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Cultural and morphological variability among *Trichoderma harzianum* and *Trichoderma asperellum* collected from chickpea growing areas of Rayalaseema Region of Andhra Pradesh

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ABSTRACT

The genus *Trichoderma* contains species of great economic importance due to their ability to act as biological control agents against a broad range of fungal plant pathogens. In the present study ten isolates of *Trichoderma* species were isolated from rhizospheric soil of chickpea growing areas of Rayalaseema Region, Andhra Pradesh. The isolates were characterized on the basis of their cultural and morphological characteristics. Out of 10 isolates, 5 isolates were identified as *T. asperellum* and 5 isolates as *T. harzianum.* Cultural Characteristics *T. asperellum* isolates were fast growing with light green to dark green fluffy granular growth, mottled with white flecks and often with inconspicuous wefts of yellow hyphae whereas *T. harzianum* isolates were relatively slow grower, with green to dark green coloured colony and effuse conidiation in different media. The size of phialides of *T. harzianum* isolates KNO 9 recorded 7.8-9.7 x 3.3-4.3 µm while the highest size of phialospores 3.5-4.0x 2.5-2.8 µm recorded by ATPU 2. The size of phialides of *T. asperellum* isolate was highest recorded in KNP 1 with 5.3-8.2 x 1.2-1.6 µm , while the size of phialospores was observed in KJ 12 with 2.1-3.5 x 1.6-2.0 µm. The chlamydospores sizes was more in KJ 12 with 9.5 - 13.3 x 8.2 - 9.4 µm.

Key words: Chickpea rhizosphere, *Cultural*, Morphological, *Trichoderma*, Variability

INTRODUCTION

Andhra Pradesh is one of the major chickpea producing states in the country with an area of 5.21 lakh ha with production of 6.76 lakh tones (Anonymous 2018). In the state, chickpea is predominantly grown in the districts of Kurnool, Kadapa and Ananatapur during *rabi* season on residual soil moisture as a winter crop. Low yield of chickpea is primarily attributed to its susceptibility to several fungal, bacterial and viral diseases. Among the economically important diseases, wilt (*Fusarium oxysporum f. sp. ciceris)*, dry root rot (*Macrophomina phaseolina*) and collar rot (*Sclerotium rolfsii*) are the major and widespread affecting chickpea cultivation

Biological control of soil-borne plant pathogens using antagonistic microorganisms is a proven success in many countries (Sivan, 1987) and is therefore becoming popular. Rhizospheric microorganisms act as an initial barrier against pathogens attacking the root and are ideal for use as bio-control agents (Reddy *et al*., 2013).

The genus *Trichoderma* is one of the most widely commercialized bio-control agents for soil-borne diseases in various crops (Pan *et al*., 2001) and hence its precise identification and characterization is essential (Lieckfeldt *et al*., 1999). *Trichoderma* have normally been defined on the basis of their morphology by many taxonomists (Rifai, 1969, Samuel 2006). As is usually the case with other fungal genera, species of *Trichoderma* too were defined originally on the basis of morphology by workers like Rifai (1969) and Bisset (1991a, b and c). The culture sporulation pattern varies within and between species. Seaby (1996) also reported that differentiation of *Trichoderma* spp. using classical microscopic features alone was

difficult since cultural morphology varied widely on different media and spore size varied significantly with incubation temperature. Moreover, variation among the isolates based on size of the phialides, and their arrangements was small. Keeping the above facts in view, the present research was undertaken to characterize the cultural and morphological variability among 10 isolates of *Trichoderma* isolated from the chickpea rhizospheric soil of Rayalaseema region.

MATERIALS AND METHODS

Isolation of Trichoderma from Rhizosphere soil sample

Soil samples were collected from different locations of Kurnool, Kadapa and Anantapur districts of Andhra Pradesh at a depth ranging 5-6 cm, by removing top 2 cm surface soil. The soil collected from rhizosphere of healthy chickpea plants was mixed thoroughly to make a composite sample. Isolation of *Trichoderma* was done by serial dilution technique. The probable colonies of Trichoderma were observed closely and picked up from petri plates containing *Trichoderma* specific media (TSM) and finally pure culture was obtained by repeated subculture. They were identified on the basis of their morphological characters (Rifai, 1969, Samuel 2006).

Cultural characteristics of T. harzianum and T. asperellum

The cultural characteristics of 10 isolates of *Trichoderma* were studied in four media, *viz.*, potato dextrose agar (PDA), oat meal agar (OMA), Cazpex Dox Agar (CZA) and Malt Extract Agar (MEA). Mycelial discs (6 mm) of young growing culture of each isolate of *Trichoderma* was inoculated at edge of the Petriplates containing solidified PDA, OMA, MEA and CZA medium seperately, and incubated at 28 ± 1°C for one week. The growth pattern of isolates of *Trichoderma* was observed daily and all distinguishing characters were recorded. The experiment was replicated thrice. The characters recorded were colony appearance, growth rate, presence or absence of pigments, hyphae and presence of any distinguishing odour.

Morphological characterization of T. harzianum and T. asperellum

Morphological/Anamorphic characterization of *Trichoderma* isolates were done by growing them in potato dextrose agar medium in order to unequivocally verify taxa and species. Morphological characteristics *viz.*, conidiophores length, width and branching pattern, phialides width at tip, middle and base, phialides length and shape, phialospores and chlamydospores shape, length and width including colouration. For morphological characterization, slide culture technique was used and incubated at 25°C with alternating 12 hr dark and 12 hr cool white fluorescent light. All conidiophores, phialides conidia and chlamydospores were measured following the methods of Lieckfeidt *et al.* (2001).

RESULTS AND DISCUSSION

Isolation and identification of Trichoderma spp.

Trichoderma cultures were isolated from soil samples collected from chickpea rhizosphere. Trichoderma isolates revealed pale or yellowish color with rapid growth, loosely arranged conidia and effused conidiation on TSM. Based on colony morphology 10 isolates were selected for further studies.

Effect of different media on growth of Trichoderma isolates

In general, there was dense mycelial growth of *Trichoderma* spp. in MEA followed by OMA, relatively less dense growth in PDA and lesser granular growth of antagonists in CZA. The sporulation was first observed in MEA, followed by OMA and PDA and sporulation was not observed in CZA. Radial growth rate of potential *Trichoderma* isolates on OMA, MEA, PDA and CZA medium were used and growth rate recorded at different intervals of time (Table 1). Out of 10 isolates, MEA and OMA medium supported faster growth rate of 89.0 mm after 72 h compared to other media. Isolates KNN4, KNPG 3, ATPP 6 and KNO 9 recorded more growth rate in four types of

Table 1. Cultural characteristics of some isolates of *Trichoderma* in OMA, MEA, PDA and CZA medium at 25 ± 2° C

Tricho-derma spp.	MEA			OMA			PDA			CZA		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
KJ-12	21.67±0.91^{j*}	50.33±1.57^{g}	76.67±1.14^{d}	19.67±1.02^{d}	55.00±1.22^{ecd}	74.00±1.05^{dbec}	29.00±0.65^{cdb}	46.67±1.46^{hi}	70.33±1.10^{c}	20.67±0.61^{edfg}	39.67±0.50^{gf}	57.33±0.72^{gh}
KNP-1	22.00±0.91^{ij}	59.00±1.57^{def}	88.67±1.14^{a}	21.00±1.02^{dc}	48.00±1.22^{fhgi}	71.67±1.05^{dbecf}	21.00±0.65^{hij}	61.33±1.46^{bc}	77.00±1.10^{c}	19.33±0.61^{efg}	34.00±0.50^{h}	67.00±0.72^{bc}
KNP-3	25.33±0.91^{hig}	60.67±1.57^{cdef}	78.67±1.14^{dc}	23.00±1.02^{dc}	44.67±1.22^{jhki}	69.67±1.05^{gef}	24.33±0.65^{ghf}	58.67±1.46^{dbec}	79.00±1.10^{c}	21.33±0.61^{edfcg}	44.00±0.50^{cab}	54.00±0.72^{ih}
KT6	26.67±0.91^{hig}	75.00±1.57^{a}	87.33±1.14^{a}	23.00±1.02^{dc}	60.33±1.22^{bc}	88.33±1.05^{a}	23.00±0.65^{ghif}	60.33±1.46^{dbc}	82.00±1.10^{bc}	19.00±0.61^{fg}	40.00±0.50^{gfe}	55.00±0.72^{igh}
KNN-2	30.33±0.91^{cedgf}	52.33±1.57^{gf}	89.00±1.14^{a}	24.00±1.02^{cdb}	49.00±1.22^{efgi}	69.67±1.05^{gef}	24.33±0.65^{ghf}	59.33±1.46^{dbc}	86.33±1.10^{ab}	21.00±0.61^{edfcg}	32.67±0.50^{h}	56.33±0.72^{igh}
KNK-1	31.67±0.91^{cedbf}	60.67±1.57^{cdef}	76.67±1.14^{d}	25.00±1.02^{cadb}	52.67±1.22^{ef}	67.33±1.05^{g}	21.33±0.65^{ghij}	40.00±1.46^{i}	86.33±1.10^{ab}	26.33±0.61^{b}	39.67±0.50^{gf}	58.00±0.72^{fge}
KNO-9	33.00±0.91^{cab}	60.00±1.57^{cdef}	85.00±1.14^{ab}	26.33±1.02^{cab}	47.67±1.22^{fhgi}	88.67±1.05^{a}	22.33±0.65^{ghij}	49.33±1.46^{hg}	87.67±1.10^{ab}	30.00±0.61^{edfg}	44.00±0.50^{cab}	58.00±0.72^{fge}
KNN-4	33.00±0.91^{cab}	63.67±1.57^{cdb}	89.00±1.14^{a}	26.33±1.02^{cab}	59.33±1.22^{bc}	68.00±1.05^{gf}	32.00±0.65^{ab}	61.00±1.46^{bc}	87.67±1.10^{ab}	19.67±0.61^{efg}	31.67±0.50^{h}	67.00±0.72^{bc}
KT-13	32.67±0.91^{cadb}	59.67±1.57^{cdef}	89.00±1.14^{a}	26.00±1.02^{cab}	50.00±1.22^{efhg}	67.67±1.05^{gf}	25.33±0.65^{gef}	57.33±1.46^{dfeg}	87.00±1.10^{ab}	24.00±0.61^{bc}	42.67±0.50^{cde}	63.00±0.72^{dc}
ATPP-6	33.67±0.91^{cab}	57.33±1.57^{gdef}	87.67±1.14^{a}	29.00±1.02^{ab}	53.00±1.22^{efd}	73.00±1.05^{dbecf}	28.00±0.65^{ced}	45.00±1.46^{hi}	88.33±1.10^{a}	20.00±0.61^{edfg}	38.00±0.50^{g}	55.67±0.7^{igh}

*Mean of three replicates

*Means with different superscripts are significantly different with p<0.05 by Tukey's HSD test

media. CZA medium supported slow growth, sparse and less sporulation of all the 10 isolates

There were clear differences in growth pattern and other cultural characters of *Trichoderma* spp. when they were grown in four types of media. The colour of *Trichoderma* colony in all media was light green to dark green, yellowish green to dark green with white tinge. More aggregated growth of *Trichoderma* spp. near the periphery region was observed. The white, yellowish or faint yellows to light green pigmentation in the reverse side of growth of *Trichoderma* spp. were also observed. Coconut odour were noticed in most of the isolates of *T. asperellum* (Table 2)

T. harzianum was found to be fast growing on all 4 different media, aerial mycelium floccose, white to greyish or yellowish in colour. pustule are flat, surface appearing granular or powdery owing to dense conidiation, exudates amber to colourless or greenish yellow, odour indistinct and hyphae hyaline. While, *T. asperellum* as rapidly growing fungus, aerial mycelium usually limited, floccose to arachnoid, reverse side of the growth was colourless to dull yellowish, some isolates with distinctive aromatic odour resembling coconut, conidiation effuse, loosely tufted, or in some isolates forming compact pustules, white at first, eventually green or brown. (Table 2&3)

The present results are in agreement with earlier findings of Singh and Kumar (2011) and Chattannavar & Hosagoudar (2012) where they have reported *T. viride* (TV 97) and *T. koningii* grew much faster i.e. @ 7.25 and 7.10 cm respectively, *T. harzianum* (Th 21) was the slowest (5.83cm). Sharma and Singh (2014) studied growth rates of the 30 *Trichoderma* isolates on PDA and all isolates were fast growing reaching a radius of 42.5 to 56.5 mm after 72 h at 25^{0}C and 20 - 37.8 mm after 72 h at 35^{0}C. Conidiation in the *T. harzianum* isolates was predominantly effuse covering the entire surface of the plates.

Our present findings are similar with the reports of Bissett (1991a-c) who characterized the *T. harzianum* as fast growing colonies, aerial mycelium floccose, white to greyish or sometimes yellowish.

Similar findings were also reported by several researchers (Pan and Bhagat, 2008; Chattannavar and Hosagoudar, 2012) that the *T. harzianum* and *T. viride* were fast growing green coloured, mycoparasitic fungi with distinct coconut aroma with 4-5 days old culture in Petriplates. The distinctive sweet or coconut odour is also produced by cultures of losely related *T. viride* and many reports of á-pyrone production by the more distantly related *T. harzianum* (Saxena *et al*., 2014;Prameela *et al*., 2012; Rajesh *et al*., 2013; Sriram *et al*., 2013; Shahid *et al*., 2014).

Morphological Characterization of T. harzianum and T. asperellum

Trichoderma isolates were characterized by following slide culture technique using half strength PDA medium. All the 10 isolates of *Trichoderma* were characterized on the basis of morphological parameters *viz*., shape, size, ornamentation and arrangement of anamorphic characters, *i.e*., conidiophores, phialides, phialospores and chlamydospores in the PDA medium. The shape and size of conidiophores (length and breadth), phialides (length: breadth: middle portion) and

Table 2. Cultural characteristics of *Trichoderma asperellum**

Isolate	Character	PDA	OMA	MEA	CZA
KNP 1	Colony	Dark green with light yellowish coloured tinge, dense mycelial growth and aerial mycelium is usually limited, floccose to arachnoids	Green to dark green colony with relatively scanty mycelial growth and whitish green ring on the colony margin	Pale yellowish or greenish fluffy growth more near the periphery region of plate, radial growth pattern with white border mark on the margin	White to pale green with suppressed growth. radial growth pattern with white border mark on the margin
	Growth rate	Rapid	Rapid	Rapid	Slow
	Pustules	More, Dark green	More, Dark green	More, Dark green	Few , dark green
	Pigment	Yellowish green	Pale yellow	Yellow	Yellow
	Odour	Coconut like aroma	Coconut like aroma	Coconut like aroma	Coconut like aroma
	Hyphae	Hyaline, smooth walled	Hyaline, smooth walled	Hyaline, smooth walled	Hyaline, smooth walled
KNP3	Colony	Greenish fluffy growth with effuse conidiation and uniform distribution on the plate	Greenish colour colony with whitish margin and effuse conidiation	Dark green, raised growth, effuse conidiation and whitish margin at the colony margin.	Dirty white to light green with suppressed growth. colony with whitish margin
	Growth rate	Rapid	Rapid	Rapid	Slow
	Pustules	Greenish to dark green	Abundant, dark green to greyish brown	Abundant, dark green to brown	Limited, dark green to brown
	Pigment	Yellow	Yellow	Dull yellow	Dull yellow
	Odour	Coconut like aroma	Coconut like aroma	Coconut like aroma	Coconut like aroma
	Hyphae	Hyaline, smooth walled	Hyaline, smooth walled	Hyaline, smooth walled	Hyaline, smooth walled
KJ 12	Colony	Light yellowish to green colony, dense mycelial growth with uniform distribution and slight fluffy growth in the periphery region.	Green with pale yellow on the upper surface of growth, slight fluffy growth and whitish border in the margin.	Pale yellowish or dark green, fluffy growth near the periphery region and radial growth pattern with whitish border at the margin.	Pale yellowish and radial growth pattern with whitish border at the margin
	Growth rate	Rapid	Rapid	Rapid	Slow
	Pustules	Abundant, dark green	Abundant, dark green to brown	Abundant, dark green to brown	Abundant, dark green to brown
	Pigment	Dull yellowish	Dull yellowish	Dull yellowish	Dull yellowish
	Odour	Coconut like aroma	Coconut like aroma	Coconut like aroma	Coconut like aroma
	Hyphae	Aerial mycelium is limited, hyaline and smooth walled	Aerial mycelium is limited, Hyaline smooth walled	Hyaline, smooth walled	Hyaline, smooth walled
KT 6	Colony	Light green with effuse conidiation, uniformly distributed throughout the plate with whitish border.	Yellowish green, sparsely distributed with concentric ring formation and white border at the colony margin.	Dull green to green, fluffy growth with uniform distribution and whitish green border at the colony margin.	Pale green, sparsely distributed with concentric ring formation and white border at the colony margin.
	Growth rate	Moderate	Moderate	Moderate	Slow
	Pustules	Abundant, dark green	Moderate, green	Abundant, brown	moderate, brown
	Pigment	Dull yellowish	Dull yellowish	Pale yellowish	Pale yellowish
	Odour	Coconut like aroma	Coconut like aroma	Coconut like aroma	Coconut like aroma
	Hyphae	Hyaline, smooth walled,	Hyaline, smooth walled,	Hyaline, smooth walled	Hyaline, smooth walled
KNN 2	Colony	Light green to green, uniform and compact growth with radial growth pattern.	Pale green to dark green colony, scanty at the centre with radial growth pattern and greenish border in the margin.	Green to dark green, dense growth with radial pattern and whitish border at the colony margin.	Light green, uniform and compact growth with radial growth pattern.
	Growth rate	Very rapid	Rapid	Very rapid	Slow
	Pustules	Fairly abundant, brown	Abundant, dark brown	Abundant, brown	Moderate , brown
	Pigment	Dull yellow	Dull yellow	Dull yellow	Dull yellow
	Odour	Coconut like aroma	Coconut like aroma	Coconut like aroma	Coconut like aroma
	Hyphae	Hyaline, smooth walled	Hyaline, smooth walled	Hyaline, smooth walled	Hyaline, smooth walled

* Data are Mean of Three Replications

Table 3. Cultural characteristics of *Trichoderma harzianum**

Isolate	Character	PDA	OMA	MEA	CZA
ATPP 6	Colony	Dark green, granular, submerged mycelial growth with more growth at the margin.	Green, submerged and whitish colour at the margin of colony.	Dark green submerged, densely growth with slightly raised at the margin.	Dull green, , submerged mycelial growth with more growth at the margin
	Growth rate	Rapid	Rapid	Rapid	Slow
	Pustules	More	Moderate	More	Less
	Pigment	Yellowish	Whitish yellow	Yellow	Whitish
	Odour	Coconut like aroma	Coconut like aroma	Coconut like aroma	Coconut like aroma
	Hyphae	Hyaline, submerged growth.	Hyaline, submerged growth.	Hyaline, submerged.	Hyaline, submerged.
KT 13	Colony	Greenish colony with fluffy growth but slow sporulation	Greenish coloured colony at centre but whitish colour at the margin with slow sporulation	Greenish coloured colony with fluffy growth and whitish colour at the margin	White coloured colony with slow sporulation
	Growth rate	Slow	Slow	Slow	Very Slow
	Pustules	Moderate	Moderate	Moderate	less
	Pigment	No pigment	No pigment	No pigment	No pigment
	Odour	Indistinct	Indistinct	Indistinct	Indistinct
	Hyphae	Hyaline mycelium with floccose.	Hyaline mycelium	Hyaline mycelium	Hyaline mycelium
KNN 4	Colony	Light green with submerged growth and sparsely distributed in the plate	Green to dark green, submerged growth and very scanty growth at the centre but it is denser at margin	Light green to green with fluffy growth at the margin but scanty growth at centre	Light green , submerged growth at the margin but scanty growth at centre
	Growth rate	Moderate	Moderate	Rapid	slow
	Pustules	More	Medium	More	less
	Pigment	Orange yellow	Creamy colour	Yellow	Yellow
	Odour	Indistinct	Indistinct	Indistinct	Indistinct
	Hyphae	Hyaline	Hyaline	Hyaline	Hyaline
KNK 1	Colony	Colonies powdery to felty, white coloured became slightly green after sporulation, no concentric ring	Whitish granular colony with no any concentric ring formation, later on turned light greenish	Whitish colony, becomes light green or greyish green after sporulation.	Whitish concentric ring formation, later on turned light greenish
	Growth rate	Moderate	Moderate	Moderate	Slow
	Pustules	No pustule	No pustule	No pustule	No pustule
	Pigment	No pigment	No pigment	No pigment	No pigment
	Odour	Indistinct	Indistinct	Indistinct	Indistinct
	Hyphae	Hyaline, smooth walled	Hyaline, smooth walled	Hyaline, smooth walled	Hyaline, smooth walled
KNO 9	Colony	Dark green, granular and submerged growth with typical whitish raised growth at the margin	Dark green, submerged with flat growth at the colony margin	Dark green, granular growth, evenly distributed on the plate, more dark green colour at the margin	light green, granular and submerged growth with concentric at margin
	Growth rate	Rapid	Moderate	Rapid	moderate
	Pustules	More	More	More	Less
	Pigment	No pigment	No pigment	No pigment	No pigment
	Odour	Indistinct	Indistinct	Indistinct	Indistinct
	Hyphae	Hyaline	Hyaline	Hyaline	Hyaline

*Data are Mean of Three Replications

phialospores were measured with the help of phase contrast stereo zoom microscope with camera attachment.

Anamorphic characteristics isolates of *T. harzianum* is presented in Table 4, which indicated that the shape of phialides varied from ampulliform to subglobose or lageniform divergent or crowded whorls of 2-5, the middle of phialides were markedly swollen and abruptly tapered towards the tip, shape of conidia were subglobose to ovoid, the conidiophores were highly branched

Table 4. Morphological Characteristics of *Trichoderma harzianum** grown on PDA

Isolate	Phialides		Phialospores		Conidiophores		Chlamydospores	
	Shape	Size (µm)	Shape	Size (µm)	Shape	Size (µm)	Shape	Size (µm)
ATPP 6	Ampulliform or lageniform abruptly tapered towards the tip of phialide.	5.3 - 9.1 x 3.2 - 4.5	Subglobose, green coloured	1.7 - 3.5 x 1.1 - 2.0	Straight, primary branches arise at nearly right angles.	6.0 - 29.0 x 3.0 - 4.2	Ellipsoid or pyriform, intercalary and terminal, light brown	10.5-12.6 x 8.0 - 9.5
KT 13	Lageniform with divergent whorls of 3-5.	3.7 - 8.4 x 2.9 - 3.9	Subglobose to obvoid.	2.0 - 3.5 x 1.2 - 2.6	Straight or flexuous, macro-nematous conidiophores, highly branched, primary branch arises at nearly right angles, bent slightly towards the apex.	5.9 – 22.6 x 3.4 - 7.2	Ellipsoid or pyriform, pale yellowish to brownish colour	9.5 - 12.2 x 6.8 - 8.7
KNN 4	Ampulliform to subglobose or lageniform with strongly swollen in the middle.	3.6 - 7.9 x 2.8 - 3.5	More obvoid, smooth walled.	1.9 - 3.0 x 1.4 - 1.8	Hyaline, smooth wall, straight or flexuous, highly branched and primary branch arises at nearly right angles.	5.5 - 34.2 x 3.0 - 6.9	Fairly abundant, intercalary and terminal, subglobose or pyriform, brownish in colour	10.2-13.0 x 7.5 - 9.3
KNK 1	Ampulliform to subglobose, markedly constricted at base but broadly swollen at middle.	3.9 - 6.8 x 2.6 - 3.4	Subglobose to obvoid.	1.7 - 2.7 x 1.3 - 1.4	Straight, highly branched with primary branches arising at nearly right angles.	6.0 - 36.2 x 2.4 - 4.9	Subglobose to ellipsoidal or pyriform, Solitary contents subhyaline to pale yellow	9.4 - 13.2 x 7.5 - 9.4
KNO 9	Ampulliform, markedly constricted at the base but broadly swollen at middle.	7.8 - 9.7 x 3.3 - 4.3	Subglobose, smooth wall, green coloured.	3.5 - 4.0 x 2.5 - 2.8	Straight or flexuous, highly branched	6.0 - 36.0 x 2.4 - 3.7	Subglobose or pyriform	10.1-12.5 x 7.5 - 9.2

*Data are the means of five replications

pyramidally at nearly right angles and the intercalary or terminal chlamydospores were subglobose to ellipsoid or pyriform. The size of phialides of *T. harzianum* isolates varied from 3.9-6.8 x 2.6-3.4 ìm (KNK1) to 7.8-9.7 x 3.3-4.3 ìm (KNO 9) while the size of phialospores varied from 1.7-2.7 x 1.3-1.4 ìm (KNK 1) to 3.5-4.0x 2.5-2.8 ìm (ATPU 2). The conidiophores sizes were varied from 5.9-22.6 x 3.4-7.2 ìm (KT 13) to 6.0-36.2 x 3.0-4.9 ìm (KNK 1). The chlamydospores sizes were varied from 9.5-12.2 x 6.8-8.7ìm (KT13) to 9.4-13.2 x 7.5-9.4 ìm (KNK 1). The rest of isolates of *T. harzianum* were with intermediate size of phialides, phialospores, conidiophores and chlamydospores**.**

Anamorphic characteristics of *T.asperellum* isolates has been presented in Table 5, revealed that phialides were of lageniform to subglobose, sometimes ampulliform to lageniform, verticillate with divergent whorls of 2-4. The shapes of phialosphores were varied from globose to ellipsoid or oblong with distinctive rough epispore walled. The conidiophores were comparatively narrow and flexuous, with primary branches at regular internodes, typically pyramidally branched and flexuous. The shapes of chlamydospores were varied from ellipsoid to oval, pyriform or globose, born intercalary and terminal. The size of phialides of *T. asperellum* isolates were varied from 5.3-8.2 x 1.2-1.6 ìm (KNP 1) to 4.7-7.8 x 1.5-1.7 ìm (KT 6), while the size of phialospores were varied from 2.1-3.5 x 1.6-2.0 ìm (KJ 12) to 1.9-2.8 x 1.7-2.1 ìm (KNP 1). The chlamydospores sizes varied from 8.7 -12.4 x 7.4 – 9.6 ìm (KNN 2) to 9.5 - 13.3 x 8.2 - 9.4 ìm (KJ12).

Identification of *Trichoderma* spp. at species level by cultural and anamorphic is important for morphological characteristics. These findings are duly supported by earlier observations (Tan Siew 2013; Shahid *et al*., 2014; Roughanian *et al*., 2013.) where they characterized different species of *Trichoderma*. spp. and also reported that *T. asperellum* and its related species are able to secrete a - pyrone, a sweet coconut like aroma. Bhagat and Pan (2010) reported that shape of phialides varied

Table 5. Morphological characteristics of *Trichoderma asperellum** grown on PDA

Isolate	Phialides		Phialospores		Conidiophores		Chlamydospores	
	Shape	Size (µm)	Shape	Size (µm)	Shape	Size (µm)	Shape	Size (µm)
KNP 1	Phialides in verticilloid of 3 or 4, lageniform to subglobose.	5.3 - 8.2 x 1.2 - 1.6	Spores rough , globose to ellipsoid or oblong.	1.9 - 2.8 x 1.7 - 2.1	Comparatively narrow and flexuous, with primary branches at regular internodes.	5.6 - 35.0 x 2.0 - 3.5	Abundant, ellipsoid to oval, intercalary and terminal, dark brown colour	9.5 - 12.7 x 7.5 - 8.9
KNP 3	Regularly paired phialides, slender irregularly bent.	4.7 - 7.8 x 1.5 - 1.7	Rough spored, globose to oval.	2.0 - 2.4 x 1.8 - 2.0	Short branches near the tip and longer ones with repeated branching at the base, without sterile hypha at the tip.	5.1 - 35.0 x 3.1 - 4.8	Ellipsoid to globose, both intercalary and terminal, dark green	9.5 - 13.3 x 7.5 - 9.3
KJ 12	Bottle shaped with rather slender and divergent whorls of 3-4.	4.0 - 7.5 x 1.3 - 1.6	Subglobose, rough spores.	2.1 - 3.5 x 1.6 - 2.0	Flexuous with pyramidally branched at regular intervals.	7.2 - 39.7 x 3.0 - 4.1	Fairly abundant, subglobose to elliptical, intercalary and terminal at short branches	9.7 - 13.8 x 8.2 - 9.4
KT 6	Lageniform, regularly paired phialides, slender irregularly bent.	4.7 - 7.8 x 1.5 - 1.7	Rough spored, globose to oval.	2.0 - 2.4 x 1.8 - 2.0	Short branches near the tip and longer ones with repeated branching at the base, not nearly or at right angles.	5.1 - 35.0 x 3.1 - 4.8	Ellipsoid, both intercalary and terminal, dark green	9.7 - 13.2 x 7.5 - 9.3
KNN 2	Verticillate, slender with open phialides, slender, abruptly tapered towards the apices.	4.5 - 8.5 x 1.3 - 1.5	Rough spored, ellipsoid.	2.2 - 3.0 x 1.8 - 2.2	Flexuous, irregularly branched, smooth walled.	4.9 - 33.4 x 2.9 - 3.7	Ellipsoid, intercalary and terminal, dark brown	8.7 - 12.4 x 7.4 - 9.6

*Data are the means of five replications

from ampulliform to subglobose or lageniform divergent or crowded whorls of 2-5; the middle of phialides were markedly swollen and abruptly tapered towards the tip; shape of conidia were subglobose to ovoid, conidiophores were highly branched pyramidally nearly at right angles in *T. harzianum* and *T. hamatum* and nearly at acute angles for *T. viride*; and the intercalary or terminal chlamydospores were subglobose to ellipsoid or pyriform. Sharma and Singh (2014) observed that conidiophores are highly branched divergent and dendritic, longest branches form near the base of the hypha and nearest the main axis. Branches toward the tip and secondary branches tended to be held at 90° with respect to the axis from which they arise in *T. harzianum.*

Chandra Sekhar *et al* (2017) the isolate, GRT-7 identified as *Trichoderma harizanum* based on the morphological characters. Colony showed dark green producing tufts or pustules fringed by sterile white mycelium, colony reverse showed dull yellowish. Conidiophores are branching and verticillate. Phialide were ampulliform and convergent. Conidia subglobose to obovoid, chlamydospore were infrequently producing and both terminally and intercalary

CONCLUSION

All isolates of *Trichoderma harzianum* initially formed white mycelia which then changed into yellowish green or dark green on its maturation. The fungus showed faster growth of 85-89 mm per 72 hr. Due to the faster colonization of mycelia, formation of white aerial mycelia and green colour colony, the characteristics made the species easier to be identified. Conidiophores of *Trichoderma harzianum* were formed as Straight or flexuous, highly branched, primary branch arises at nearly right angles, bent slightly towards the apex. Whereas, *T. asperellum* growth was recorded moderate 78-88 mm/72 hr. The colony of *T.asperellum* forms less white pustules mycelium compare to *T. harzianum*. The conidiation was predominantly effused and powdery. Most of the conidiophores of *T. asperellum* were formed symmetrically paired along the main branches and axis. The conidiophores branching patterns was broad, verticillate, and almost 90° angle.

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