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Research article

EFFECT OF BIO-CONTROL AGENTS ON RADIAL GROWTH OF *SCLEROTIUM ROLFSII IN VITRO*

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ABSTRACT: To know the efficacy of bio-control agents on growth of *Sclerotium rolfsii* causing stem rot of groundnut, an *in vitro* study was conducted in the Department of Plant Pathology, Agricultural College, Bapatla. For this, three *Trichoderma* spp., two *Pseudomonas fluorescens* isolates and one *Bacillus subtilis* were selected as biocontrol agents. We observed that, there is a significant difference among all the treatments. The lowest radial growth (1.9 cm) and highest per cent inhibition (79.26%) were recorded with *T. viride* PDBC isolate followed by *T. harzianum* 4 (2.3 cm and 74.81%) and *Trichoderma harzianum* PDBC (2.6 cm and 70.74%) isolates, respectively. Among the bacterial isolates, lowest radial growth (4.6 cm) and highest per cent inhibition (48.89%) of *S. rolfsii* were recorded with *Bacillus subtilis* PDBC isolate. But the highest radial growth (7.6 cm) and lowest per cent inhibition (15.56%) was recorded with *P. fluorescens* SPA1 isolate.

Key words: Biocontrol, *Sclerotium rolfsii, Trichoderma* and *Bacillus subtilis*

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%. Groundnut productivity is affected by several abiotic and biotic stresses, which include poor soil fertility, leaf spots, virus diseases and collar rot and stem rot. Stem rot incited by *Sclerotium rolfsii* Sacc. is one of the major production constraints of groundnut (*Arachis hypogaea* L.) 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 and causing severe damage to crop with yield losses of over 25 per cent (Mayee and Datur, 1998).

MATERIAL AND METHODS

In order to find out the antagonistic effect of different antagonistic microorganisms on radial growth of *S. rolfsii,* we conducted dual culture experiment. For this, *T. viride*, *T. harzianum*, and *Bacillus subtilis* isolates collected from Project Directorate of Biological Control (PDBC), Bangalore and native isolates includes one isolate of *T. harzianum* (Th4) and two isolates of *Pseudomonas fluorescens* (SPA1 and BN 1) were used as bio-control agents.

Effect of bio-control agents on radial growth of *S. rolfsii:*

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 *T. viride* 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. In case of bacterial isolates, bacteria were inoculated as a line by streaking on one edge of a 9 cm diameter Petri plate containing PDA medium, pH 6.1 and incubated at 30^0C. Each treatment was replicated three 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.

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Inhibition of growth was calculated by using the formula given below.

$$\text{Per cent inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

RESULTS AND DISCUSSION

Results from the Table 1 revealed that, there is a significant difference among all the treatments. The lowest radial growth (1.9 cm) was recorded with *T. viride* PDBC followed by *T. harzianum* 4 (2.3 cm) and *T. harzianum* PDBC (2.6 cm), respectively. Among the bacterial isolates, lowest radial growth (4.6 cm) of *S. rolfsii* was observed with *B. subtilis* PDBC isolate. But the highest radial growth (7.6 cm) was observed with *P. fluorescens* SPA1 isolate.

The highest per cent inhibition (79.26%) was recorded with *T. viride* PDBC followed by *T. harzianum* 4 (74.81%) and *T. harzianum* PDBC (70.74%), respectively. Among the bacterial isolates, highest per cent inhibition (48.89%) of *S. rolfsii* was recorded with *B. subtilis* PDBC isolate. But the lowest per cent inhibition (15.56%) was recorded with *P. fluorescens* SPA1 isolate (Fig. 1&2 and Plate 1&2).

Table 1. Effect of bio-control agents on radial growth of *S. rolfsii*

S. No.	Bio-control agent	On 5th day	
		Growth (in cm)	Inhibition (%)
1	*Trichoderma viride* PDBC	1.90	79.26
2	*Trichoderma harzianum* PDBC	2.60	70.74
3	*Trichoderma harzianum* 4	2.30	74.81
4	*Bacillus subtilis* PDBC	4.60	48.89
5	*Pseudomonas fluorescens* SPA1	7.60	15.56
6	*Pseudomonas fluorescens* BN1	7.30	18.52
7	Control (*Sclerotium rolfsii* alone)	9.0	--
	CV (%)	2.88	
	CD at 5%	0.11	

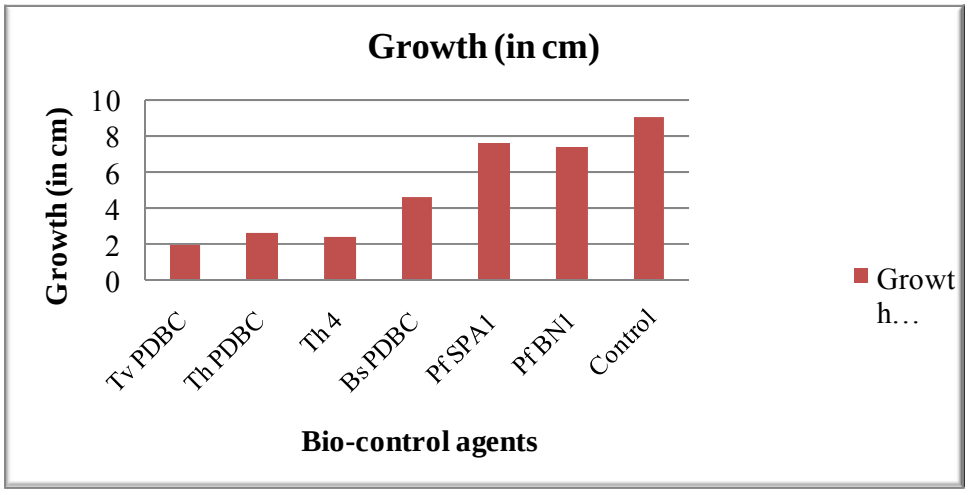

Fig. 1: Effect of bio-control agents on radial growth of *Sclerotium rolfsii* (Growth)

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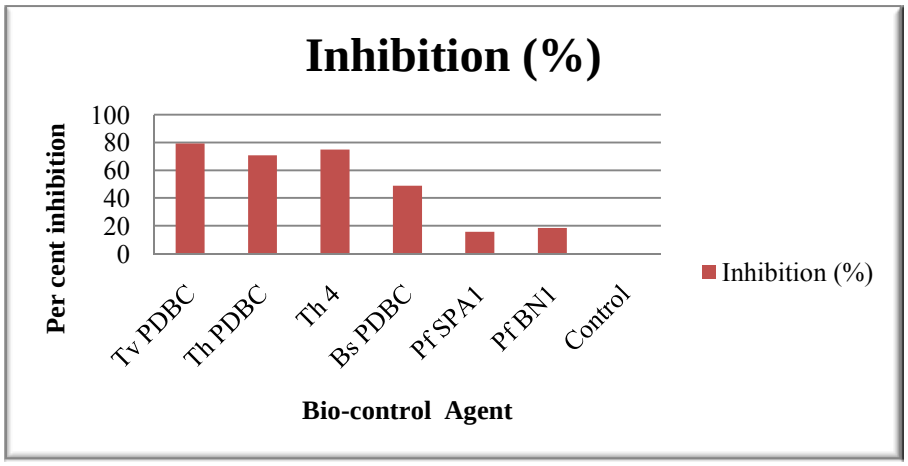

Fig. 2: Effect of bio-control agents on radial growth of *Sclerotium rolfsii* (Per cent inhibition)

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

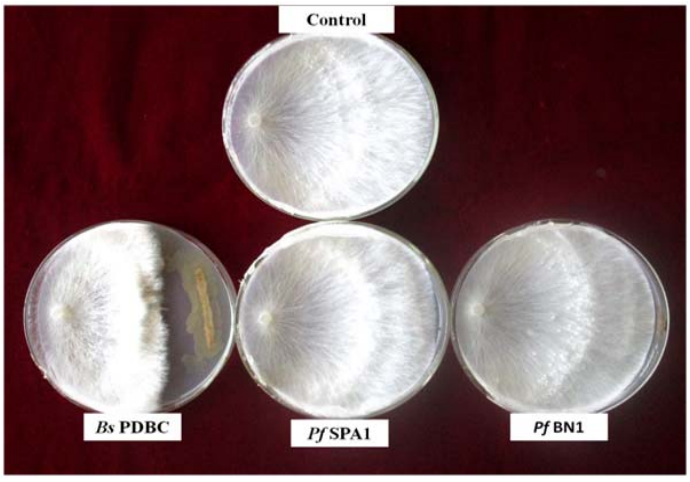

Plate 2. Effect of bacterial antagonists on radial growth of *Sclerotium rolfsii*

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It was also found that the colonies of *T. harzianum* grew faster than those of *S. rolfsii* in agar plates in the present study. It may be assumed that the rapid growth of *T. harzianum* may give it an advantage in the competition with pathogenic fungi for space and nutrients. Five days after incubation, *T. viride* over grown on *S. rolfsii*. In case of *T. harzianum* PDBC isolate, a clear inhibition zone was observed at interaction site and later this zone was extended towards *S. rolfsii.* This may be due to production of antibiotics or chitinase enzymes by antagonistic *Trichoderma* isolates.

Our results were supported by earlier workers. Elad *et al.* (1983) 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. Varadharajan *et al.* (2006) reported that isolate 1 of *T. viride* (69.4%) and *P. fluorescens* (64.4%) were inhibitory to radial growth and sclerotial formation of *S. rolfsii.* The culture filtrate of *T. viride* isolate 1 inhibited the growth of the pathogen (87.05%) as well as the sclerotial germination (62.5%) to a greater extent. Rangeshwaran and Prasad (2000) reported that, highest disease suppression was exhibited with *P. fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19) (60 and 63% disease freeplants). Manjula *et al.* (2004) reported *T. viride* pq 1 reduced the mortality of seedlings by >70.0% compared to control. *T. viride* pq 1 inhibited the external growth of seed carried *S. rolfsii* and its radial growth in dual cultures by 50.0% and 58.0%, respectively. *T. viride* pq 1 produced extracellular chitinase but not β-1,3- glucanase. Ganesan *et al,* (2006) reported that *T. harzianum* showed around 57% of inhibition against *S. rolfsii* in the dual culture method.

Khonga *et al.* (1998) reported that, *S. rolfsii* produced only 16 sclerotia when paired with *T. harzianum* compared to 224 sclerota in the control plates. When the 16 sclerotia were plated on fresh PDA, they failed to germinate and only *T. harzianum* was recovered. Observations under the microscope showed that the hyphae of *T. harzianum* coiled around the hyphae of *S. rolfsii* and the coiling was sparse. Incase of spore germination method, the germination of sclerotia was completely inhibited when the spore concentration of $4X10^6$ of *T. harzianum* used. *T. harzianum* significantly reduce the incidence of damping-off and showing low number of lesion in both pot culture and field experiments.

REFERENCES

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.

Ganesan S, Ganesh Kuppusamy R and Sekar R (2007). Integrated Management of Stem Rot Disease (*Sclerotium rolfsii*) of Groundnut (*Arachis hypogaea* L.) Using *Rhizobium* and *Trichoderma harzianum* (ITCC - 4572). Turkish Journal of Agriculture and Forestry, 31: 103-108.

Khonga E B, Kaunda C C and Hillocks R J (1998). Biocontrol of *Sclerotium rolfsii* Sacc. in peanuts (*Arachis hypogaea* L.) by *Trichoderma harzianum* Rifai in Malawi. Malawi Journal of Science and Technology, 4: 51-62.

Manjula K, Kishore G K, Girish A G and Singh S D (2004). Combined application of *Pseudomonas fluorescens* and *Trichoderma viride* has an improved biocontrol activity against stem rot in groundnut. Plant Pathology Journal, 20: 75-80.

Mayee C D and Datar V V (1988). Diseases of groundnut in the tropics. Review of Tropical Plant Pathology, 5: 169-198.

Rangeshwaran R and Prasad R D (2000). Biological control of Sclerotium rot of sunflower. Indian Phytopathology, 53: 444-449.

Varadharajan, Karthikeyan, Ambalavanan, Sankaralingam, Sevugaperumal and Nakkeeran (2006). Biological control of groundnut stem rot caused by *Sclerotium rolfsii* (Sacc). Archives of Phytopathology and Plant Protection, 39: 3(16): 239-246.