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Research Article

IN VITRO ANTAGONISM BETWEEN PHYTOPATHOGENIC FUNGI *SCLEROTIUM ROLFSII* AND *TRICHODERMA* STRAINS

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Abstract

Six *Trichoderma* strains (collected from IARI, New Delhi and MTCC, Chandigarh) were tested for their ability to inhibit soil born pathogen of groundnut mainly *Sclerotium rolfsii* (causing stem rot on groundnut). Morphological observations of *Trichoderma* strains as well as phytopathogenic fungi *S. rolfsii* were made from culture grown at 28 0C for about one week on PDA media. The radial growth, fastest growth and coiling of test fungus of various *Trichoderma* strains on plant pathogenic fungi *S. rolfsii* were examined under LEICA phase contrast binocular light microscope. In vitro percent growth inhibition of S.rolfsii by various *Trichoderma* strain was recorded at 5 days after inoculation at 28 ºC in the 90 cm petriplates. Results obtained from the antagonism study indicated that *Trichoderma* viride (NBAII Tv 23) inhibited 61% growth of phytopathogenic fungi *S. rolfsii* followed by *T. harzianum* (NBAII Th1) (55% growth inhibition of pathogen). This suggested that among different *Trichoderma* strains, *T. viride* was the best bio-control agent to inhibit in vitro growth of phytopathogen *S.rolfsii* which otherwise cause stem rot disease in groundnut.

Key words: Antagonism; soil borne pathogens; *Trichoderma*; biocontrol agent

Introduction

Groundnut (*Arachis hypogaea* L.) is the most important oil seed crop of India. Groundnut occupies the third place in regard to acreage and in production which provides a rich source of high-quality edible oil (45–50%), easily digestible protein (23–25%), minerals and vitamins. Plant disease cause losses of agricultural crops every year. The distribution of several phytopathogenic fungi such as *Sclerotium rolfsii*, *Pythium*, *Phytophthora*, *Rizoctonia* and *Fusarium* has spread during last few years due to changes introduced in farming, with detrimental effects on crops of economic importance. Chemical compounds have been used to control plant disease but it has adverse effect that creates health hazards for humans and other non-target organisms. The development of safer and environmentally feasible plant disease control alternative has become a top priority. In this context, biological control becomes an urgently needs for modern agriculture. Fungi of the genus *Trichoderma* are potential biocontrol agents of several soil born phytopathogens (Benitez *et al*., 2004; Hassan *et al*., 2014). Moreover, all *Trichoderma* isolates exhibited inhibition to the mycelial growth of all pathogens. This could be due to the production of diffusible components, such as lytic enzymes or water-soluble metabolites Anees *et al*. (2010). *Trichoderma* strains are free-living fungi that are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environments. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. Among the plant pathogens, *S. rolfsii* were found to be cause for the stem rot disease in Groundnut. *Trichoderma* have been considered to play an important role in the biological control of stem rot disease. The main objective of this study was to evaluate the interaction between the antagonists *Trichoderma* strains and fungal pathogen *S. rolfsii* (Chet and Baker, 1981; Papavizas, 1985; Chet, 1987; Kumar and Mukerji, 1996; Hassan *et al*., 2013) and the possibility of mycoparasitism through examination of the coiling pattern during antagonism using light microscopy.

Materials and Methods

S. rolfsii was isolated from groundnut plants showing typical symptoms of stem rot were collected from university fields in Junagadh Agricultural University, Junagadh. Strain of *T. harzianum* (NBAII Th1), *T. hamantum* (NBAII Tha – 1), *T. virens* (NBAII Tvs 12) and *T. viride* was (NBAII Tv 23) collected from Indian Agriculture Research Institute (IARI), plant pathology department, New Delhi and two other strains *T. koningii* (MTCC 796) & *T. pseudo koningii* (MTCC 2048) was collected from MTCC, Chandigarh, India. Collected *Trichoderma* strains and isolated pathogen from stem rot of groundnut was sub-cultured on potato dextrose agar slants and kept at 28 ± 1 ºC for seven to eight days for good growth. Such

slants were preserved under refrigeration at 20 ºC. This culture was sub-cultured at regular intervals and was used for further studies.

For each pathogen - *Trichoderma* interaction, a clean slide was placed in petri plates (9 cm) and sterilized. Then a small amount of autoclaved melted potato dextrose agar was spread over the slide to make a thin PDA film on the slide. The 5 mm discs of one week old growing colonies cut from the margin of each pathogen and *Trichoderma* strains were placed on the opposite sides of the slide 3 cm apart on the PDA surface. Then a few ml of double distilled water was added to the plate to prevent drying and then incubate at 25 ± 1 ºC for a week. At the end of incubation period, meeting area of *Trichoderma* – pathogen hyphae was observed under a light microscope for the presence of coiling structures for wall disintegration (Hajieghrari *et al*., 2008).

Six strains of *Trichoderma* were screened against test pathogen in dual culture methods using potato dextrose agar (PDA)(Dennis and Webster, 1971). The radial growth of the pathogen in dual culture and control plates was measured after seven day incubation at 28 ± 1 ºC temperatures. The percent inhibition of mycelial growth of pathogen was calculated according to formula suggested by Watanabe (Watanabe, 1984).

% Growth inhibition = [(C-T)/C] X 100
Where, C = colony diameter of pathogen in control
T = colony diameter of pathogen in inhibition plate

Results and Discussion

Morphological observation

Pigmentation of varying shades was recorded in some strains of *Trichoderma*. Generally white myceliya of *Trichoderma* had many aerial mycelia and its gradually turn to green colour. Yellow pigmentation was shown in *T. koningii* and dark green colours were observed in *T. hamntum.* Similar results were obtains with *Trichoderma* strains often can be readily identified to genus by a distinctive morphology that includes rapid growth, bright green or white conidial pigments and a repetitively branched, but otherwise poorly defined conidiophore structure (Bissett, 1991). Sclerotia begin to develop after 7-8 days mycelial growth of *S .rolfsii*. Initialy a felty white appearance, sclerotia quickly melanize to dark brown coloration. Narsimha rao also reported that, fungus *Sclerotium rolfsii* showed characteristics with white, radiating abundant mycelial growth on the affected portion of plant and tuber. Initially sclerotia exhibited white colour later turn to chocolate brown (Narasimha, 2000).

In vitro percent growth inhibition of pathogen – *S. rolfsii*: The first apparent physical contact between *Trichoderma* strains and its host *S. rolfsii*, occurred within 2-3 days after inoculation (DAI), followed by growth inhibition. Growth inhibition of *S. rolfsii* during *in vitro* interaction with bio-control agents *Trichoderma* at 5 and 10 DAI was depicted in (Fig. 1 and Fig. 2). Percent growth inhibition of pathogen (*S. rolfsii*) was higher in T_6 (61%) antagonist followed by T_1 (55%), T_3 (50%), T_2 (50%), and T_4 (44%) at 5 DAI (Fig. 1). Further, mycoparasitism of antagonists were observed upto 10 DAI (Fig. 1). Thus, it was observed that T_6 antagonist (i.e. interaction between *T. viride* NBAII Tv 23 and pathogen *S.rolfsii*) have a better growth inhibition of test fungus followed by T_1 (*T. harzianum* NABII Th 1× *S.rolfsii*) compared to other *Trichoderma* strains (Table 1).

Evaluation of coiling pattern at 10 DAI

Trichoderma strains tested in this work had a marked significant variation in inhibition of pathogen growth. Maximum pathogen growth inhibition occurred in interacting with *T. viride* NBAII Tv 23 (T_6 antagonist). At 10 DAI, *T. viride* NBAII Tv 23 completely destroyed the host and sporulated (Fig. 3). This process occurred at different intensities depending on the *Trichoderma* strains. suggesting that various antagonists have different mechanisms of host recognition (Silva *et al*., 2004). Microscopic study showed that *T. viride* NBAII Tv 23 was capable of overgrowing and degrading *S. rolfsii* mycelia, coiling around the hyphae with apressoria and hook-like structures (Fig. 2). Formation of apressoria-like structures enabled the hyphae of *Trichoderma* strains to firmly attach to the surface of its host mycelium. However, some antagonists (*T. koningi* MTCC 796, *T. hamatum* NABII Tha 1) used different mechanism against *S. rolfsii* just touched the hyphae without coiling, while, *T. pseudo koningii* showed spore around pathogen not attached to hyphae (Fig. 3). Variability in antagonistic potential among the different strains of *Trichoderma* against different pathogen have been reported (Bhagat and Pan, 2007). Science has reported that the *Trichoderma* strains inhibited mycelial growth of *S. rolfsii* to various degrees, with 52% of strains expressing an average inhibition, varying between 45% and 55%. So, *Trichoderma* strains are very useful as biocontrol agent (Khattabi *et al*., 2004). Efficient coiling process followed by a substantial production of hydrolytic enzymes was reported by dos Reis Almeida *et al*. (2007). The interaction between fifteen isolates of *T. harzianum* and the soil-borne plant pathogen, *Rhizoctonia solani*, was studied by light microscopy and transmission electron microscopy (TEM). *Trichoderma* attaches to the pathogen with cell-wall carbohydrates that bind to pathogen lectins (Howell, 2003; Hassan *et al*., 2014). Once *Trichoderma* is attached, it coils around the pathogen and forms the appresoria. The following step consists of the production of pathogenesis related enzymes and peptaibols.

From above results, it can be concluded that the maximum (61%) growth inhibition of test pathogen with *T. viride* (NBAII Tv 23) followed by *T. harzianum* (NBAII Th1) (55%) was found. So, *Trichoderma* strains are very useful as biocontrol agent. It also can be concluded that the tested *Trichoderma* strains reduced the growth of all the three soil borne pathogens significantly and, therefore, can be incorporated for integrated disease management of soil borne plant pathogens. The degree of antagonism varied between and within strains of *Trichoderma* against the soil borne plant pathogen.

Table 1: Percent inhibition of six strains of *Trichoderma* against plant pathogen *Sclerotium rolfsii*.

Strains of Fungi	Radial mycelial growth (cm)	Percent inhibition
T. harzianum (NBAII Th1),	4.1	55
T. hamantum (NBAII Tha – 1),	4.3	50
T.virens (NBAII Tvs 12)	5.1	50
T. viride (NBAII Tv 23)	4.1	44
T. koningii (MTCC 796)	3.9	50
T. pseudo koningii (MTCC 2048)	4.9	61
Sclerotium rolfsii	4.3	--
C.D at 5 %	0.149	
S.E.(m)	0.049	
C.V%	1.919	

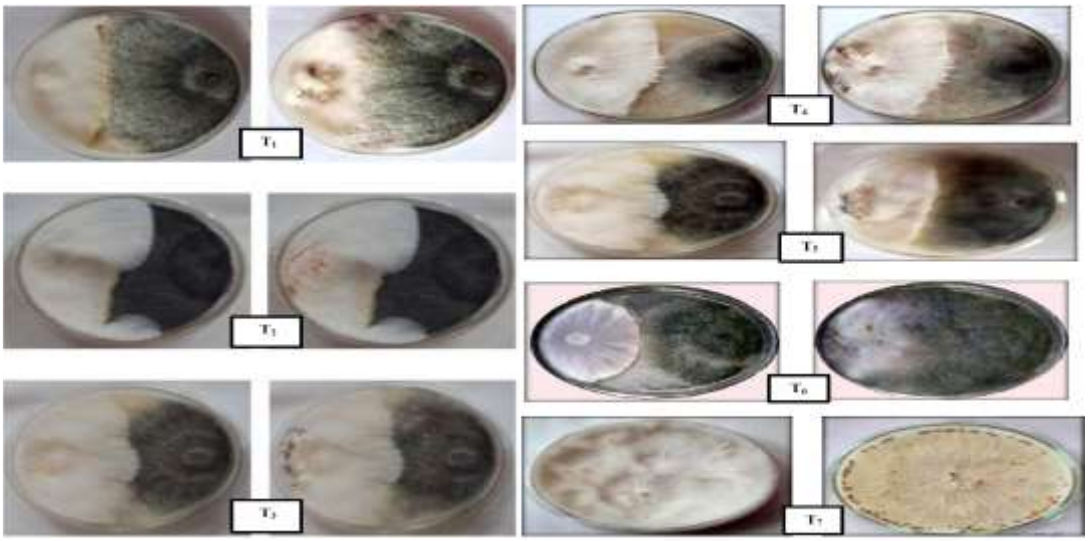

Fig 1: Antagonism between *Trichoderma* strains and *S. rolfsii* at 5 days and 10 days after inoculation in PDA medium. T_1) *T. harzanium X S. rolfsii*; T_2) *T. hamantum* X *S. rolfsii;* T_3) *T. koningii X S. rolfsii*; T_4) *T. Pseudo koningii X S. rolfsii*; T_5) *T. virens X S. rolfsi;*, T_6) *T. viride X S. rolfsii*.

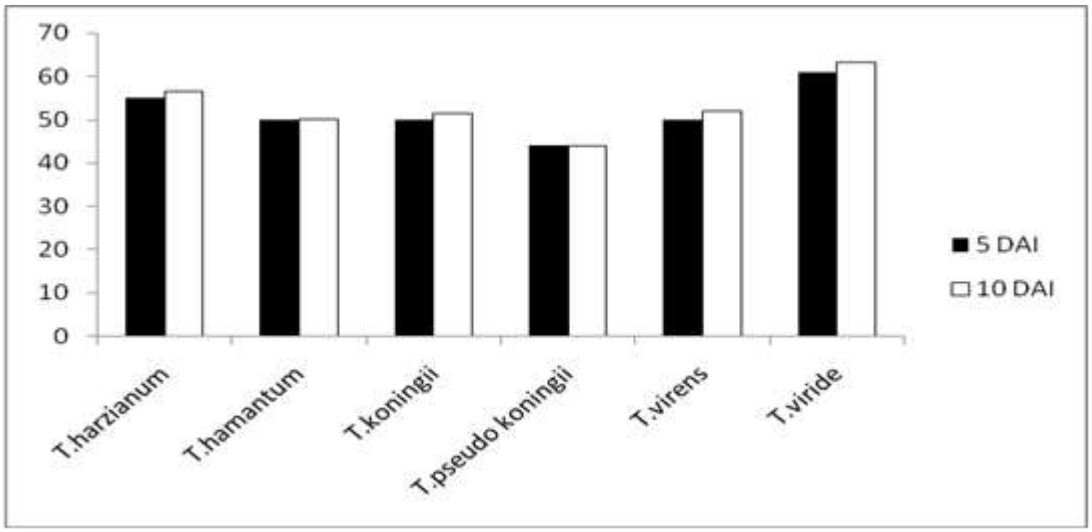

Fig 2: Growth inhibition of *Trichoderma* strains during *in vitro* antagonism with *Sclerotium rolfsii* at 5 and 10 days.

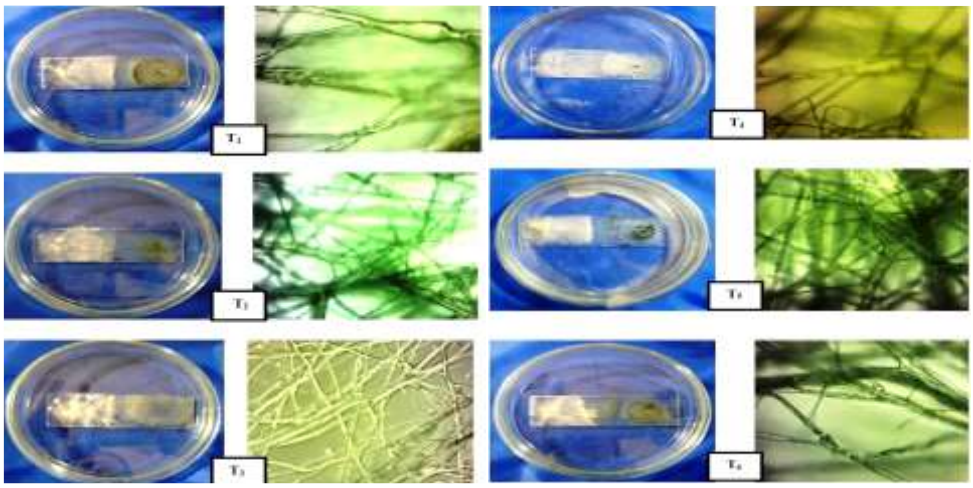

Fig 3: Coiling of mycelia on pathogen (*Sclerotium rolfsii*) by slide culture method. T_1) *T. harzanium X S. rolfsii*; T_2) *T. hamantum* X *S. rolfsii;*T_3) *T. koningii X S. rolfsii*; T_4) *T. Pseudo koningii X S. rolfsii*; T_5) *T. virens X S. rolfsi;*, T_6) *T. viride X S. rolfsii*.

References

Anees M, Tronsmo A, Hermann V, Hjeljord LG, Heraud C and Steinberg C (2010) Characterization of field strains of Trichoderma antagonistic against Rhizoctonia solani. *Fungal Biology* **114**: 691-701. DOI: 10.1016/j.funbio.2010.05.007

Hajieghrari B, Torabi-Giglou M, Mohammadi MR and Davari M (2008) Biological potantial of some Iranian Trichoderma isolates in the control of soil borne plant pathogenic fungi. *African Journal of Biotechnology* **7**: 967-972.

Benitez T, Rincon AM, Limon MC and Codon AC (2004) Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology* **7**:249-260.

Bhagat S and Pan S (2007) Mass multiplication of *Trichoderma harzianum* on agricultural byproducts and their evaluation against seedling blight of mung bean and collar rot of groundnut. *Indian J. Agricltural Science* **77**(9): 583-588.

Bissett J (1991) A revision of the genus *Trichoderma*. II. Infra generic classification. *Canadian J. Botany* **60**: 2357-2372. DOI: 10.1139/b91-297

Chet I (1987) *Trichoderma* - application, mode of action, and potential as a bio-control of soil-borne plant pathogenic fungi. In: Chet I (eds) Innovative Approaches to Plant Disease Control. Wiley, New York, pp. 137-160.

Chet I and Baker R (1981) Mechanism of biological control of pre-emergence damping-off of pea by seed treatment with *Trichoderma* spp. *The American Phytopathological Society* **71**: 286- 290. DOI: 10.1094/Phyto-71-286

Dennis C and Webster J (1971) Antagonistic properties of species-groups of *Trichoderma*: III. Hyphal interaction. *Transactions of the British Mycological Society* **57**: 363-369. DOI: 10.1016/S0007-1536(71)80050-5

dos Reis Almeida FB, Cerqueira FM (2007) Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: evaluation of coiling and hydrolytic enzyme production. *Biotechnology letters* **29**: 1189. DOI: 10.1007/s10529-007-9372-z

Hassan MM, Gaber A and El-Hallous EI (2014) Molecular and Morphological Characterization of *Trichoderma harzianum* from different Egyptian Soils. *Wulfenia Journal* **21**: 80-96.

Hassan MM, El-Awady MA, Lakhani HN and El-Tarras AE (2013) Improvement of Biological Control Activity in *Trichoderma* Against Some Grapevine Pathogens Using Protoplast Fusion. *Life Science Journal* **10**(4): 2275-2283.

Howell CR (2003) Mechanisms Employed by *Trichoderma* Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. *Plant Diseases* **87**: 4-10. DOI: 10.1094/PDIS.2003.87.1.4

Khattabi N, Ezzahiri B, Louali L and Oihabi A (2004) Antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfsii* : screening of efficient isolates from Morocco soils for biological control. *Phytopathologia Mediterranea* **43**: 332-340.

Kumar RN and Mukerji KG (1996) Integrated disease management future perspectives, In: Mukerji KG, Mathur B, Chamala BP and Chitralekha C (Eds.) Advances in Botany. APH Publishing Corporation, New Delhi, pp. 335-347.

Narasimha Rao S (2000). Biological control of wilt of potato caused by *Sclerotium rolfsii* Sacc. M.Sc. (Agri.) thesis, University of Agricultural Sciences, Dharwad, pp – 41- 42.

Papavizas GC (1985) *Trichoderma* and *Gliocladium*: Biology, Ecology, and Potential for Biocontrol, *Annual Review Phytopathology* **23**:23-54. DOI: 10.1146/annurev.py.23.090185.000323

Silva RN, da Silva SP, Brandão RL and Ulhoa CJ (2004) Regulation of N-acetyl-beta-D- glucosaminidase Produced by *Trichoderma harzianum*: Evidence That Camp Controls Its Expression. Research in Microbiology, 155 (8): 667-671. DOI: 10.1016/j.resmic.2004.05.012

Watanabe N (1984) Bulletin of Faculty of Agriculture, Maiji University, Japan **66**: 45