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ISOLATION AND IDENTIFICATION OF NATIVE ANTAGONISTIC TRICHODERMA SPP. FROM RHIZOSPHERE OF GROUNDNUT, REDGRAM AND TOMATO

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ABSTRACT
A total of 27 rhizosphere soil samples were collected from healthy plants present in groundnut, redgram and tomato fields of Chittoor district. A total of nine *Trichoderma* spp. were obtained from 27 soil samples on *Trichoderma* selective medium on 7th day after inoculation. The isolates were designated as GRT-1 to GRT-5 for the five isolates of *Trichoderma* collected from groundnut rhizosphere soils, RRT-1 to RRT-2 for the two isolates collected from redgarm and TRT-1 to TRT-2 for the two isolates collected from tomato crop. *Trichoderma* spp. were characterized based on growth rate, GRT-2, GRT-3, RRT-2 and TRT-2 were categorized as very fast growing; GRT-1, RRT-1 and TRT-1 as fast growing and GRT-4, GRT-5 as medium growing. These nine *Trichoderma* isolates were grouped. *Trichoderma* isolates GRT-2, GRT-4, GRT-5 were grouped under *Trichoderma virens*, RRT-1 and GRT-3 as *Trichoderma harzianum*. TRT-2 and RRT-2 as *Trichoderma asperillum*. Isolates GRT-1 and TRT-1 identified individually as *Trichoderma longibrachiatum*, *Trichoderma pseudokoningii*

KEY WORDS: Rhizosphere, *Trichoderma* spp., antagonistic.

INTRODUCTION

Trichoderma spp. commonly available in soil and root ecosystems has gained immense importance since last few decades due to biological control ability against several plant pathogens.The potential of *Trichoderma* spp. as biocontrol agents of plant diseases was first recognised in early 1930's. Fungal spp. belonging to the genus *Trichoderma* is worldwide in occurrence and belongs to division Ascomycota, subdivision Pezizomycotina, class Sordariomycetes and order Hypocreales. *Trichoderma* is characterized as rapidly growing colonies bearing tufted or postulate, repeatedly branched conidiophores with lageniform phialides and hyaline or green conidia born in slimy heads (Shalini and Kotasthene, 2007) and can be easily isolated from soil preferably in acidic soils with pH 3.5 to 4.5. The success of *Trichoderma* strains as biocontrol agents is due to their high reproductive capacity, ability to survive under very unfavourable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi, and efficiency in promoting plant growth and defense mechanisms. These properties have made *Trichoderma* a ubiquitous genus present in any habitat and at high population densities. Several advantages of using *Trichoderma* in managing soil borne plant pathogens are reported by different workers such as, ecofriendly (Gaur *et al*., 2005), effective in managing diseases caused by soil borne plant pathogens which cannot be easily controlled by chemicals (Manoranjitham *et al*., 2001), ease and cost effective mass Culturing of antagonists (Gaur *et al*., 2005 and Das *et al*., 2006), growth promoting effect (Pan and Bhagat, 2007) and long lasting effective disease management (Sarojini *et al*., 2007). Several strains of *T. viride* had a significant reducing effect on plant diseases caused by pathogens such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium aphanidermatium*, *Fusarium oxysporum*, *F. culmorum* and *Gaeumannomyces graminis* var. *tritici* under greenhouse and field conditions (Bassim *et al*., 1999). Soil borne pathogens causing diseases like collar rot, stem rot, root rot, damping off and wilt in crops like Ground nut, Redgram and Tomato. Root colonization ability of *Trichoderma* spp, is another important properties which make them strong competitive colonizer in the rhizosphere of plant and protect the root infection by pathogens. Thus in present study we concentrated on isolation, identification of *Trichoderma* isolates existing in Ground nut, Redgram and Tomato fields in few regions of Chittoor.

MATERIALS & METHODS

Isolation and Identification of Trichoderma Species

Soil samples were collected from 27 rhizosphere soil samples from different cropping systems such as groundnut, redgram and tomato fields in Chittoor district, Andhra Pradesh. Among 27 soil samples, 13 samples from groundnut, 7 samples from redgram and 7 samples from tomato rhizosphere region were collected. For rhizospheric soil, plants were gently uprooted, soil tightly adhering the roots was collected, mixed and composite mixture of soil of the region was obtained. The pH of soil was determined in 1:2 (soil: water) ratio, keeping 30 minutes of

equilibration. Collected soil samples were air dried for 4 hour and isolation was done by serial dilution technique. Trichoderma Selective Medium (TSH) was used for identification of the isolates of *Trichoderma* (Elad and chet, 1983). One ml of soil suspension was taken with the help of 5ml sterilized pipette and poured on the Petri- plate seeded with TSM. The plates were incubated at 28 + 2°C for 5 days. Observation on the appearance of colonies was recorded from 3rd to 5th day. Individual colonies were picked up and maintained in pure culture for further study. *Trichoderma* species were identified and examined under compound microscope on the basis of their cultural and morphological character (Park *et al.*, 2005) and were maintained on PDA slants at 4°C for subsequent studies.

RESULTS & DISCUSSION

A total of 27 rhizosphere soil samples were collected from healthy plants present in groundnut, redgram and tomato fields of Chittoor district. Among 27 soil samples, 13 samples from groundnut, 7 samples from redgram and 7 samples from tomato rhizosphere region were collected. From these soil samples, five *Trichoderma* isolates were isolated from soils collected from groundnut rhizosphere region, two from redgram and two from tomato (Table 1 and 2, Fig1). The isolates were designated as GRT-1 to GRT-5 for the five isolates of *Trichoderma*

TABLE 1. Soil Samples collected from rhizosphere region of groundnut, redgram and tomato fields from different mandals in Chittoor district

S. No	Name of the mandal	Name of the village	crops	No of soil samples collected
1	Irala	Tellagundlapalli	Groundnut	2
			Redgram	1
2	Rompicherla	Peddagottigallu	Groundnut	1
			Redgram	1
			Tomato	1
3	Vadamalpeta	Devaraju kandriga	Groundnut	2
			Redgram	1
4	Pakala	Pachipala palli	Groundnut	3
5	Ramachandrapuram	Ramapuram	Groundnut	1
			Tomato	2
6	Rompicharla	Bommaiahgari palle	Groundnut	1
			Redgram	1
			Tomato	1
7	Pulicherla	Gaddamvaripalle	Groundnut	1
			Redgram	1
			Tomato	1
8	Pileru	Agraharam	Tomato	1
			Groundnut	2
9	Tirupati rural	Peruru	Redgram	2
			Tomato	1
		Total		27

TABLE 2. List of *Trichoderma* spp. isolated from soil samples collected from different mandals in Chittoor Dist.

S. No	Isolate No	Name of the Mandal	Name of the Village
1	GRT-1	Irala	Tellagundlapalli
2	GRT-2	Ramachandrapuram	Ramapuram
3	GRT-3	Rompicharla	Bommaiahgari palle
4	GRT-4	Pileru	Agraharam
5	GRT-5	Pulicherla	Gaddamvaripalle
6	RRT-1	Vadamalpeta	Devaraju kandriga
7	RRT-2	Rompicharla	Bommaiahgari palle
8	TRT-1	Rompicherla	Peddagottigallu
9	TRT-2	Tirupati rural	Peruru

GRT = Groundnut Rhizosphere *Trichoderma*, RRT = Redgram Rhizosphere *Trichoderma*, TRT = Tomato Rhizosphere *Trichoderma*

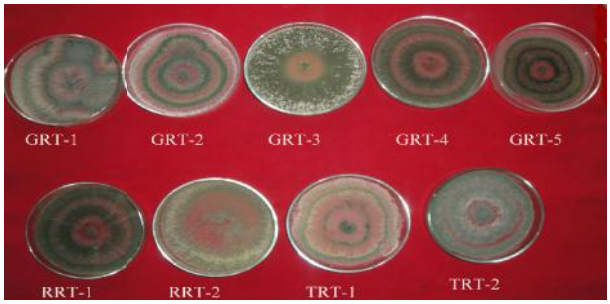

FIGURE 1. Pure cultures of *Trichoderma* isolates from rhizosphere region of groundnut, tomato and redgram

Pure cultures of *Trichoderma* isolates from rhizosphere region of groundnut, tomato and redgram collected from groundnut rhizosphere soils, RRT-1 to RRT-2 for the two isolates collected from redgarm and TRT-1 to TRT-2 for the two isolates collected from tomato crop. In similar study several scientists reported the isolation of *Trichoderma* spp. from rhizosphre soils of different crops. Gajera *et al.* (2011) isolated 12 isolates of three strains (*T. virens, T. viride* and *T. harzianum*) from rhizosphere soils of groundnut crop. Sundaramoorthy and Balabaskar (2013) isolated fungal native antagonists from tomato rhizosphere soils by serial dilution technique using *Trichoderma* selective medium and identified as *T. hamatum, T. harzianum, T. koningi, T. longiconis* and *T. viride*. Depending on the growth rate on PDA medium, nine isolates of *Trichoderma* spp. were categorized into three groups, *viz*., very fast, fast, and medium in radial growth. It is evident from Table 2 that the isolates GRT-2, GRT-3, TRT-2, RRT-2 with 90 mm diameter radial growth within 48 h were categorized as very fast growing and the isolates, GRT-1, RRT-1, TRT-1 which exhibited 90 mm in diameter within 72 h were categorized as fast growing, whereas the isolates GRT-4 and GRT-5 exhibited 90 mm in diameter within 96h were categorized as medium growing isolates. Singh *et al.* (2006) 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 and were categorised as medium, fast and very fast growing. Nine *Trichoderma* isolates were identified according to the identification key (Rifai, 1969) based on branching of conidiophores, shape of the phialides, emergence of phialospores, and shape of phialospores.. These nine *Trichoderma* isolates were grouped in to different isolates. *Trichoderma* isolates GRT-2, GRT-4, GRT-5 were grouped under *Trichoderma virens,* RRT-1 and GRT-3 as *Trichoderma harzianum*. TRT-2 and RRT-2 as *Trichoderma asperillum*. Isolates GRT-1 and TRT-1 identified individually as *Trichoderma longibrachiatum, Trichoderma pseudokoningii* respectively. Muthukumar and Pratibha Sharma (2011) used morphological description for characterization and grouping of *Trichoderma* isolates.

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