International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Online International Journal Available at http://www.cibtech.org/jfav.htm
2012 Vol. 2 (1) January-April, pp.134 -141/ Basha et al.
Research Article

BIOCONTROL POTENTIAL OF INDIGENOUS *PSEUDOMONAS* SPP. AGAINST *SCLEROTIUM ROLFSII* CAUSING STEM ROT OF GROUNDNUT

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ABSTRACT
The antagonistic potential of sixty isolates of *Pseudomonas* spp. isolated from rhizophere soils of three districts of Andhra Pradesh was determined. When tested *in vitro*, the bioagents ANT5 (100%), ANT11 (100%), KDP6 (100%), TPT15 (100%) and TPT17 (100%) were predominantly aggressive in inhibiting the mycelial growth of the pathogen in dual culture. The isolate TPT15 was highly compatible with mancozeb (99.50%). In pot culture studies, integrated seed treatment with potential BCA (TPT15) + seed treatment with fungicide mancozeb (T6) was highly effective and found to be superior which recorded least PDI (12.63%) and increased plant growth parameters *viz.*, plant height (24.80 cm), maximum shoot (12.0 g) and root dry weight (1.90 g) when compared to other treatments. The present study was a successful attempt in selection of broad-spectrum and fungicide tolerant potential biocontrol agent TPT15 which can be a useful component of biological control of stem rot disease of groundnut.

***Key Words*:** *Sclerotium rolfsii, Stem rot, Groundnut, Bioagents*

INTRODUCTION
Groundnut (*Arachis hypogea* L.) is one of the important oilseed crops in India which is affected by many devastating diseases. Among them, stem rot of groundnut caused by *Sclerotium rolfsii* Sacc., is a serious soil borne disease causes severe damage to the crop with yield losses of over 25 per cent (Mayee, and Datar, 1988). The yield loss caused by pathogen infection generally is 25%, but sometimes it reaches 80 - 90% (Grichar, and Bosweel, 1987). The pathogen produces sclerotia which over winter in soil and on plant debris and can survive in a long period causing disease in the following season (Punja, 1985). Thus, the control of the disease is very difficult. Biological control of plant diseases is an important area, which needs attention since most of the hazardous inputs added into the agricultural system are in the form of plant protection chemicals. Studies aimed at replacing chemical pesticides with environmentally safer methods are currently being a greater importance at this juncture. The biological control of soil-borne pathogens with antagonistic bacteria, particularly *Pseudomonas* spp. belonging to plant growth promoting rhizobacteria, has received prominent attention because of the dual role of these bacteria in plant-growth promotion and disease control (Zehnder, *et al.*, 2001). The genus *Pseudomonas* has been heterogenous since Migula first named it in 1894 (Migula,1894). He designated and described the species associated with the genus in 1895 (Migula, 1895). *Pseudomonas* is Gram-negative, strictly aerobic, polarly flagellated rods. Soil pseudomonads possess a variety of promising properties, which make them better biocontrol agents (Cook, 1993). They are aggressive colonizers of the rhizosphere of various crop plants, and have a broad spectrum anatagonistic activity plant pathogens, such as antibiosis (Cartwright, *et al.*, 1995; Rosales, *et al.*, 1995), siderophores production (Winkelmann, and Drechsel, 1997) and nutrition or site competition (Bull *et al.*, 1991). The importance of bacteria and fungi as sources of valuable bioactive metabolites is very well established for more than half a century. As a result, over 120 of the most important medicines (penicillins, cyclosporin A, adriamycine, etc.) in use today are obtained from microorganisms (Alanis, 2005).The objective of the current research was aimed to identify the fungicide

compatible potential antagonistic *Pseudomonas* spp. from Rayalaseema region of Andhra Pradesh for the biological control of soil-borne pathogen *Sclerotium rolfsii* causing stem rot of groundnut.

MATERIALS AND METHODS

Isolation of pathogen and biocontrol agents

The pathogen was isolated from the groundnut plants showing typical symptoms of stem rot disease by tissue segment method (Rangaswami, 1999). The pathogen was purified by single hyphal tip method and identified based on its mycelial and sclerotial characteristics through standard mycological keys (Barnett and Hunter, 1972). Sixty rhizosphere soil samples were collected from thirty locations from three districts in Rayalaseema region, Andhra Pradesh, India. *Pseudomonas* spp. was isolated on Hi-Veg *Pseudomonas* isolation agar (Himedia, Mumbai) and identified using microbiological and biochemical tests as listed in Bergey's Manual (Unnnamalai, and Gnanamanickam, 1984).

Screening for potential Pseudomonas spp.

The antagonistic potential of *Pseudomonas* isolates was assessed by dual culture method (Kishore, *et al.* 2005b). A nine mm PDA culture disc of the pathogen was cut individually from seven day old culture and placed at one side on the sterilized PDA plates. Simultaneously, the actively growing biocontrol agents were streaked on the opposite side. Four replications of each treatment and suitable controls were maintained. The plates were incubated at room temperature (28 ± 2°C) for seven days and the mean diameter of the mycelial growth was measured. The results were expressed in terms of per cent inhibition of the mycelium over control.

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

where,
I = Per cent reduction in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatments

Compatibility studies

Native potential *Pseudomonas* isolates were tested for their compatibility with the fungicides commonly recommended for soil drenching *viz.,* copper oxychloride (0.2%), mancozeb (0.2%), carbendazim (0.1%) and thiophanate methyl (0.1%) by spectrophotometric method (Kishore, *et al.* 2005b) under *in vitro.*

Talc based formulations

The highly potential and compatible antagonistic isolate was multiplied on Hi-Veg *Pseudomonas* isolation agar (Himedia, Mumbai). A loopful of bacterium was inoculated into the broth and incubated in rotary shaker at 150 rpm for 48 h at room temperature (28 ± 2°C). The bacterium grown in the broth was formulated using talc as carrier material (talc: liquid broth culture of antagonist @ 2:1 w/v) with 10 g of carboxy methyl cellulose (CMC) per kilogram of carrier material as adhesive and fried to 8-10% moisture under shade and packed in white polythene bags, sealed and stored at 27± 2°C (Vidyasekaran and Muthamilan, 1995).

Green house studies

The pathogen was mass multiplied on sterilized sorghum seeds (Umamaheswari, *et al.,* 2002). Antagonistic bacterium was mass multiplied on nutrient broth and incubated at 28 ± 2°C for two days. The bacterial suspension was added to soil in the pots @ 20 ml kg-1 of soil (Gogoi, *et al.,* 2002). The potential biocontrol agent and compatible fungicide were evaluated under greenhouse conditions against the pathogen. The sandy loamy soils and the groundnut variety TCGS-888 was used for the experiment with the following treatments (Table 1).

Table 1: List of treatments against *S.rolfsii* under green house conditions

S.No.	Treatment No.	Treatment
1.	T_1	Soil application with potential biocontrol agent
2.	T_2	Soil drenching with compatible fungicide
3.	T_3	$T_1 + T_2$
4.	T_4	Seed treatment with potential BCA
5.	T_5	Seed treatment with fungicide
6.	T_6	$T_4 + T_5$
7.	Control	Inoculated with pathogen alone

Design : CRD Replications: 03

Groundnut seeds were treated with talc based formulation of TPT15 @ 4g per kg of seed were used for sowing. Observations on percent disease incidence (PDI) shoot and root length, dry weight of shoots and roots were recorded.

Statistical analysis

Completely Randomized Design (CRD) was used for radial growth, percent disease incidence, poisoned food technique and dual cultural technique. Two-way CRD was used for spectrophotometric method (Gomez, 1984).

RESULTS AND DISCUSSION

***Antagonistic potential of Pseudomonas* Spp.**

Plants develop an enhanced defensive capacity against a broad spectrum of plant pathogens after colonization of the rhizosphere soil by selected strains of nonpathogenic biocontrol bacteria. The use of biocontrol agents for increasing yield and inducing resistance against various plant pathogens is an emerging, ecofriendly and economically attractive approach in the modern system of sustainable agriculture. Of all the sixty bioagents screened for its antagonistic activity, the potential antagonistic isolates ANT5 (100%), ANT11 (100%), KDP6 (100%), TPT15 (100%) and TPT17 (100%) completely inhibited the mycelial growth of the pathogen and found to be superior compared to other isolates against the test pathogen, *S.rolfsii* in dual culture (Table 2). Similar results were obtained for *Pseudomonas* spp. inhibiting the growth of plant pathogens by Brion, and Genevieve (1999). This effect is the result of production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide. Competitive exclusion of pathogens as the result of rapid colonization of the rhizosphere by *Pseudomonas* spp. may also be an important factor in disease control. On the other hand, induced systemic resistance (ISR) might be another mechanism for achieving biological control of plant diseases by *Pseudomonas* spp. (Van Loon, *et al.*, 1998). The isolates ANT8 and ANT16 were found to be least effective which are able to control the pathogen growth upto the extent of 26.25% and 29.4% respectively.

Compatibility antagonistic Pseudomonas with fungicides

The six potential antagonistic isolates *viz.*, ANT5, ANT11, KDP6, TPT15 and TPT17 which proved effective in dual culture were further used for fungicide compatibility study. The antagonist TPT15 was highly compatible with mancozeb (99.50%), followed by carbendazim (92.88%), thiophanate methyl (86.80%) and copper oxychloride (3.8%) was found to be less compatible by spectrophotometric method (Table 3). Statistical analysis revealed that there was a significant difference between the compatibility of mancozeb and copper oxychloride. Similar findings were observed by Vidyasekharan, and Muthamilan (1995) that carbendazim was not inhibitory to *P.fluorescens*.

Table 2: *In vitro* evaluation of antagonistic *Pseudomonas* against *S. rolfsii* by dual culture technique

S.No.	Antagonistic isolates	*Mycelial growth (mm)	Percent inhibition over control	S.No.	Antagonistic isolates	*Mycelial growth (mm)	Percent inhibition over control
1.	ANT1	39.43	56.18	**31.**	KDP11	55.75	38.05
2.	ANT2	16.85	81.27	**32.**	KDP12	61.29	31.9
3.	ANT3	59.90	33.44	**33.**	KDP13	9.15	89.83
4.	ANT4	21.47	76.14	**34.**	KDP14	36.15	59.83
5.	**ANT5**	**0.00**	**100**	**35.**	KDP15	55.68	38.13
6.	ANT6	17.83	80.18	**36.**	KDP16	8.25	90.83
7.	ANT7	29.12	67.64	**37.**	KDP17	24.13	73.18
8.	ANT8	66.37	26.25	**38.**	KDP18	57.80	35.77
9.	ANT9	18.55	79.38	**39.**	KDP19	12.47	86.14
10.	ANT10	49.63	44.85	**40.**	KDP20	53.18	40.91
11.	**ANT11**	**0.00**	**100**	**41.**	TPT1	10.15	88.72
12.	ANT12	57.10	36.55	**42.**	TPT2	61.75	31.38
13.	ANT13	15.60	82.66	**43.**	TPT3	46.33	48.52
14.	ANT14	19.60	78.22	**44.**	TPT4	20.11	77.65
15.	ANT15	44.54	50.51	**45.**	TPT5	30.12	66.53
16.	ANT16	63.54	29.4	**46.**	TPT6	43.76	51.37
17.	ANT17	22.29	75.23	**47.**	TPT7	17.15	80.94
18.	ANT18	21.36	76.26	**48.**	TPT8	31.33	65.18
19.	ANT19	38.36	57.37	**49.**	TPT9	41.89	53.45
20.	ANT20	13.14	85.4	**50.**	TPT10	22.15	75.38
21.	KDP1	9.11	89.87	**51.**	TPT11	35.48	60.57
22.	KDP2	27.63	69.3	**52.**	TPT12	39.17	56.47
23.	KDP3	19.20	78.66	**53.**	TPT13	52.11	42.1
24.	KDP4	47.25	47.5	**54.**	TPT14	15.16	83.15
25.	KDP5	17.28	80.8	**55.**	**TPT15**	**0.00**	**100**
26.	**KDP6**	**0.00**	**100**	**56.**	TPT16	18.18	79.8
27.	KDP7	36.14	59.84	**57.**	**TPT17**	**0.00**	**100**
28.	KDP8	56.14	37.62	**58.**	TPT18	29.56	67.15
29.	KDP9	10.43	88.41	**59.**	TPT19	25.40	71.77
30.	KDP10	11.91	86.76	**60.**	TPT20	18.29	79.27
	Control	90.00					

CD (5%) = 1.5287; SEm± = 1.2753
** Mean of three replications*

Table 3: Compatibility of the potential antagonistic isolate TPT15 with different fungicides

Fungicides	Concentration	*Optical Density (OD) at 600 nm				
		ANT5	ANT11	KDP6	TPT15	TPT17
Carbendazim	(0.1%)	0.955	0.903	0.619	0.926	0.828
Thiophanate methyl	(0.1%)	0.935	0.969	0.577	0.866	0.920
Mancozeb	(0.2%)	0.926	0.612	0.733	1.002	0.708
Copper oxychloride	(0.2%)	0.794	0.174	0.017	0.038	0.118
Control	-	1.054	0.935	0.908	0.997	0.926
CD (0.05)	-	0.2468	1.000	0.605	1.008	0.721
SEm±	-	1.8725	1.035	1.027	1.038	1.151

** Mean of three replications*

Table 4: Biological control of stem rot of groundnut with potential *Pseudomonas* antagonist TPT15 in pot culture studies

*Treatment No.	Treatment	Per cent Disease Incidence (%)	Plant height(cm)	Root length (cm)	Dry weight of each plant (g)	
					Shoot	Root
T_1	Soil application with potential biocontrol agent	37.20	16.0	9.80	8.0	0.51
T_2	Soil drenching with compatible fungicide	42.40	18.0	8.60	8.0	0.63
T_3	T1+T2	28.0	19.50	10.40	11.0	0.91
T_4	Seed treatment with potential biocontrol agent	41.80	20.50	10.20	9.90	0.60
T_5	Seed treatment with fungicide	49.50	21.0	9.50	7.80	0.50
T_6	T4+T5	12.63	24.80	12.0	12.0	1.90
T_7	Inoculated control	90.75	12.0	5.0	4.0	0.40
CD (5%)		1.6081	0.4925	0.2815	0.8203	0.3594
SEm±		2.0185	1.3941	1.2640	0.4086	0.2962

***Mean of three replications*

Pot culture studies

The fungicide tolerant potential antagonist isolate TPT15 was used in green house studies. From the data (Table 4) it is evident that all the treatments were significantly superior over control in reducing the per cent disease incidence. Different *Pseudomonas* strains having potential to reduce the disease incidence under greenhouse and field conditions and increase the plant growth were studied by Ramamoorthy, *et al.,* 2002b; Silva, *et al.,* 2004; Girish, and Umesha, 2005 and Ji, *et al.,* 2006. Maximum reduction was observed in treatment T6 (seed treatment with potential BCA + seed treatment with fungicide) in which PDI of 12.63 per cent was recorded when compared to treatment T7 inoculated control (90.75%). Fluorescent pseudomonad strains found to be effective against *S.rolfsii* were also evaluated by Patil, *et al.,* (1998) under greenhouse conditions for their effects on groundnut and on collar rot incidence. *Pseudomonas* strains are well adapted to in soil are being investigated extensively for use in applications that require release and survival of bacteria in the soil. The per cent disease incidence was reduced to maximum extent and effect of different treatments on plant growth parameters *viz.,* plant height, root length, shoot dry weight and root dry weight of groundnut in each of the treatment are recorded and presented in Table 4. Maximum plant height (24.80 cm) was recorded in treatment T6 (seed treatment with potential BCA+seed treatment with fungicide) and minimum was recorded in inoculated control (12.0 cm). The treatment T6 stimulated the plant growth and development when compared to inoculated control. *Pseudomonas* spp. suppress plant diseases by protecting the seeds and roots from fungal infection were also reported (O' Sullivan *et al.,* 1992). The maximum root length was also recorded in treatment T6 (1.90 cm) followed by treatment T4 (0.60 cm) and the least root length (0.40 cm) was recorded in inoculated control. The dry weight of shoot was recorded maximum in treatment T6 (12.0 g) followed by treatment T3 (11.90 g) and the least (4.0 g) recorded in inoculated control. Maximum root weight (1.90 g) was recorded in treatment T6 and least (0.45 g) was recorded in inoculated control. The present findings are supported by other workers according to whom the integration of biocontrol agent with compatible fungicide gave significantly higher disease control in several crops than obtained by either biocontrol agent (or) fungicide alone (Sawant, and Mukhopadhay, 1990). From the above results it is evident that, integrated seed treatment with potential BCA (TPT15) + seed treatment with fungicide mancozeb (T6) is highly effective and found to be superior which recorded least PDI, maximum shoot and root dry weight when compared to other treatments.

Biological suppression was proved to be a reliable component of integrated management of phytopathogenic fungi following greenhouse (Paulitz, and Belanger, 2001) and also field demonstration of several biocontrol agents. However, variations in the performance of biocontrol agents resulting from interactions with the native microflora, environmental conditions, and nutrient availability that affect the root colonization (Weller, 1988) remain as a major bottleneck for large-scale use of the available biocontrol agents. In the present study, the enhanced growth of plants might be due to the combined use of biocontrol agents and low dose of compatible fungicides. Integration of biocontrol agents with other disease management options, with identifiable differences in their mechanisms of action, has improved disease protection and the activity spectrum of biocontrol agents (Jetiyanon, and Kloepper, 2002).

Thus, the present findings exhibit the potential antifungal activity of the *Pseudomonas* antagonist TPT15 and the possibility of using this candidate against *S.rolfsii* causing stem rot of groundnut which would benefit to replace or supplement chemical use is extremely important. Further research is focused on identifying the potential antagonist by molecular approach and to evaluate in different agro-climatic regions of Andhra Pradesh.

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