INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-4, Oct-Dec-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 10th August -2013 *Revised: 07th Sept-2013* *Accepted: 12th Sept-2013*

Research article

EVALUATION OF *TRICHODERMA* SPP. AGAINST *SCLEROTIUM ROLFSII IN VITRO*

G. Darvin, I. Venkatesh and G. Nagarjuna Reddy

Department of Plant Pathology, Agricultural College, Bapatla, Guntur (District), A.P, Pin-522101.

Email:darvin.agri@gmail.com

ABSTRACT: To evaluate the effect of *Trichoderma* spp. on radial growth of *Sclerotium rolfsii,* three species of *Trichoderma viz., Trichoderma viride, Trichoderma harzianum* and *Trichoderma longibrachiatum* were selected. The results from this experiment revealed that *T. viride* (TvL), *T. harzianum* 4 (Th 4) and *T. harzianum* 14 (Th14) isolates were found effective and showed lowest radial growth of 3.50 cm and highest per cent inhibition (56.25%) of *S. rolfsii* but were statistically on par with each other. The highest radial growth (4.23 cm) and lowest per cent inhibition (47.1%) were recorded with *T. longibrachiatum* (Tl2). Results from non-volatile assay indicated that irrespective of concentration, culture filtrate of *T. viride* (TvL) was found to be most effective; recorded lowest radial growth and highest per cent inhibition followed by T. harzianum 14 (Th14) and T. harzianum 4 (Th4).

Key words: *Ticoderma, Sclerotium rolfsii, In vitro*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the important oilseed crops of the world. In total agricultural production in India, groundnut's contribution is 11.6%. In India, the area sown under groundnut 2009-10 is 5.48 M ha with a production of 5.43 M t. Gujarat is a major producer (1.43 M ha and 2.0 M t). In Andhra Pradesh the crop is cultivated in 1.02 M ha with a production of 0.8 M t and productivity of 728 kg/ha (Annual Report, Ministry of Agriculture, Government of India, 2010-2011).

Groundnut productivity is affected by several abiotic and biotic stresses, which include poor soil fertility, leaf spots, viral diseases, collar rot and stem rot. Stem rot incited by *Sclerotium rolfsii* Sacc. is one of the major production constraints of groundnut in majority of the tropical and subtropical countries.

S. rolfsii is known to occur in many groundnut growing areas of India including Andhra Pradesh. *S. rolfsii* is a soil borne pathogen which is prevalent where high temperature coupled with high humidity causing severe damage to crop with yield loss of over 25 per cent (Mayee and Datur, 1998).

MATERIAL AND METHODS

Dual culture

In order to find out the antagonistic effect of different species of *Trichoderma viz., Trichoderma viride, Trichoderma harzianum* and *Trichoderma longibrachiatum,* against radial growth of *Sclerotium rolfsii*, dual culture experiment was conducted.

The semi-synthetic medium, Potato Dextrose Agar (PDA) was prepared as per the standard protocol and sterilized in an autoclave. Sterilized potato dextrose agar medium, melted and cooled at 45^0C, was poured aseptically into sterilized Petri dishes. Mycelial discs of 5 mm diameter from the edge of actively growing culture of *S. rolfsii* and isolates of *Trichoderma* spp. were separately cut with the help of a sterilized cork borer and the two discs were simultaneously placed on the periphery about 1 cm from the edge of the Petri dishes (9 cm diameter) on opposite sides. Each treatment was replicated four times and experiment was conducted in completely randomized design. The petri dishes containing potato dextrose agar medium inoculated with the pathogen alone served as control.

All the Petri dishes were incubated at room temperature of 25 ± 1^0C. Seven days after incubation, the colony diameter of the pathogen was measured and the per cent inhibition of *S. rolfsii* was calculated by adopting the following formula.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

$$I = \frac{C - T}{C} X\ 100$$

Where,

I= Per cent Inhibition

C = Radial growth in control

T= Radial growth in treatment

Three effective *Trichoderma* isolates were selected from the above experiment for further studies i.e. determination of non-volatile compounds produced by *Trichoderma* spp.

Effect of non-volatiles on radial growth of S. rolfsii:

Collection of culture filtrates of bioagents

To obtain the culture filtrates of bioagents, the bioagents were grown on the potato dextrose broth (PDB). PDB was prepared as per the standard protocol. Fifty ml of broth was transferred to each of 250 ml conical flasks and sterilized at 1.1 kg/cm^2 pressure for 15 minutes. These flasks were allowed to cool. Separate sets of flasks were maintained for each bioagents. Five mm disc of each *Trichoderma* spp. were inoculated separately to the respective sets of flasks. The flasks were incubated at 27 ±1^{0}C. After seven days of incubation the culture was filtered through Watman No. 42 filter paper. Thus the culture filtrates of each bioagents were collected separately in different sterilized container and utilized for further studies.

Assaying of culture filtrate of bioagents to S. rolfsii

Required quantity of culture filtrate was added to sterilized and cooled PDA medium to get desired concentrations viz., 5%, 10% and 15% of the selected bio-agents. Twenty ml of such poisoned PDA medium was poured in to the sterilized Petri plates and allowed to cool. On these plates a 3 mm culture discs were placed at the centre of the plate. After inoculation plates were incubated in inverted position at 27 ± 1^{0}C for a period of one week and the observations were taken on the radial growth of the pathogen and per cent inhibition was calculated by using following formula.

$$I = \frac{C - T}{C} X\ 100$$

RESULTS AND DISCUSSION

Dual culture technique

Among the *Trichoderma* spp., *T. viride* (TvL), *T. harzianum* 4 (Th 4) and *T. harzianum* 14 (Th14) isolates were found effective and showed lowest radial growth of 3.50 cm and highest per cent inhibition (56.25%) of *S. rolfsii* but, were statistically on par with each other. These were followed by *T. harzianum* (ThL) (3.77 cm & 52.92%) and *T. harzianum* 22 (Th 22) (3.83 cm & 52.08%) of radial growth and per cent inhibition, respectively. The highest radial growth (4.23 cm) and lowest per cent inhibition (47.1%) were recorded with *T. longibrachiatum* (Tl2) (Table 1& Fig 1).

Table 1. Effect of *Trichoderma* spp. on radial growth of *S. rolfsii*

S.No:	*Trichoderma* isolate	Growth (in cm)*	Inhibition (%)*
1	*Trichoderma viride* (Tv L)	3.50	56.25
2	*Trichoderma harzianum* (Th L)	3.77	52.92
3	*Trichoderma harzianum* 22 (Th 22)	3.83	52.08
4	*Trichoderma harzianum* 4 (Th4)	3.50	56.25
5	*Trichoderma harzianum* 14 (Th14)	3.50	56.25
6	*Trichoderma longibrachiatum* 2 (Tl 2)	4.23	47.1
7	Control	8.00	--
CV (%)	3.29		**CD at 5%** 0.13

*** Mean of four replications**

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

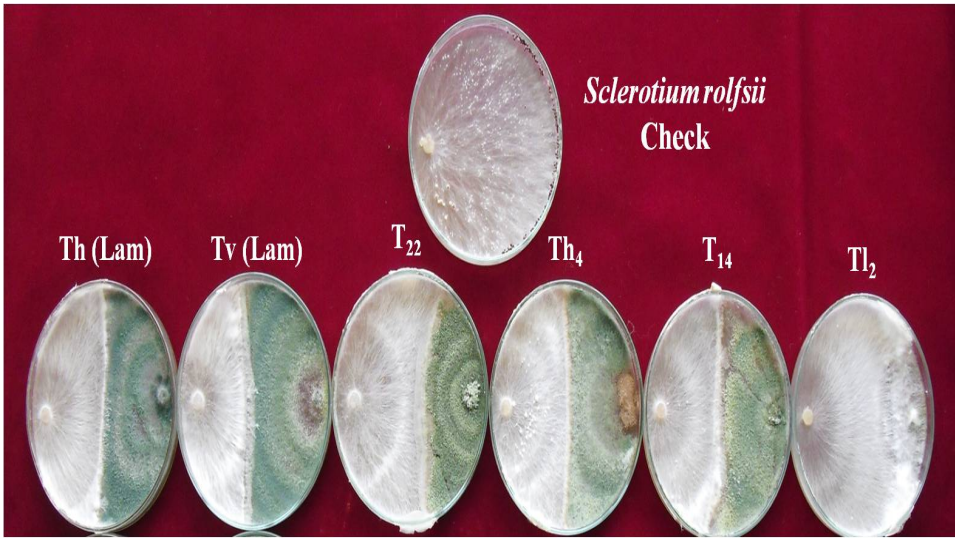

Fig. 1. Effect of *Trichoderma* spp. on radial growth of *Sclerotium rolfsii*

A clear inhibition zone was observed on the 6th day after the inoculation in *T. viride* treatment and later the growth of *T. viride* was observed on *S. rolfsii.* This may be attributed to the faster growing ability of *Trichoderma* spp. than *S. rolfsii* in agar plates. These results were supported with earlier workers. Rapid growth of *T. harzianum* may give it an advantage in the competition with pathogenic fungi for space and nutrients (Barbosa *et al*., 2001). The inhibition was supported by Elad *et al.* (1983), who reported that *Trichoderma* spp. attached to the *S. rolfsii* either by hyphal coils, hooks, or appressoria. Lysed sites and penetration holes were found in hyphae of the pathogenic fungi, following removal of parasitic hyphae and high β-(1,3) glucanase and chitinase activities were detected in dual agar cultures when compared with fungus alone. Suppression of *Macrophomina phaseolina* by overgrowth of *Trichoderma* spp., colonies in the culture medium accompanied by hyphal coiling, hyphal abnormalities, reduction in sclerotial production and lysis of hyphae and sclerotia was reported (Malathi, 1996). Bhuiyan *et al.,* (2012) reported that, *T. harzianum* (TH)-18 showed the highest (83.06%) reduction of the radial growth followed by TH-2 (74.19%).

Effect of non-volatiles on radial growth of *S. rolfsii:*

Among the biocontrol agents, irrespective of concentration, culture filtrate of *T. viride* (Tv L) was found to be the most effective; recorded the lowest radial growth of 3.37 cm, and it was significantly superior to other treatments. It was followed by *T. harzianum* 14 (Th14) (5.60 cm). Highest radial growth was found in the culture filtrate of *T. harzianum* 4 (Th4) (15.03 mm), indicating the least inhibition on the radial growth of *S.rolfsii* (Table 2 & Fig 2).

Table 2. Effect of culture filtrate on radial growth of *S. rolfsii*

Bio-control agent	Concentration (%)						Mean
	5 %		10 %		15 %		
	Growth (cm)*	Inhibition (%)*	Growth (cm)*	Inhibition (%)*	Growth (cm)*	Inhibition (%)*	
Trichoderma viride (Tv L)	5.40 (2.42)	40.0	4.70 (2.28)	47.8	0.0 (0.71)	100.0	3.37 (1.80)
Trichoderma harzianum 14 (Th14)	7.07 (2.75)	21.5	5.4 (2.43)	40.0	4.33 (2.20)	51.9	5.60 (2.46)
Trichoderma harzianum 4 (Th4)	7.50 (2.83)	16.7	5.53 (2.46)	38.5	4.53 (2.24)	49.6	5.86 (2.51)
Control	9.00		9.00		9.00		9.00
Mean	6.66(2.67)		5.21(2.39)		2.96(1.72)		

	SEm	SEd	CD	CV
Biocontrol	0.014	0.020	0.041	1.84
Concentration	0.014	0.020	0.041	
Interaction	0.024	0.034	0.094	

Values in parenthesis are square root transformed values
*** Mean of three replications**

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

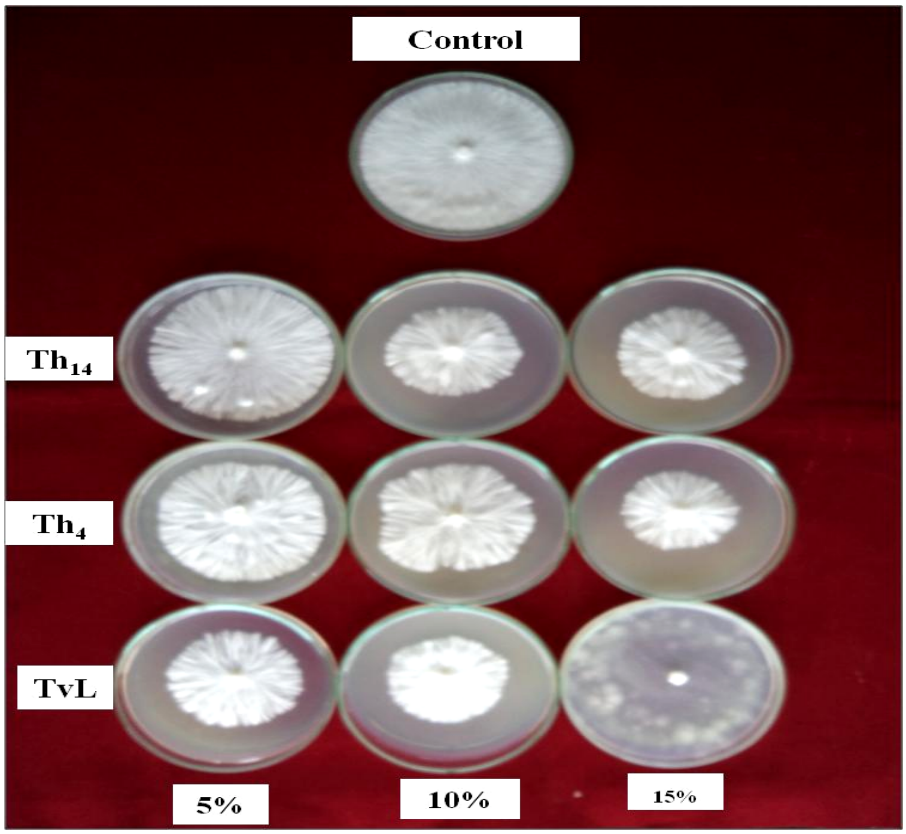

Fig. 2. Effect of culture filtrate of *Trichoderma* spp. on radial growth of *Sclerotium rolfsii*

A significant increase in inhibition of mycelia growth was observed with increase in the concentration of the culture filtrate. Irrespective of the biocontrol agents, concentration of the extracts at 15% was found to be most effective against mycelia growth, recording the lowest radial growth (2.96 cm), followed by the concentration at 10% (5.21 cm) and concentration at 5% (6.66 cm). Interactions between *Tricoderma* spp. and concentration of culture filtrate were found to be significant. The inhibitory effect of culture filtrate on the development *S.rolfsii* was influenced by the concentration at which it was used. Similarly the differences in the inhibition of radial growth due to differences in the concentrations of extracts were affected by the culture filtrate. Our results are inconformity with the earlier workers. Agrawal *et al.* (1978) showed that culture filtrate of the *T. harzianum* inhibited the growth of pathogen. Upadhyay and Mukhopadhyay (1983) observed that the culture filtrate of the isolates of *T. harzianum* reduced the growth of *S. rolfsii* and recorded the per cent inhibition ranged from 5.67 to 50.67.

Pande (1985) found that culture filtrate of *T. viride* retarded the growth of *S. rolfsii.* The effect was slightly higher when culture was applied to seeds than mixed with soil under green house condition. Sharma (1994) observed the reduction in sclerotial germination in culture filtrate of *T. harzianum, T. virens and T. viride* and concluded that the inhibitory activity of antagonists might be due to diffusible metabolites secreted by them.

Subhendu Josh and Pan (2004) observed that at 2% concentration of culture filtrate of *T. viride* inhibited maximum mycelial growth. At 10 per cent concentration, *T. viride* showed complete inhibition of *Rhizoctonia solani* mycelia.

Kapil and Kapoor (2005) observed that the culture filtrates of *Trichoderma* spp. inhibiting the mycelial growth and sclerotial germination of *Sclerotinia sclerotiorum.* Maximum inhibition in mycelial growth and sclerotial germination (45.65%) were observed due to culture filtrates of *T. viride* (93.33%). The antifungal effect of the culture filtrate was attributed because of the viridin, as a fungistatic metabolite produced in the culture of *Trichoderma.*

Studies using metabolites of the bioagents Brain and Mc Gowan (1945), described a second highly fungistatic antibiotic, viridian produced by *T. viride*. Dennis and Webster (1971) studied the production of non-volatile (diffusible) antibiotic substance by *Trichoderma* spp. by an "agar layer technique". They noticed that, many isolates produced the non-volatile antibiotics active against a range of fungi. The ability to produce such substances varied between the isolates. The susceptibility of pathogenic fungi was also varied widely.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Papavizas (1981) found that several UV-induced mutants of *T.harzianum* which produced two unidentified metabolites and one was heat stable. Mathur and Bhatnagar (1994) observed the production of non-volatile substances by *T. viride* in agar culture that inhibited completely both the growth and pycnidial production of *M. phaseolina.*

REFERENCES

Agrawal S C, Khare, M N and Agrawal P S (1978). Biological control of *Sclerotium rolfsii* Sacc. Causing color rot of lentile. Indian Phytopathology, 30: 176- 179.

Annual Report (2010-2011). Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India. http:/www. agricoop.nic.in.

Athur K and Bhatnagar K (1994). Effect of volatile and non volatile substances produced by *Trichoderma viride* on stem blight pathogen of cowpea. Journal of Biological Control, 8: 64-65.

Barbosa M A, Rehn G K, Menezes M and L R Mariano (2001). Antagonism of *Trichoderma* species on *Cladosporium herbarum* and their enzymatic characterization. Braz. Journal of Microbiology, 32: 98-104.

Bhuiyan M A H B, Rahman M T and Bhuiyan K A (2012). *In vitro* screening of fungicides and antagonists against *Sclerotium rolfsii.* African Journal of Biotechnology, 11(82): 14822-14827.

Brian P W and Gowav J C (1945). Viridin, a injury fungicidal substrate produced by *Trichoderma viride*. Nature, 156: 144-145.

Dennis C and Webster J (1971). Antagonistic properties of species of groups of *Trichoderma* I. Production of non-volatile antibiotics. Transactions of Brurish Mycological Society, 57: 25-29.

Elad Y, Chet I, Boyle P and Henis Y (1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* – scanning electron microscopy and fluorescence microscopy. Phytopathology, 73: 85-88.

Kapil R and Kapoor A S (2005). Management of white rot of pea incited by (*Sclerotinia sclerotiorum*) using *Trichogramma* spp. and biopesticides. Indian Phytopathology, 58(1): 10-17.

Malathi P (1996). Biocontrol of groundnut (*Arachis hupogaea* L.) dry root rot caused by *Macrophomina phaseolina* (Tassi.) Gold. *Ph D Thesis*, Tamil Nadu Agricultural University, Coimbatore, India.

Mayee C D and Datur V V (1998). Diseases of groundnut in the tropics. Review of Tropical Plant Pathology, 5: 85-118.

Pande A (1985). Biocontrol characteristics of some moulds. Biovigyanam, 11: 14-18.

Papavizas G E (1981). Survival of *Trichoderma harzianum* in soil and in peanut and rhizosphere. Phytopathology, 72: 121-125.

Sharma B K (1994). Efficacy of biocontrol agents for the control of chickpea stem rot. Journal of Biological Control, 8: 115-117.

Subhendu Josh and Pan S (2004). Stable mutants of *Trichoderma virens* for biological control of *R. solani* that causes seedling blight in greengram. Journal of Myocology and Plant Pathology, 34: 476-479.

Upadhyay J P and Mukhopadhyaya A N (1983). Effect of non volatile and volatile antibiotics of *Trichoderma harzianum* on the growth of *Sclerotium rolfsii.* Indian Journal of Mycology and Plant Pathology, 13: 232-233.