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BIO EFFICIENCY OF CERTAIN BIO CONTROL AGAINST FOR THE MANAGEMENT OF STEM ROT OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) CAUSED BY *SCLEROTIUM ROLFSII* (SACC.)

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Abstract

Groundnut (*Arachis hypogea* L.) the king of oilseeds is popularly called as wonder nut, poor men's cashew nut. The crop is affected by various diseases caused by fungi, bacteria and viruses. In India among the soil-borne fungal diseases of groundnut, stem rot caused by *S. rolfsii* is a potential threat to production and is of considerable economic significance for groundnut grown under irrigated conditions. This disease causes severe damage during any stage of crop growth, and yield losses over 25%. 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. Hence, an attempt was made bio efficacy of certain biocontrol against for the management of stem rot of groundnut. The results revealed that out of seven media, all the isolates of *S. Rolfsii* varied in their ability to produce sclerotia on PDA medium. The maximum sclerotial number of 346 per nine mm culture disc were produced by the isolate SR_1 which was also found as the most virulent isolate. Among the 10 isolates of *T. viride* and *P. fluorescens* tested, the isolate Tv_3 and Pf_5 effectively inhibited the mycelial growth and sclerotia of *S. rolfsii* under *in vitro* conditions. The combination of culture filtrate of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) recorded the maximum germination, shoot and root length of groundnut.

Key words: Groundnut, *Sclerotium rolfsii , Trichoderma viride* and *Pseudomonas fluorescens*

Introduction

Groundnut (*Arachis hypogea* L.) the king of oilseeds is popularly called as wonder nut, poor men's cashew nut, earth nuts ,goober peas, monkey nuts and pig nuts. It is belongs to the family of Fabaceae, subfamily *Papilionaceae* and it contains they available source of all nutrients. In India it's grown under rain fed as well as irrigated conditions. It is a legume which thrives best in tropical climate and requires 20°C to 30°C temperature, 50-75 cm rainfall. Well drained light sandy loams, red, yellow and black soils are well suited for its cultivation. India is the second largest producer of groundnut after China. It is grown in 24.70 million hectares worldwide contributing 1.63 metric tonnes of pod yields. India, groundnut was cultivated in 4.56 Million hectares with a productivity of 0.98 metric tons and 4.47Million metric tons per hectare of production in(2015-16)(World Agricultural Production, 2017). Groundnut rich in energy (567calories per 100g), its seed contain 45-50%rich source of high-quality edible oil, 27-33% easily digestible protein as well as essential minerals and vitamins. Groundnut oil is composed of mixed glycerides and contains high proportion of unsaturated fatty acids, in particular, oleic (50-65%) and linoleic acids (18-30%) (El Naim *et al*., 2010). The flavonoids secreted by the ground nut root increase the growth of symbiotic and non-symbiotic nitrogen fixing bacteria, root nodules and nitrogen uptake by plants (Solaiman *et al*., 2014). The crop is affected by various diseases caused by fungi, bacteria and viruses. In India among the soil-borne fungal diseases of groundnut, stem rot caused by *S. rolfsii* is a potential threat to production and is of considerable economic significance for groundnut grown under irrigated conditions. This disease causes severe damage during any stage of crop growth, and yield losses over 25% have been reported by Mayee and Datar (1988). The symptoms of stem rot produced by *S. rolfsii* on

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groundnut plants under field conditions were characterized by formation of deep brown lesion on the stem region of the plant just near the ground followed by yellowing of groundnut leaves than by loss of vigour and premature death. The infected plant showed poor root growth and rotting of the stem region. Soon after this, the lesion was covered by a radiating white mycelium with the rotting underneath it. In later stages of infection, light deep brown spherical or round sclerotial bodies were formed, which adhered around the infected stem region and such bodies were produced abundantly on stem. Kernels were infected in the advanced stage of plant growth; such kernels were small and shriveled in size (Abid, 2011). 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.

Materials and Methods

Isolation of native antagonists from rhizosphere soil Trichoderma spp.

Groundnut rhizosphere soil samples collected from ten different locations were used for the isolation of *Trichoderma* spp isolates by soil dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). These *Trichoderma* spp. cultures were purified by single hyphal tip method and used for the studies. Micrometric measurements of conidia were done by mounting four days old culture stained with lactophenol cotton blue and observed under high power of research microscope.

In vitro testing of fungal antagonists

The antagonistic activity of bio control agents against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile Petri dish containing 15 ml of sterilized and solidified PDA medium a 6 mm mycelial disc obtained from one five days old culture of Trichoderma spp. was placed under aseptic conditions. Similarly, at the opposite end approximately 75 mm away from the *Trichoderma* culture disc, a six mm mycelial disc obtained from seven days old culture of *S. rolfsii a* was placed and incubated. A control was maintained by inoculating *S .rolfsii* alone at one end of the Petri dish. The plates were incubated at room temperature (28 ± 2°C) for seven days. The radial growth (in mm) of the pathogen and the test antagonists and the extent of the inhibition zones (in mm) developed between the two colonies were measured. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula (Vincent, 1927).

$$\text{Percent inhibition (I)} = \frac{C \quad T}{C} \times 100$$

Where, C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual plate

I - inhibition Per cent

Based on the dual culture technique the effective *T. viride* were identified and used for further studies.

Preparation of the culture filtrates of *T. viride*

The effective *Trichoderma* spp. isolates were grown for 10 days at room temperature (28 ± 2°C) in Erlenmeyer flasks containing 50 ml of sterilized potato dextrose broth. The cultures were filtered under vacuum through bacteriological filter to remove the mycelium and spores. The filtrate thus obtained was used for the studies.

Effect of culture filtrates on the mycelial growth *of S. rolfsii* (SR_1)

The culture filtrates of the antagonists were separately incorporated into sterile PDA medium at 5, 10 and 15 per cent by adding the calculated quantity of the culture filtrates to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The amended media were transferred to sterile Petri dishes separately @ 15ml and allowed to solidify. Each plate was inoculated at the centre with a seven days old (six mm) PDA culture disc of. *S. rolfsii*. Three replications were maintained for each treatment. The diameter of the mycelial growth (in mm) of *S. rolfsii* was measured when the mycelial growth fully covered the control plates.

Isolation of native bacteria from rhizosphere soil of groundnut plants

Bacterial isolates were collected from groundnut rhizosphere soil samples collected from ten different locations were used for the isolation after removing the loosely adhering soil from freshly excised roots, root segments (1g) were taken and suspended in 10 ml sterile distilled water to get 10^{-1} dilution. Serial dilutions were made to get dilutions up to 10^{-6}. One ml of 10^{-5} and 10^{-6} dilution were pipetted out into sterile Petri plate and 15 ml of King's B medium (King *et al.,*1954) was added and rotated clockwise and anticlockwise. The plates were incubated at room temperature (28 ± 2°C) for 48 hours for development of bacterial colonies. The Identified isolates were designated as *P. fluorescens* Pf_1 to Pf_8.

In vitro testing of bacterial antagonists

The antagonistic activity of bio control agents against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile Petri dish containing 15 ml of sterilized and solidified PDA medium a 6 mm mycelial disc of pathogen obtained from seven day old culture of *S. rolfsii* was placed at 1.5 cm away from the margin of the petri dish. Similarly, one cm long streak was gently made onto the medium using 48 h old bacterial isolates just opposite to pathogenic culture at equidistance under aseptic conditions. A control was maintained by inoculating *S. rolfsii* alone at one end of the Petri dish. The plates were incubated at room temperature (28 ± 2°C) for 48 h. The radial growth (in mm) of the pathogen and the test antagonists and the extent of the inhibition zones (in mm) developed between the two colonies were measured after incubation. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula (Vincent, 1927)

$$\text{Per cent inhibition (I)} = \frac{C \quad T}{C} \times 100$$

Where, C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual plate

I - inhibition Per cent

Preparation of the culture filtrate of *P. fluorescens*

The effective *P. fluorescens* isolates were inoculated into Erlenmeyer flasks containing 50 ml of sterile King's B broth and Nutrient agar medium, respectively and kept on a rotary shaker at 100 rpm for 48 h. Then the cultures were filtered through bacteriological filter under vacuum and the filtrates thus obtained were used for the studies.

Effect of culture filtrates on the mycelial growth of *S. rolfsii* (SR_1)

The culture filtrates of the antagonists were separately incorporated into sterile PDA medium at 5, 10 and 15 percent by adding the calculated quantity of the culture filtrates to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The amended media were transferred to sterile Petri dishes separately @ 15 ml and allowed to solidify. Each plate was inoculated at the centre with a seven day old (6 mm) PDA culture disc of *S. rolfsii*. Three replications were maintained for each treatment. The diameter of the mycelial growth (in mm) of *S. rolfsii* was measured when the mycelial growth fully covered the control plates.

Compatibility test between selected antagonists

Dual culture technique

Compatibility among *T. viride,* and *P. fluorescens* was tested by following the dual culture technique (Dennis and Webster, 1971) and observed for the mycelial over growth of *T. viride* onto the PGPR isolates without forming any inhibition zone.

Results and Discussion

Effect of different solid media on radial growth and sclerotia formation of *S. rolfsii*

Effect on radial growth

Maximum radial growth (90.0 mm) was recorded on PDA medium (Table1) followed by Richard's agar medium and Czapek's Dox agar which recorded 80.50 mm and 75.30mm radial growth, respectively. Least radial growth (40.66 mm) of the test fungus was recorded on Coon's agar medium. The radial growth recorded on Carrot agar medium and Yeast extract agar were 70.00 and 50.83 mm respectively. The fungus produced apprised to fluffy type of growth and dull white to white pigmentation on all the media tested. This indicates that maximum growth of *S. rolfsii* was supported by PDA medium. Potato dextrose agar was best for the radial growth and sclerotial production of *S. rolfsii,* as stated by Akram *et al.,* (2007), Rajalakshmi *et al.,* (2006) and Nene and Sheila (1995). Chaurasia *et al.,* (2013) also reported that potato-dextrose medium was most suitable for mycelial growth and sclerotia production of *S. rolfsii.* Similar growth PDA medium of *S. rolfsii* was observed by several workers (Zape *et al*., 2013; Shiva Kant Kushwaha, 2016). These earlier reports add value to the present observations.

In vitro antagonism of *Trichoderma viride* against *S. rolfsii*(SR_1)

In general all the native *T. viride* isolates significantly inhibited the mycelial growth of *S. rolfsii* (Table2). However, among the isolate Tv_3 showed the maximum growth inhibition of *S. rolfsii* up to 76.04 per cent respectively. This was followed by the isolates Tv_4 and Tv_8 in the decreasing order the least growth inhibition of pathogen (56.64 %) was exhibited by the isolates Tv_1. The results are in agreement which early workers (Darvin *et al*., 2013; Padmaja *et al*., 2013; Pan *et al*., 2013; Swathi *et al*., 2015; Dwivedi and Ganesh Prasad, 2016**;** Hirpara *et al*., 2017).

Effect of culture filtrate of *T. viride* on the mycelia growth of *S. rolfsii*(SR_1)

The results presented in (Table 3) showed that all

Table 1: Effect of different solid media on mycelial growth and sclerotia formation of *S. rolfsii* (SR_1)

S. No.	Name of the medium	Mycelial growth (mm)			Type of colony	Pigmentation	Degree of clerotia formation (After 15 days)
		72h	96 h	120 h			
1	Potato dextrose agar	43	65.00	90.00	Apprised	White	Good
2	Czapek's Dox agar	35	50.50	75.30	Fluffy	Dull white	Poor
3	Richard's agar	40	56.16	80.50	Fluffy	Dull white	Poor
4	Yeast extract agar	29	37.00	50.83	Apprised	White	Fair
5	Coon's agar	25	32.16	40.66	Fluffy	White	Fair
6	Carrot agar	30	40.83	70.00	Fluffy	White	Fair
	S.Ed CD (0.05)	1.212.43	1.302.81	1.493.21			

the *T. viride* isolates significantly inhibited the growth of *S. rolfsii* when compared to control and generally an increase in the concentration of the culture filtrate showed enhanced inhibition on the mycelial growth of the pathogen. Among the isolates tested, the isolate Tv_3 was found to be most inhibitory to the growth of *S. rolfsii* by recording the least mycelial growth with 23.33, 19.26, 10.33 and 0.00mm at 10, 20, 30 and 40 percent concentration of the culture filtrate, respectively in poison food technique. Similarly, Siddanagour (2005) reported reduction in mycelial growth of *S. rolfsii* when PDA amended with culture filtrates *Trichoderma spp*. Culture filtrates of *Trichoderma* spp inhibited the mycelial growth and sclerotial germination of *S. sclerotiorum* (Kapil and Kapoor 2005). Vengatesh (2013) reported that culture filtrate of isolates -I_2 (THA) recorded complete inhibition of *S. rolfsii at* 15% concentration. The cell free culture filtrate of *T. viride* and *T. harzianum* showed 100 per cent mycelial growth inhibition at 60 and 80 per cent conc. against *S. rolfsii* (Swathi *et al.,* 2015). These earlier reports are in line with the present findings.

Table 2: *In vitro* antagonism of *T. viride* against *S. rolfsii* (SR_1).

S. No.	Isolates number	Mycelial growth(mm)	Per cent inhibition over control
1	Tv1	39.02	56.64
2	Th2	30.02	66.64
3	Tv3	21.56	76.04
4	Tv4	25.31	71.87
5	Tv5	38.00	57.77
6	Tv6	28.52	68.31
7	Th7	37.21	58.65
8	Tv8	26.66	70.37
9	Tv_9	31.38	65.13
10	Tv_{10}	35.98	60.02
11	Control	90.00	—
	S.EdC d(0.05)	0.811.70	

In vitro inhibition of mycelial growth of *S. rolfsii* (SR_1) by native *P. fluoresens* isolates

The results presented in (Table 4) revealed varying degree of antagonism by the isolates of *Pseudomonas* against *S.rolfsii*. Among the *Pseudomonas* isolates, Pf_5 produced significantly the maximum inhibition zone (13.00mm) and minimum mycelial growth (21.01mm) accounting for 77.76 percent reduction on the mycelial growth of *S. rolfsii* over control. This was followed by isolate Pf_{10} which recorded an inhibition zone of 12.00mm accounting for 72.11 percent reduction on the mycelial

Table 3: Effect of culture filtrate of *Trichoderma viride* on the mycelia growth of *S. rolfsii* (SR_1).

S. No.	Isolates Number	Mycelial growth (mm) Conc. of culture filtrate (%)				Mycelial growth (mm) Conc. of culture filtrate (%)			
		10	20	30	40	10	20	30	40
1	Tv1	26.32	23.45	19.45	11. 50	70.75	73.94	78.38	87.22
2	Th2	30.00	25.34	20.37	12.33	66.66	71.84	77.36	86.03
3	Tv3	23.33	19.26	10.33	0.00	74.07	78.06	88.52	100
4	Tv4	27.89	21.34	16.20	05.78	69.01	76.28	82.00	93.57
5	Tv5	36.66	30.00	25.32	19.32	59.26	66.66	71.86	78.53
6	Tv6	28.66	25.35	19.34	13.83	68.15	71.83	78.51	84.63
7	Th7	33.33	29.00	24.66	15.45	62.96	67.77	72.06	82.83
8	Tv8	25.65	20.99	15.89	07.90	71.05	76.67	82.34	91.22
9	Tv_9	27.87	24.35	19.34	17.43	69.03	72.94	78.51	80.63
10	Tv_{10}	26.32	22.76	17.98	15.35	70.75	74.71	80.02	82.94
11	Control	90	90	90	90	—	—	—	—
	S.EdCD (0.05)	0.811.71	1.41 2.97	0.771.61	0.971.79				

growth over control. The isolate Pf_4 was the least effective among *Pseudomonas* isolates as it recorded the minimum inhibition zone.

A similar observations on variation in antagonistic efficacy between isolates were recorded by several workers. Manjunatha *et al*., (2012) reported that *P. fluorescens* showed maximum inhibition of mycelial growth of *S. rolfsii* through dual plate technique. Pastor *et al*., (2010) reported that *Pseudomonas* cf. *monteilii* showed highest antagonistic active against *S. rolfsii*. Similarly, Prasada Babu and Paramageetham (2013) reported that *P. fluorescens* isolate PATPT6 was found to be potential antagonist against *S. rolfsii*. Sab *et al*., (2014) reported that *P. fluorescens* inhibited growth of *S. rolfsii* in dual culture.

Effect of culture filtrate of *P. fluorences* on the mycelial growth of *S. rolfsii*(SR_1)

The mycelial growth of *S. rolfsii* was found reduced with an increase in the concentration of culture filtrates of all the isolates of the antagonists tested and the reduction was significantly the maximum in the case of *Pseudomonas* isolate Pf_5 with 21.66,19.00,10.00 and 0.00 mm at 10, 20, 30 and 40 percent concentration of the culture filtrate respectively as against the maximum growth of 90 mm in the control in poison food technique (Table 5). Similarly Chanutsa *et al.,* (2014) reported that the isolates culture filtrate of three bacteria completely inhibited the growth of *S. rolfsii*. The antifungal metabolites produced by *P. fluorescens* might be attributed as the reason for the reduction in the growth of pathogen. Several studies indicated the production of lytic enzymes which was correlated with antagonistic potential of *P. fluorescens* against various soil born e plant pathogen (Velazhahan *et al.,* 1999). Culture filtrate of *P. fluorescens* was the most effective in inhibiting the mycelial growth of *S. rolfsii* (Revathy and Muthusamy, 2003). Culture filtrate of *P. fluorescens* isolates I_7 total inhibited mycelial growth of *S. rolfsii* at a concentration of 15% *in vitro* (Venkatesh 2013).

Table 4: *In vitro* inhibition of mycelial growth of *S. rolfsii* (SR_1) by native *P. fluorescens* isolates.

S. No	Isolates number	Mycelial growth (mm)	Per cent inhibition over control
1	Pf_1	30.89	65.67
2	Pf_2	27.00	70.00
3	Pf_3	29.72	66.97
4	Pf_4	32.00	64.44
5	Pf_5	21.01	77.76
6	Pf_6	25.10	72.11
7	Pf_7	31.50	65.00
8	Pf_8	30.47	70.58
9	Pf_9	29.67	67.03
10	Pf_{10}	25.10	72.11
11	Control	90.00	—
	S.EdCD(0.05)	0.831.76	

Testing the compatibility between *T. viride* and *P. fluorescens* isolates (Dual culture)

The most effective isolates (Table6) identified in the present investigations *viz., T. viride* (Tv_3) and *P. fluorescens* (Pf_5) alone were tested for compatibility among them for to be used in combination for managing stem rot pathogen. The results showed that *T. viride* (Tv_3) isolates grew over *P. fluorescens* (Pf_5) isolates without any inhibition zone thus indicating the compatibility. Several researchers have suggested that an important prerequisite for the desired effectiveness of strains appears to be compatibility of the co inoculated microorganisms in order to establish better and more

Table 4: Effect of culture filtrate of *P. fluorences* on the mycelia growth of *S. rolfsii* (SR_1).

S. No.	Isolates Number	Mycelial growth (mm) Conc. of culture filtrate (%)				Percent increase over cntrol (mm) Conc. of culture filtrate (%)			
		10	20	30	40	10	20	30	40
1	Pf_1	29.66	25.56	20.00	8.33	67.04	71.6	77.77	90.74
2	Pf_2	41.33	38.34	35.10	26.00	54.07	57.04	61.00	71.11
3	Pf_3	29.66	25.33	20.30	15.21	67.04	71.85	77.44	83.01
4	Pf_4	25.45	22.34	16.45	11.30	71.72	75.17	81.72	87.44
5	Pf_5	21.66	19.00	10.00	0.00	75.93	78.88	88.88	100
6	Pf_6	23.70	20.43	11.34	8.76	73.66	77.03	87.04	90.26
7	Pf_7	39.33	35.23	30.33	22.00	56.03	60.85	66.03	75.55
8	Pf_8	36.66	31.31	27.50	19.45	59.26	65.21	69.44	78.38
9	Pf_9	33.33	27.54	24.66	19.45	62.96	69.04	72.06	78.38
10	Pf_{10}	40.33	37.89	34.00	25.50	55.18	57.09	62.22	71.66
11	Control	90	90	90	90	—	—	—	—
	S.Ed CD (0.05)	0.811.70	0.811.71	0.761.61	0.781.65				

Table 6: Testing the compatibility between isolates *P. fluorescens* (Pf_5) and *T. viride*(Tv_3) (liquid medium).

Antagonist	Number of bacterial cells × 10 -6	Percent reduction over control	Mycelial dry weight(mg)	Percent reduction over control
T. viride (Tv3)	—	—	473.25	—
P. fluorescens (Pf5)	52.32	—	—	—
T. viride (Tv3) + *P .fluorescens* (Pf5)	48.26	7.75	463.65	20.02
S.Ed CD(0.05)	0.721.76	—	—	—

consistent disease suppression (Venkatesh, 2013, Alizadeha *et al.,* 2013). The results observed in the present study corroborates with these earlier reports.

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