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IDENTIFICATION OF *TRICHODERMA* SPECIES BASED ON MORPHOLOGICAL CHARACTERS ISOLATED FROM RHIZOSPHERE OF GROUNDNUT (*ARACHIS HYPOGAEA* L)

Y. Chandra Sekhar[1], S. Khayum Ahammed[2], T.N.V.K.V. Prasad[3] and R. Sarada Jayalakshmi Devi[4]

[1]Department of Plant Pathology, S.V. Agricultural College, Acharya N G Ranga Agricultural University, Tirupati – 517 502

[2]Regional Agricultural Research Station, Nandyal, ANGRAU, Andhra Pradesh

[3]Nanotechnology laboratory, Institute of Frontier Technology, Regional Agricultural Research Station, Acharya N G Ranga Agricultural University, Tirupati – 517 502

[4]University Librarian, Acharya N G Ranga Agricultural University, Lam, Guntur - 522034

E-mail: chandu.008y@gmail.com

Abstract: Taxonomic identification of ten isolates of *Trichoderma* spp. upto species level were done based on colony colour, morphology included maximum radial growth was recorded in isolates GRT-3, GRT-4 and GRT-9 at 5th day after inoculation (90.00mm) at growth rate of 30.00mm/day. Were as least radial growth rate was observed in case of GRT-7 (73.00) with growth rate of 24.33mm/day. Microscopic observation was done by using Labomed LX 400 microscope. Species-level identification of *Trichoderma* isolates was done based on the formation of chlamydospores, conidiophores and phialides characters, shape of conidia as the main characters to identify the species. The identified strains are *Trichoderma viride* (GRT-1, GRT-6 and GRT-9), *Trichoderma koningii* (GRT-2, GRT-5 and GRT-8), *Trichoderma* sp (GRT-3), *Trichoderma reeseii* (GRT-4), *Trichoderma harizanum* (GRT-7), *Trichoderma aureoviride* (GRT-10).

Keywords: Groundnut rhizosphere, *Trichoderma* sp., identification.

Introduction

Trichoderma species are economically important for their production of industrial enzymes (cellulases and hemicellulases), antibiotics and their action as biocontrol agents against plant pathogens based on various mechanisms such as the production of antifungal metabolites, competition for space and nutrients and mycoparasitism (Howell, 2003). The ecological importance of this genus, particularly of its mycelium, is to take part in the decomposition of plant residues in soil. Mycoparasitic *Trichoderma* strains are able to recognize the host hyphae, to coil around them, develop haustoria and penetrate the cell wall of the host (Abdullah, 2007). Characterization of the antagonistic effect of *Trichoderma* species is the first step in utilizing the full potential of *Trichoderma* species for specific anti-plant pathogenic applications. Competitiveness is based on rapid growth and the production of various asexual generated conidia and chlamydospores (Chet *et al*., 1998). The ability to

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promote growth and induce resistance in plants is a mechanism which has also been described for members of this genus (Harman, 2006). Several species of *Trichoderma* were used as biological control agents against soil borne plant pathogenic fungi (Kucuk and Kivank, 2003). The advantage of using *Trichoderma* in managing soil borne plant pathogens are eco-friendly, effective, ease of mass culturing with less cost of production and growth promoting effect. However, commercialization of *Trichoderma* for its utility in field crops could not be achieved successfully. A series of abiotic and biotic parameters have an influence on the biocontrol efficacy of *Trichoderma*.

Material & methods

Isolation of native antagonistic *trichoderma* spp. from rhizosphere of groundnut

Composite soil samples were collected from rhizosphere of healthy plants in groundnut field and shade dried. Serial dilution technique (Johnson and Curl, 1972) was used to isolate *Trichoderma* spp**.** from rhizosphere of groundnut. Antagonistic microflora were isolated on *Trichoderma* Selective Medium. One ml of final dilution of soil suspension was poured on to sterilized petriplates and then medium was poured at lukewarm stage. Plates were rotated gently to get uniform distribution of soil suspension in the medium. The plates were incubated at 28 $\pm$ 1^0C and observed at frequent intervals for the development of colonies. Three days old colonies of *Trichoderma* isolates were picked up and purified by single hyphal tip method. A total of ten *Trichoderma* spp. isolates were identified based on mycological keys described by Barnett *et al*. (1972) and used for further studies.

Results and discussion

Categorization of *Trichoderma* isolates based on radial growth

Among the ten isolates of *Trichoderma* spp. considerable variations were observed regarding total growth and growth rate (Table 1). The maximum radial growth was recorded in isolates GRT-3, GRT-4 and GRT-9 at 5^{th} day after inoculation (90.00mm) at rate of 30.00mm/day. Isolate GRT-2 recorded growth of 88.00mm at the rate of 29.33mm/day. The total growth rate of remaining isolates in decreasing order as GRT-6 (87.70mm), GRT-1 (85.30mm), GRT-5 (84.30mm), GRT-10 (83.30mm) GRT-8 (82.70mm).Among all the isolates the least total growth rate was observed in case of GRT-7 (73.00) with growth rate of 24.33mm/day (Fig. 1a & b)

S. No	Isolate	Total radial growth (mm)	Growth rate (mm/day)
1	GRT-1	85.30	28.43
2	GRT-2	88.00	29.33
3	GRT-3	90.00	30.00
4	GRT-4	90.00	30.00
5	GRT-5	84.30	28.10
6	GRT-6	87.70	29.23
7	GRT-7	73.00	24.33
8	GRT-8	82.70	27.56
9	GRT-9	90.00	30.00
10	GRT-10	83.30	27.76

Table 1. Total radial Growth & growth rate of different isolates of *Trichoderma* spp., on PDA media at 5^{th} day after inoculation

Fig1a. Colony growth of different isolates of *Trichoderma* on PDA medium at 5th day after inoculation

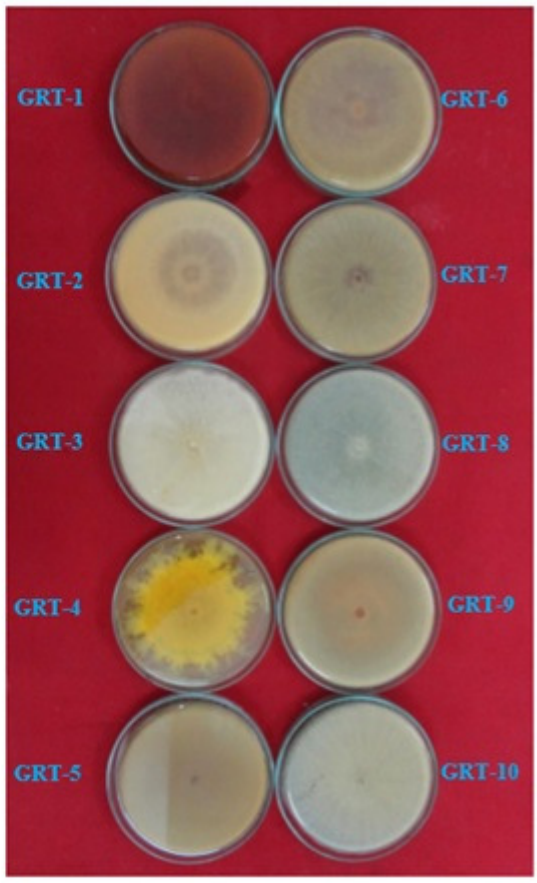

Fig1b. Colony growth of different isolates of *Trichoderma* spp, on PDA medium at 5th day after inoculation (reverse)

Singh *et al.* (1996) obtained twenty seven isolates of *T. harzianum* from soil samples collected randomly from fallow agricultural fields throughout the Punjab and studied their growth rate on PDA medium.

Identification of *Trichoderma* spp. based on morphological Characters

Antagonistic fungal colony had key characteristics that can be used to identify them as *Trichoderma*, including growth pattern, growth rate and colour. Species-level identification of *Trichoderma* isolates was done based on the colour of the colony, formation of chlamydospores, conidiophores and phialides characters, shape of conidia as the main characters to identify the species (Gams and Bisset, 1998). Taxonomic identification of ten isolates of *Trichoderma* spp. upto species level were done based on colony morphology and microscopic observation was done by using Labomed LX 400 microscope (Table 2)

Isolates GRT-1, GRT-6 and GRT-9, colony showed dark green to dark bluish green sporulation, colony reverse was amber or uncoloured. conidiophore usually long, infrequently

branched, verticillate conidiophores. Phialides frequently paried, lageniform convergent(GRT-6 and GRT-9) or divergent (GRT-1). Conidial shape was globose to ellipsoidal. Formation of chlamydospore infrequent or frequent producing terminally and intercalary. Based on these features these isolates were identified as *Trichoderma viride* (Fig .2)

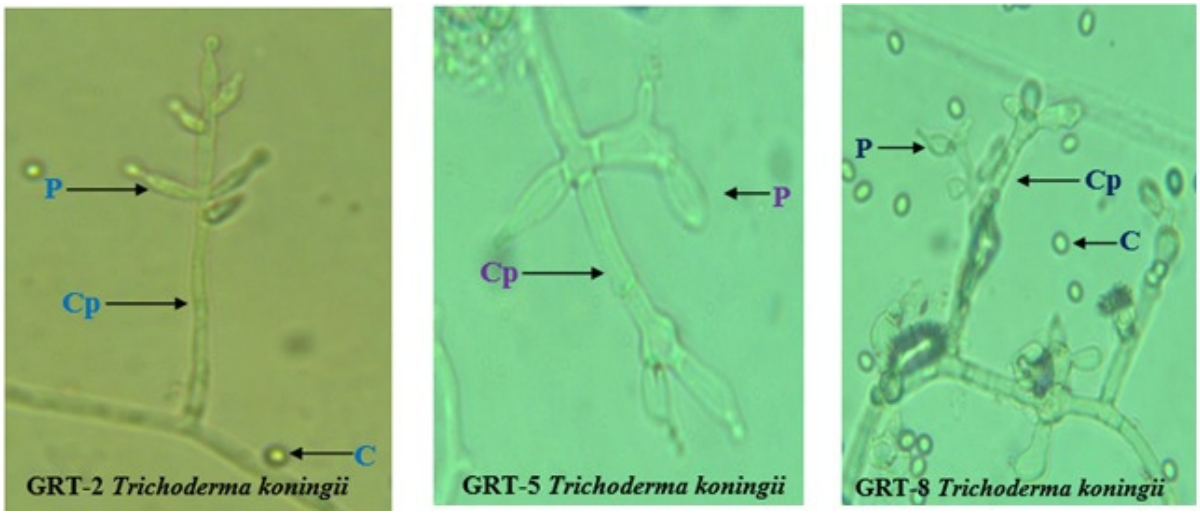

Fig 2. *Trichoderma viride* showing Conidiophores (Cp), Phialides (P), Conidia (C).

S. No	Isolate	Colony colour	Colony Reverse colour	Conidiophores character	Phialide character	Conidia shape	Chlamydospore formation
1	GRT-1	Dark green	Amber	Long, infrequently branched, verticillate	Frequently paired, lageniform, divergent	Globose to ellipsoidal	Infrequent, terminal & intercalary
2	GTR-2	Dull green to bluish green	Colourless	Broad, verticillate, frequent branching	Lageniform, divergent, terminal philaid more elongeated	Sub cylindrical to narrow ellipsoidal	Frequent , intercalary & terminally
3	GRT-3	White	White	-	-	No conidia	Abundant, terminal & intercalary
4	GRT-4	Scattered in minute tufts, pale yellow-green	Pale yellowish	Rarely branched , verticillate	Cylindrical or slightly inflated, divergent	Elliposoidal	Frequently, intercalary & terminally
5	GRT-5	Dell green to bluish green	Pale yellowish	Broad, frequently, branching, verticillate	Ampulliform, Divergent	Sub cylindrical	Infrequent, intercalary & terminally
6	GRT-6	Dark bluish green	Uncoloured	Infrequent branching, verticillate	Lageniform, Convergent	Globose to ellipsoidal	Frequently intercalary & terminally

7	GRT-7	Dark green producing tufts or pustules fringed by starile white mycelium	Dull yellowish	Frequent branching, verticillate	Ampulliform, convergent	Sub globose to obovoid	Infrequent, internally & terminally
8	GRT-8	Dull green to bluish green	Pale yellowish	Narrow verticillate, frequent branching	Ampuliiform, divergent	Sub cylindrical to narrow ellipsoid	Infrequent, intercalary & terminally
9	GRT-9	Dark bluish green	Uncoloured	Infrequent branching, verticillate	lageniform, convergent	Globose to ellipsoidal	Infrequent, intercalary & terminally
10	GRT-10	Compute dull green tufts or pustules	Discoloured	Frequent branching, pyramidal structure	Lageniform, Divergent	Obovoid	Frequently, intercalary & terminally

Table 2. Morphological Characters of *Trichoderma* spp, isolates under Microscope

Isolates GRT-2, GRT-5 and GRT-8, colony showed dull green to bluish green sporulation, colony reverse colourless to pale yellow, conidiophores broad or tnarrow,verticillate branching frequently, phialides showed lageniform or ampulliform, shape divergent and terminal phialidemore elongated. Conidia shape was sub cylindrical to narrow ellipsoid. Formation of chlamydospore infrequent or frequent producing intercalary and terminally. Based on these characters this isolates were identified as *Trichoderma koningii* (Fig 3).

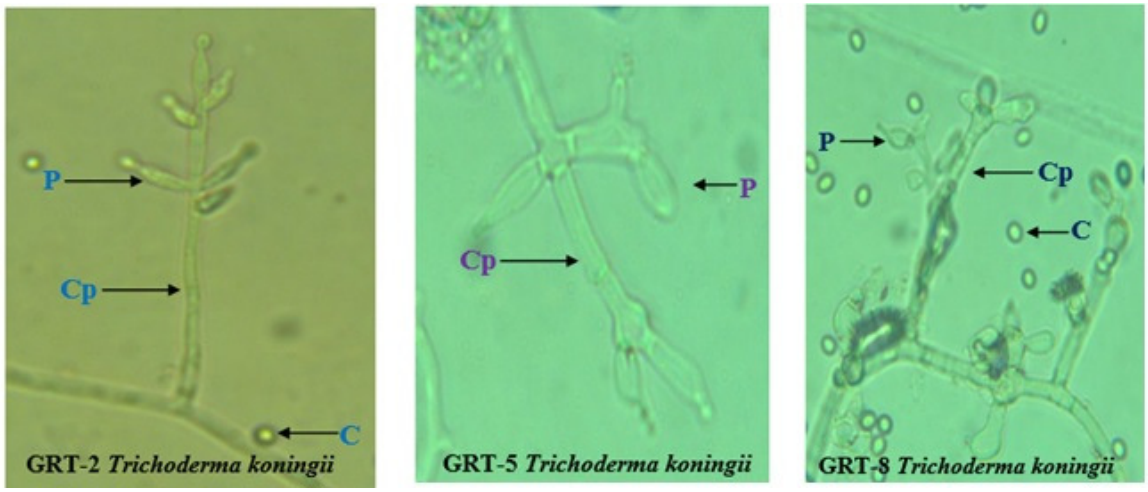

Fig 3. *Trichoderma koningii* showing Conidiophores (Cp), Phialides (P), Conidia (C)

Isolate GRT-3, colony and colony reverse were white in colour, this isolate formed abundant Chlamydospores producing both terminally and intercalary. Based on these characters this isolate cannot be identified at species-level and identified as *Trichoderma* sp. (Fig 4a)

Isolate GRT-4, colony showed scattered in minute tufts, pale yellowish-green in colour, colony reverse was pale yellowish in colour. Conidiophores characters rarely branched and

verticillate. Phialides shape cylindrical or slightly inflated and divergent phialides. Conidia shape was ellipsoidal. Formation of chlamydospore frequently producing both in terminally and intercalary. Based on these characters this isolate was identified as *Trichoderma reeseii* (Fig. 4b)

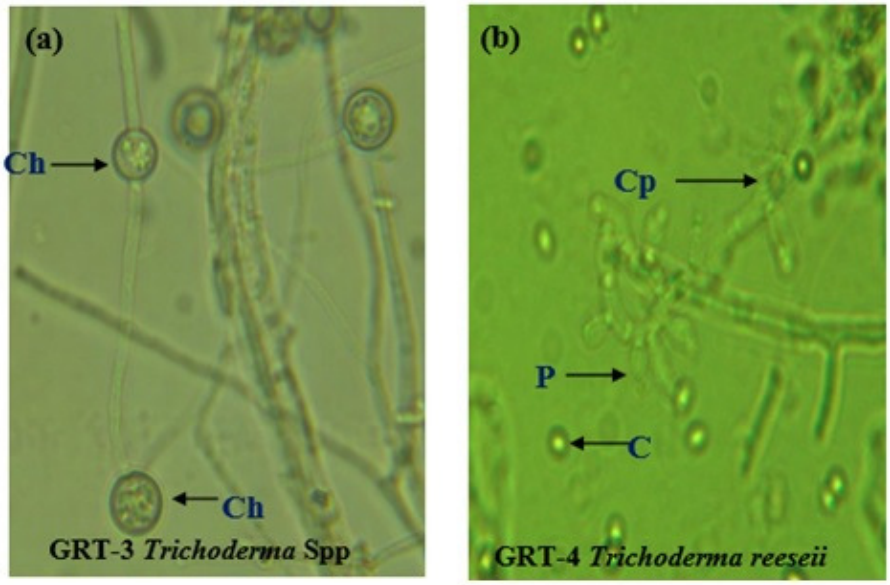

Fig 4a & b. *Trichoderma* spp showing Chlamydospores (Ch) and (b) *Trichoderma reeseii* showing Conidiophores(Cp), Phialides (P), Conidia (C).

Isolate GRT-7, colony showed dark green producing tufts or pustules fringed by sterile white mycelium, colony reverse showed dull yellowish. Conidiophores character frequent branching and verticillate. Phialide were ampulliform and convergent. Conidia subglobose to obovoid shape. Formation of chlamydospore infrequent and producing in both terminally and intercalary. Based on these characters this isolate was identified as *Trichoderma harizanum* (Fig. 5a).

Isolate GRT-10, colony showed compute dull green tufts or pustules, colony reverse was discoloured, Conidiophores character frequently branching and pyramidal structure, phialides was lageniform shape and divergent, conidia Shape was obovid. Formation of chlamydospore frequently and producing in both terminally and intercalary. Based on these characters this isolate was identified as *Trichoderma aureoviride* (Fig. 5b)

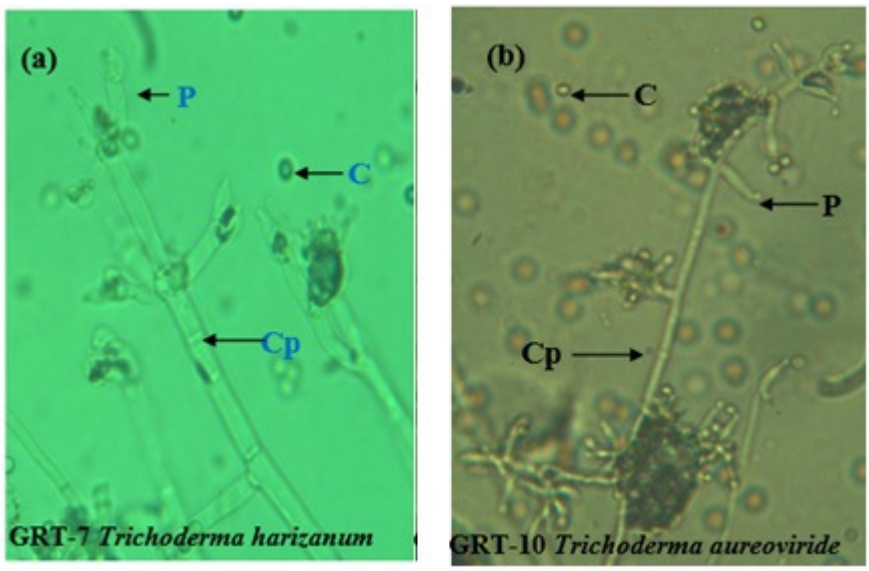

Fig 5a & b *Trichoderma harzianum* showing Chlamydospores (Ch) and (b) *Trichoderma aureoviride* showing Conidiophores(Cp), Phialides (P), Conidia

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