm) (Table 2).

Cultural characteristics of *Trichoderma viride*

TvFB1

The growth of isolate TvFB1 on PDA was light greenish with fluffy growth and concentric ring wth dense growth at the margin of the colony, on OMA it showed lime green coloured colony with scanty mycelia growth with concentric ring at the margin of the colony and on TSM dark green colony with concentric ring. The growth was found to be highest on PDA followed by OMA and TSM. The pustules were light green on PDA and OMA and dark green on TSM. Dull yellow pigment was found on PDA, OMA and TSM. The characteristics of hyphae were hyaline in all the three media and also coconut like aroma produces in all the three media.

TvFB5

The growth of isolate TvFB5 on PDA was light greenish colour with concentric ring at the margin of the colony, on OMA it showed lime green colour with irregular colony and on TSM, dark green colony with sparse growth. The growth was found to be maximum on PDA followed by OMA and TSM. The pustules were dark green with whitish pigment on PDA, TSM and OMA. The characteristic of hypha were hyaline without any significant aroma in all the three media.

TvFB6

The growth of isolate TvFB6 on PDA was dark greenish fluffy more dense growth towards the periphery, on OMA it showed light green colour colony with sparse mycelia growth and on TSM, dark green colony with sparse growth. The growth was found to be very rapid on PDA, rapid on OMA and slow on TSM. The pustules were dark green on PDA, TSM and light green on OMA. A dull yellow pigment was found on PDA and OMA and whereas whitish on TSM. The characteristic of hyphae were hyaline without any significant aroma in all the three media.

TvFB8

The growth of isolate TvFB8 on PDA was light greenish colour with sparse growth, on OMA it showed lime green colour with sparse growth and on TSM light green colony with sparse growth. The growth was found to be very fast on PDA, fast on OMA and slow on TSM. The pustules were light green with whitish pigment in all the media.The characteristic of hyphae were hyaline without any aroma in all the three media.

TvFB10

The growth of isolate TvFB10 on PDA was light greenish fluffy with sparse growth, on OMA it showed light green colour colony with sparse growth and on TSM, light green colony with concentric ring. The growth was found to be very fast on PDA, fast on OMA and slow on TSM. The pustules were dull yellow on PDA, moderate light green pustules on OMA and TSM. A dull yellow pigment was found on PDA, OMA and TSM. The characteristic of hyphae were hyaline without any aroma in all the three media.

Cultural characteristics of *Trichoderma harzianum*

ThrFB2

The growth of isolate ThrFB2 on PDA was whitish greenish colour with concentric ring near the periphery, on OMA it showed compact whitish green colour colony with concentric rings and on TSM, sparse whitish green colour colony with concentric ring. The growth was found to be highest on PDA followed by OMA and TSM. The pustules were showed powdery or granular owing to dense conidiation on PDA and whitish green powdery mass on OMA and TSM. A dull yellow pigment was found on all the media. The characteristics of hyphae were hyaline without any significant aroma in all the three media.

ThrFB4

The growth of isolate ThrFB4 on PDA was dark green colour with dense growth, on OMA it showed dark green colour with double layer colony and on TSM, light green colour colony with dense growth more on periphery. The growth was found to be highest on PDA followed by OMA and TSM. The pustules were showed dark green on PDA and OMA and light green on OMA. A dull yellow pigment was found on PDA, OMA and TSM. The characteristics of hyphae were hyaline without any significant aroma in all the three media.

ThrFB7

The growth of isolate ThrFB7 on PDA was yellowish green colour with dense, on OMAit showed dark green colour with dense growth and on TSM, whitish green colony with sparse growth. The growth was found to be very fast on PDA, fast on OMA and slow on TSM. The pustules were yellow on PDA, dull yellow OMA and whitish green on OMA. A yellow pigment was found on PDA, dull yellow OMA and whitish on TSM. The characteristic of hyphae were hyaline without aroma in all the three media.

ThrFB9

The growth of isolate ThrFB9 on PDA was dark green colour

Table 1: Location, latitude, longitude and elevation of areas from where *Trichoderma* isolates were isolated

Sl.no.	Location	Latitude	Longitude	Elevation(ft)
1.	Salam	24°48′23.50"N	93°51′05.30′′E	2566
2.	Kongba	24°46′52.58"N	93°57′48.00"E	2561
3.	Thoubal	24°39′08.64"N	93°59′27.08"E	2562
4.	Basikhong	24°45′34.70′′N	93°56′92.10′′E	2525
5.	Samurao	24°40′21.86"N	93°56′12.71"E	2547
6.	Lilong	24°43′09.26"N	93°56′19.69"E	2559
7.	Khoijuman	24°36′54.04"N	93°47′26.17"E	2546
8.	Khurai	24°50′00.26"N	93°58′23.60"E	2570
9.	Nambol	24°42′02.06"N	93°50′09.98"E	2529
10.	Thangmeiband	24°49′09.26"N	93°56′18.31"E	2571

Table 2: Comparision of growth of *Trichoderma* isolates in different culture media**

Sl.no.	*Trichoderma* isolates	Radial growth (cm) on different media			
		PDA	OMA	TSM	Mean
1.	TvFB1	6.93*	5.33	5.33	5.86
2.	ThrFB2	7.23	5.00	5.43	5.88
3.	ThmFB3	8.36	6.86	5.96	7.06
4.	ThrFB4	8.53	7.86	5.83	7.40
5.	TvFB5	7.70	6.86	6.50	7.02
6.	TvFB6	7.56	5.93	5.43	6.30
7.	ThrFB7	8.36	7.26	6.53	7.38
8.	TvFB8	7.50	7.20	6.40	7.03
9.	ThrFB9	9.00	9.00	7.40	8.46
10.	TvFB10	8.40	8.50	7.23	8.04
	Mean	7.95	6.98	6.20	
S.E.(d) ±		0.18	0.10	0.31	
C.D. at (5%)		0.38	0.21	0.65	

**PDA-Potato Dextrose Agar, TSM-*Trichoderma* Specific Media and OMA- Oat Meal Agar; *Mean of three replications, T = *Trichoderma* isolates, M = Media

Table 3: Anamorphic characteristics of *Trichoderma* isolates*

Sl.no.	*Trichoderma* isolates	Phialides(μm)	Phialospores (μm)	Conidiophores(μm)
1.	TvFB1	4.1-7.8x 1.3-1.7	1.6-2.4x1.8-2.2	4.7-28.6x3.0-4.2
2.	ThrFB2	5.7-8.4x2.6-3.7	2.0-3.4x1.6-2.7	5.3-27.1x2.4-4.7
3.	ThmFB3	5.0-6.7x2.7-2.8	4.1-5.2x1.9-2.7	5.9-35.1x3.2-4.6
4.	ThrFB4	4.2-7.8x2.3-3.4	1.9-2.9x1.5-2.4	5.1-26.8x2.2-4.9
5.	TvFB5	4.3-8.4x1.3-1.5	2.2-2.8x1.7-2.1	4.9-32.4x2.7-3.6
6.	TvFB6	4.0-7.8x1.3-1.6	1.8-2.4x1.7-2.2	4.7-28.6x3.0-4.1
7.	ThrFB7	3.9-7.5x2.3-3.2	1.8-3.2x1.3-2.6	4.8-29.7x4.7-5.8
8.	TvFB8	4.0-7.5x1.3-1.6	2.1-3.5x1.6-2.0	7.0-38.8x3.0-3.9
9.	ThrFB9	3.7-8.4x2.6-3.7	2.0-3.5x1.2-2.6	5.2-26.7x2.1-4.6
10.	TvFB10	4.5-8.4x1.2-1.5	2.0-3.0x1.8-2.2	4.8-33.3x2.9-3.6

*Mean of 50 replications

with dense growth and on OMA it showed dark green colour with compact growth and on TSM, whitish green colony with sparse growth. The growth rate was found to be fast on PDA followed by OMA and TSM. The pustules were yellow green on PDA, dark green powdery mass OMA and whitish green on TSM. A yellow pigment was found on PDA, dull yellow on OMA and whitish on TSM. The characteristic of hyphae were hyaline without any aroma in all the three media.

Cultural characteristics of *Trichoderma hamatum*

ThmFB3

The growth of isolate ThmFB3 on PDA was whitish green to greenish colour with highly fluffy growth and compact colony, on OMA it showed compact whitish green colour, compact colony and on TSM, sparse whitish green colour colony with concentric ring. The growth was found to be highest on PDA followed by OMA and TSM.The pustules were showed dark green on PDA and TSM and light green powdery mass on OMA. A pale yellow pigment was found on PDA, where as white pigment was found on OMA and TSM.

Anamorphic characterization of *Trichoderma* isolates

Anamorphic characteristics of *Trichoderma*i solates *viz.*, conidiophores length and width, phialides length and width, phialospores length and width were studied by growing them in potato dextrose agar medium and results are presented in table 3. It is evident from the table that size of*T. Viride* phialides ranged from 4.0-8.4x1.2-1.7μm while phialospores ranged from1.6-3.0x1.6-2.2 and conidiophores ranged from 4.2-38.8x2.7-4.2 μm. The size of *T. hamatum* phialides ranged from 5.0-6.7x2.7-2.8 μm while phialospores ranged from 4.1-5.2x1.9-2.7 μm and conidiophores ranged from 5.9-35.1x3.2-4.6 μm. The size of *T. harzianum* phialides ranged from 3.7-8.4x2.3-3.7μm while phialospores ranged from 1.8-3.5x1.2-2.7 μm and conidiophores ranged from 4.8-29.7x2.1-5.8 μm.

Results of the present findings showed variation in the growth, cultural and anamorphic characters viz., size of phialides, phialospore and conidiophores among the isolates and also species level. Identification of the *Trichoderma* spp. based on cultural characters is in conformity with the findings of Bissett (1991a-c) who characterized the *T. harzianum* as fast growing colonies, white to greyish or sometimes yellowish exudates colourless to amber or greenish yellow, odour indistinct or faintly earthy, and hyphae hyaline. Similarly, it was also found that *T. viride* as rapidly growing fungus, aerial mycelium usually limited, side of the growth was colourless to dull yellowish, some isolates with distinctive aromatic odour resembling coconut and *T. hamatum* was characterized as moderately rapid growing fungus, white to grayish mycelium, indistinct odour, hyaline hyphae. These findings are duly supported by earlier observations (Rifai, 1969; Domsch *et al.*, 1980, Bissett, 1991a-c; Samuel, 2006) where they characterized different species of *Trichoderma*. They have also reported that *T. viride* and its related species are able to secrete á - pyrone, a sweet coconut like aroma. The present findings may give some of the picture regarding the presence of types of *Trichoderma* spp at cultural and morphological level.

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