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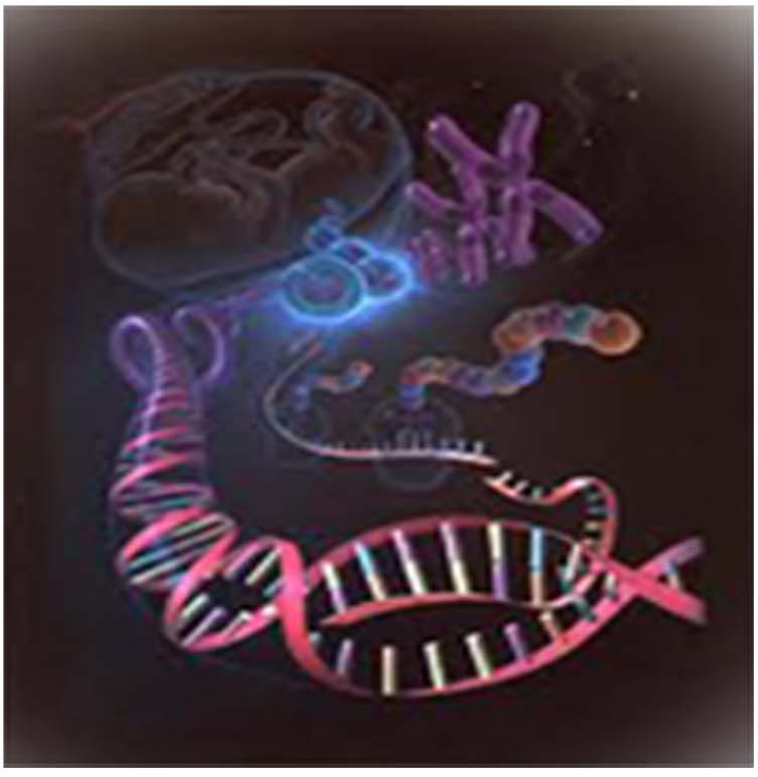

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Research Paper

EFFECT OF POTENTIAL BIOCONTROL AGENTS AGAINST *SCLEROTIUM ROLFSII* CAUSING STEM ROT OF GROUNDNUT

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Total of 40 antagonists were isolated from groundnut rhizosphere and root endophytes of Regional agricultural research station of tirupati region. When tested *in vitro*, the potential biocontrol agents GRE-9 (100%) and GRB-16 (85%) were predominantly aggressive in inhibiting the mycelial growth of the pathogen in dual culture against *Sclerotium rolfsii.* Among the fungicides tested, Mancozeb was most compatible with all antagonists.

Keywords: Groundnut, Stem rot, *Sclerotium rolfsii*, Biocontrol agents

INTRODUCTION

Groundnut (*Arachis hypogaea L.*) is a major legume and oil seed crop in India, covering nearly half of the area under oilseeds. It is grown in over 100 countries with a total estimated area of 21.8 m. ha and with production of 28.5 MT. In India it is grown over an area of 4 lakhs/hectare, with an annual production of 5.5 million tonnes and yield was 1007 kg/hectare in the year 2009-10 (Economic survey 2010-11). *Sclerotium rolfsii* Sacc. is an important soil-borne pathogen and causes disease in numerous crops including peanut (Punja, 1988; Krupa and Dummergues, 1979). The loss of yield caused by pathogen infection generally is 25%, but sometimes it reaches 80-90% in some cases (Grichar and Bosweel, 1987). The disease causes damage on root and stem of plant. The pathogen produces sclerotia which overwinter 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. Different control methods have been investigated before including cultural practices (Gurkin and Jenkins, 1985; Punja *et al*., 1986), chemical control (Bowen *et al*., 1992; Minton *et al*., 1993; Culbreath *et al*., 1992), biological control (Elad *et al*., 1984; Papavizas and Lewis, 1989; Latunde-Dada, 1993; and integrated control (Bicici *et al*., 1994; Cilliers *et al*., 2003). It is widely recognised that biological control of plant pathogens is the most important alternative for the future and can be exploited within the frame work of integrated disease management. The present study is mainly focussed to study and to isolate and identify potential antagonists of *S.rolfsii* and their

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compatibility with the commonly used fungicides.

MATERIALS AND METHODS

Isolation and Identification of Pathogen

The pathogen was isolated from stem rot infected groundnut plants showing typical symptoms of stem rot disease by tissue segment method (Rangaswami and Mahadevan, 1999).

The pathogen was identified based on mycelial and sclerotial characters based on standard mycological keys (Barnett and Hunter, 1972) and maintained on Potato dextrose agar (Dhingra and Sinclair, 1995) for further studies.

Isolation of Root and Endophytes

For isolation of root endophytes and rhizosphere bacteria, 5 g of root were surface sterilized for 5 min. with 70.0 per cent ethanol and homogenized in 20 ml of sterilized phosphate buffer (20mM Na2HPO4+20mM NaH2PO4) using mortar and pestle. Appropriate dilutions (10-4 for fungi and 10-6 for bacteria) of these suspensions were plated on PDA and NA for the isolation of fungi and bacteria respectively. The plates were incubated for 72 hr at 28±2°C (Kishore *et al*., 2005).

Screening of Native Potential Biocontrol Agents by Dual Culture Technique

The antagonistic activity of microflora isolates against *S.rolfsii* was determined by dual culture technique (Johnson *et al*., 1959). Mycelial discs measuring 6 mm diameter from four day old cultures of both fungal antagonist and the test pathogen were placed at equidistant on sterile Petri plate containing PDA medium. One day old cultures of bacteria were streaked on opposite side of the pathogen on PDA medium. The Petri plates were then incubated at 28 ± 2°C. Three replications were maintained in each treatment. Suitable controls were kept without antagonist. Growth of antagonists, pathogen and zone of inhibition were measured after recording full growth in control plate. Percent inhibition of mycelial growth of test pathogen was calculated

Efficacy and Compatibility of Native Potential Antagonists with Different Fungicides Under *In Vitro*

The selected native potential biocontrol agents were tested for their compatibility with the fungicides generally recommended for soil drenching viz., copper oxychloride (0.1%), mancozeb (0.2%), carbendazim (0.2%), thiophanate methyl (0.1%), Hexaconazole (0.1%) and Propiaconazloe (0.1%). Fungal isolates were tested for their compatibility by poisoned food technique (Nene and Thapliyal, 1993) and spectrophotometric method (Kishore *et al*., 2005) for bacterial isolates under *in vitro*.

Statistical Analysis

Completely Randomized Design (CRD) was used for radial growth percent disease incidence, poisoned food technique and dual cultural technique (Gomez and Gomez,1984)

RESULTS AND DISCUSSION

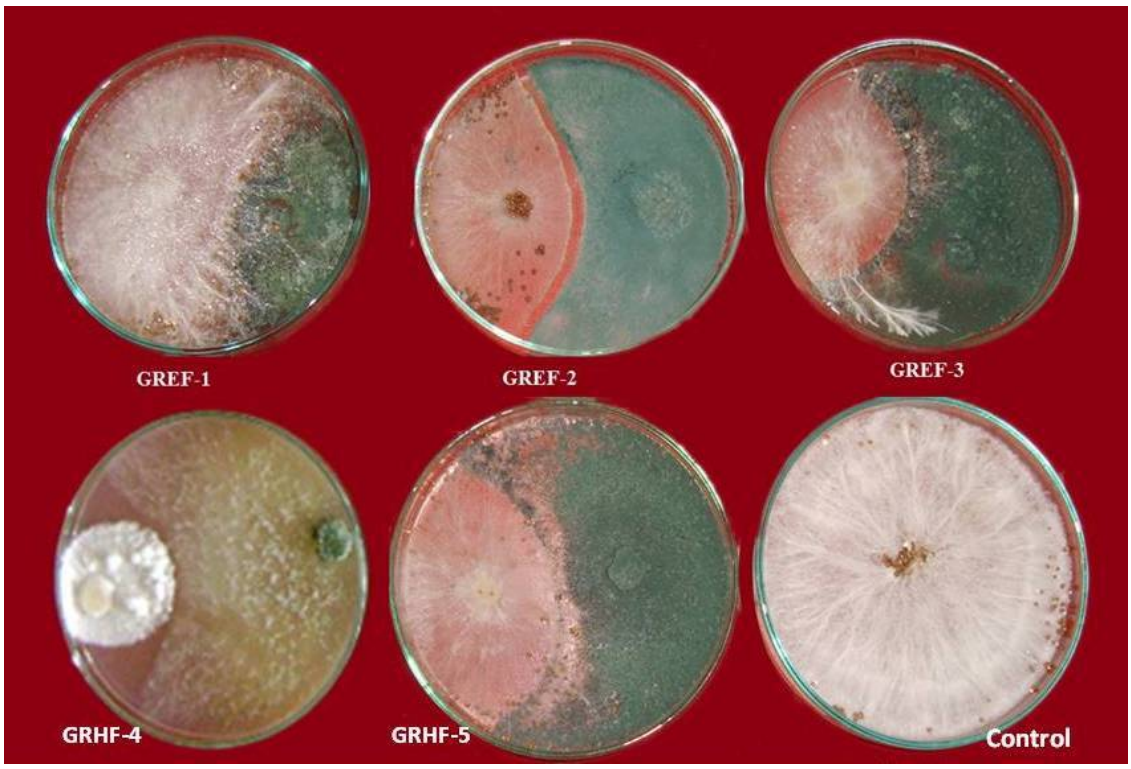
A total of 40 antagonists were obtained from groundnut rhizosphere bacteria and root endophytes. 5 fungi and 40 bacterial isolates were obtained. The bacteria was designated as GRE 1 to GRE 20 (from Groundnut Root endophytes) and GRB 1 to GRB 20 (from Rhizosphere soil) similarly the fungi were named as GREF and GRBF and these were identified as *Trichoderma* spp. in which GRHF 75% from rhizosphere was found to be superior when compared to other isolates in inhibiting the growth of *S.rolfsii* in dual culture (Table 1 and Figure 1). Fungi in the genus

Table 1: *In Vitro* Efficacy of Rhizosphere and Root Antagonistic by Trichoderma Isolates Against *S. rolfsii* in Dual Culture Technique

Isolate	*Linear Growth of *S. rolfsii* (cm)	Percent of Inhibition of Mycelial Growth of *S. rolfsii*
GREF-1	4	55.55 (48.18)
GREF-2	4.5	50 (45.00)
GREF-3	5	44.44 (41.80)
GRHF-4	2.2	75.55 (60.36)
GRHF-5	4.8	46.66 (43.08)
Control	9	0
CD (0.05)	-	7.99
S.Em±	-	2.59

Note: * Mean of three replications.

Figure 1: *In Vitro* Efficacy of Rhizosphere and Root Antagonistic *Trichoderma* Isolates Against *S. Rolfsii* in Dual Culture



Trichoderma have been known since 1920s for the ability to act as biocontrol agents against plant pathogens (Harman, 2006). In case of 40 bacterial isolates GRE 9 Completely inhibited (100%) followed by GRB 16 (85%) the growth of pathogen (Table 2).

Table 2: In Vitro Evaluation of the Antagonistic Activity of Bacterial Root Endophytes and Rhizosphere Bacteria by *S. Rolfsii* in Dual Culture Technique

Isolate	*Linear Growth of *S. rolfsii* (cm)	Percent Inhibition of Mycelial Growth of *S. rolfsii*	Isolate	*Linear Growth of *S. rolfsii* (cm)	Percent Inhibition of Mycelial Growth of *S. rolfsii*
GRE-1	2.1	77.77 (61.86)	GRB-1	2.1	77.77 (61.86)
GRE-2	2.2	75.55(60.36)	GRB-2	4.3	52.22 (46.27)
GRE-3	2.0	76.66 (61.11)	GRB-3	3.8	57.77 (46.89)
GRE-4	3.7	58.88 (46.53)	GRB-4	3.0	65.55 (54.05)
GRE-5	3.1	66.66(54.73)	GRB-5	3.1	66.66 (54.73)
GRE-6	2.8	68.89 (56.09)	GRB-6	2.6	71.11(57.48)
GRE-7	2.43	72.96 (58.83)	GRB-7	3.5	61.11 (51.26)
GRE-8	2	77.78 (61.88)	GRB-8	2	77.78 (61.88)
GRE-9	**0.0**	**100 (90.00)**	GRB-9	3.2	64.44 (53.39)
GRE-10	2.83	68.52 (55.87)	GRB-10	2.83	68.52 (55.87)
GRE-11	3.96	55.93 (48.41)	GRB-11	3.9	55.93 (48.41)
GRE-12	3.13	65.19 (53.84)	GRB-12	3.1	65.19 (53.84)
GRE-13	2.5	72.22 (58.19)	GRB-13	2.5	72.22 (58.19)
GRE-14	3.4	62.00 (51.94)	GRB-14	3.4	62.00 (51.94)
GRE-15	3.6	60 (50.76)	GRB-15	3.6	60 (50.76)
GRE-16	2.2	75.55 (60.36)	**GRB-16**	**1.3**	**85.55 (67.65)**
GRE-17	3.0	66.66 (54.73)	GRB-17	3.0	66.66 (54.73)
GRE-18	4.0	57.23 (49.34)	GRB-18	2.03	77.41 (61.66)
GRE-19	2.43	72.96 (58.83)	GRB-19	3.3	63.33 (52.73)
GRE-20	1.4	84.44 (66.76)	GRB-20	1.8	80.00 (63.43)
Control	9	0	Control	9	0
CD(0.05)	-	10.21	CD(0.05)	-	7.97
S.Em±	-	3.46	S.Em±	-	2.70

Note: * Mean of three replications.

The use of biological control methods to reduce disease incidence caused by plant pathogens is continually being developed and is being used in variety of crops. By including biocontrol agents for controlling *S.rolfsii* as a part of integrated disease management will help in

managing disease as well as delaying the development of tolerance/resistance to fungicides. The bacterial isolate GRE 9 (Table 3) was more compatible with mancozeb followed by carbendazim, Copper oxychloride. Similar observations were made by Vidhyasekharan and Muthamilan (1995) and reported that carbendazim was not inhibitory to *P. fluorescens*. The fungal isolate GRHF4 (Table 4 and Figure 2) was more compatible with Mancozeb followed by copper oxy chloride. Similar results were obtained by Vijayaraghavan and Abraham (2004) observed that mancozeb was compatible with *T.viride.* Among all the fungicides tested Mancozeb was found to be more compatible. The present study revealed that the pathogen *S.rolfsii* developed tolerance against mancozeb when compare to other fungicides.

Table 3: *In Vitro* Evaluation of the Compatibility of the Potential Antagonistic Bacterial Isolate Gre-9 With Different Fungicides

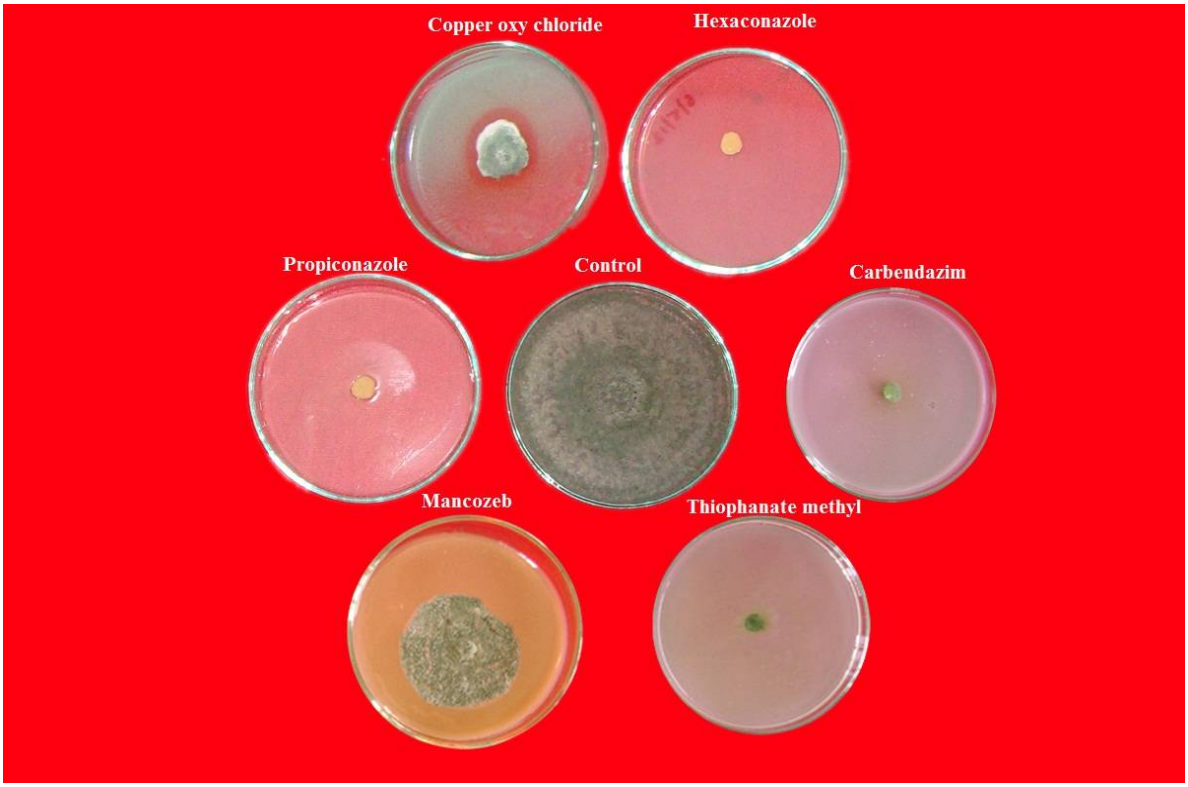
Fungicide	Concentration	*Growth of Bacterial Isolate (OD at 600nm)
Mancozeb	0.2%	1.45
Carbendazim	0.2%	0.92
Copper oxychloride	0.1%	0.86
Thiophanate methyl	0.1%	0.67
Hexaconazole	0.1%	0.78
Propiaconazole	0.1%	0.80
Control	-	1.56
CD (0.05)	-	0.27
SEm±	-	0.096

Note: * Mean of three replications.

Table 4: *In Vitro* Evaluation of Compatibility of Trichoderma Isolate GRHF-4 With Different Fungicides in Poisoned Food Technique

Fungicide	Concentration	*Growth of Trichoderma (mm)	Per cent inhibition over control
Mancozeb	0.2%	4.7	48.01
Copper oxychloride	0.2%	2.1	77
Carbendazim	0.1%	0	100
Thiophanate methyl	0.1%	0	100
Propiaconazole	0.1%	0	100
Hexaconazole	0.1%	0.1	99
Control		9.0	-
CD(0.05)		-	5.52
SEm±		-	1.82

Figure 2: *In Vitro* Evaluation of Compatibility of Tricoderma Isolate GRBF-4 With Different Fungicides in Poisoned Food Technique



CONCLUSION

The results revealed that GRE -9 has showed 100% inhibition against the pathogen *(S.rolfsii).* The bacterial isolate GRE 9 was more compatible with mancozeb under poison food technique. In future the work will be carried on integrated disease management to reduce inoculum thresh hold levels and to suppress the disease for the purpose of higher yields.

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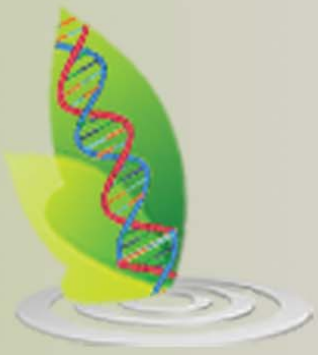

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