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Integrated Management of Stem Rot Disease (*Sclerotium rolfsii*) of Groundnut (*Arachis hypogaea* L.) Using Rhizobium and *Trichoderma harzianum* (ITCC - 4572)

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Abstract: Soil-borne plant pathogenic fungi cause heavy crop losses all over the world. With variable climate from region to region, most crops grown in India are susceptible to diseases caused by soil-borne fungal pathogens. Among tropical and subtropical land crops, groundnut (*Arachis hypogaea* L.) is an important oil seed crop, providing vegetable oil as human food and oil cake meal as animal poultry feed. A large number of diseases attack groundnut plants in India; of these, stem rot (collar rot) caused by *Sclerotium rolfsii* is the most common disease. Certain well-studied chemical pesticide management strategies are available for reducing damage by *S. rolfsii*, but increasing awareness about the health hazards and environmental problems due to the use of chemical pesticides resulted in the development of Integrated Pest Management. In the present study, integrated management of stem rot disease of groundnut using a combined application of *Rhizobium* and *Trichoderma harzianum* (ITCC – 4572) was performed. The results indicated that the application of these native micro-organisms successfully decreases the stem rot incidence and also increases the growth of the groundnut plants. The plant growth promoting activity and disease control ability of these microbial agents are discussed.

Key Words: *Rhizobium, Trichoderma harzianum*, IPM, stem rot, *Sclerotium rolfsii*, groundnut

Introduction

The green revolution has led to intensified agriculture to meet the ever increasing demands for food and fibre, which is practised at great cost to the environment, resulting in continuous damage of natural ecosystems, ground water and food-stuff pollution and other environmental degradation. Indiscriminate use of chemical pesticides and fertilisers in modern agriculture has resulted in the development of several problems such as pesticide resistance in pests, resurgence of target and non-target pests, destruction of beneficial organisms like honey bees, and chemical residues in food, feed and fodder. Awareness about the health hazards and environmental problems due to the continuous use of pesticides resulted in the development of Integrated Pest Management (IPM). Keeping this in view, studies have been initiated to include crop protection and growth promotion using the native micro-organisms, as a component of IPM.

Groundnut (*Arachis hypogaea* L.) is an important annual oil seed crop (Brown, 1999). A large number of diseases attack groundnut in India (Mayee, 1987; Mayee and Datar, 1988; Ganesan and Sekar, 2004a). The majority are caused by fungi and several of them are yield reducers in certain regions and seasons (Mayee, 1995). Among the soil-borne fungal diseases of groundnut, stem rot caused by *S. rolfsii* is a potential threat to groundnut 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 majority of work done on plant disease biocontrol was related to soil-borne diseases using either bacteria or fungal antagonists. Among bacteria, *Pseudomonas* and *Bacillus* spp. were widely used. However, the use of antagonistic fungi, especially *Trichoderma* and *Gliocladium* spp., has been more extensive than their

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bacterial counterparts (Ganesan et al., 2003; Ganesan, 2004; Ganesan and Sekar, 2004a, 2004b). Bacteria isolated from the rhizosphere and belonging to a wide variety of genera have the potential to suppress diseases caused by a diversity of soil-borne plant pathogens. Some symbiotic N_2 fixing *Rhizobium* strains not only fix atmospheric N_2 in the nodules but also show an antagonistic effect against soil-borne pathogens (Tu, 1978; Chakraborty and Purkayastha, 1984; Chakraborty and Chakraborty, 1989; Muthamilan and Jeyarajan, 1996; Deshwal et al., 2003; Bardin et al., 2004).

In the present work an attempt was made to determine the plant growth promoting ability and control of stem rot disease in groundnut by combined application of an antagonistic *Rhizobium* and *T. harzianum* in the soil.

Materials and Methods

Isolation and maintenance of micro-organisms

Groundnut (*Arachis hypogaea* L.) plants showing stem rot symptoms were collected from Pallapatty village crop field, Madurai district, Tamil Nadu, India (lat 9°5'-10°5'N, long 77°81'-78° -2'E). The isolation of the pathogen from diseased plants was performed on Potato Dextrose Agar (PDA) medium and identified according to their morphology and colony characteristics. The pathogenicity of the isolated *S. rolfsii* was studied in a pot culture experiment as described by Singh and Thapliyal (1998).

Different strains of *Rhizobium* were isolated from root nodules of groundnut by standard dilution plate technique on Yeast Extract Mannitol Agar (YMA) medium. They were screened for their antagonistic activity against the pathogen (*S. rolfsii*) by dual culture method as described by Rangeswaran and Prasad (2000). Isolates which showed significant antagonistic activity were identified by using methods described in Bergey's Manual of Systematic Bacteriology 8th edt. (1984) and tested for their ability to establish nodule formation in groundnut seedlings. Pure cultures were maintained on YMA medium. The biocontrol agent *Trichoderma harzianum* (ITCC-4572) was obtained from the Indian Type Culture Collection (ITCC), New Delhi, and the pure culture was maintained on PDA medium.

Interaction among *Trichoderma harzianum* and *Rhizobium* and the pathogen *Sclerotium rolfsii* was studied by dual culture method as described by Rangeswaran and Prasad (2000). Culture filtrate of *Rhizobium* was prepared as described by Amer et al. (1997) and the effect of this filtrate on the pathogen was studied as described by Jariwala et al. (1991). Volatile activity of *Rhizobium* on *Sclerotium rolfsii* was tested according to Dennis and Webster (1971).

The inhibition percentage was calculated using the following formula:

Inhibition Percentage (%) = $A_1 - A_2 / A_1 \times 100$, where, A_1 is area covered by fungi in the control, and A_2 is area covered by fungi in the dual culture.

Mass culture of pathogen *(Sclerotium rolfsii)* and biocontrol agent (*T. harzianum*)

Sorghum grains (250 g) soaked overnight in 2% sucrose solution were collected and sterilised in autoclavable bottles at 121 ºC for 20 min. Sterilised sorghum grains were inoculated with mycelium of *Sclerotium rolfsii* and *T. harzianum*, taken from the margin of actively growing cultures using a cork borer (10 mm) and incubated at room temperature (28 ± 2 ºC) for 15 days.

Mass culture of *Rhizobium*

Rhizobium isolates were inoculated in YMA broth separately and incubated in room temperature (28 ± 2 ºC) for 48 h. The log phase cultures were used for the pot experiment.

Pot culture assay of biocontrol agents

Biocontrol of *S. rolfsii* using *T. harzianum* and *Rhizobium* was studied in vivo by pot culture method (Dubey, 1998). Surface sterilised healthy looking seeds of groundnut were sown (5 seeds/pot) in 16-cm diameter plastic pots containing sterilised sand and red soil (2:1 ratio). After 15 days, 5 g of pathogen, 5 g of *T. harzianum* (4×10^4 conidia ml^{-1}) and 20 ml of *Rhizobium* (9×10^6 cfu ml^{-1}) were added per pot at 2-cm depth of soil around the stem. The following treatments were carried out in triplicate.

T_1 - control (without any inoculant); T_2 - *Sclerotium rolfsii*; T_3 - *Rhizobium* Isolate (RI) -2; T_4- RI-3; T_5 - *Trichoderma harzianum*; T_6 - RI-2 + *S. rolfsii*; T_7 - RI-3 + *S. rolfsii*; T_8 - *T. harzianum* + *S. rolfsii*; T_9 - *T. harzianum* + RI-2 + *S. rolfsii*; T_{10} - *T. harzianum* + RI-3 + *S. rolfsii*; T_{11} - RI-2 + RI-3 + *S. rolfsii*; T_{12} - RI-2 + RI-3 + *S. rolfsii* + *T. harzianum*; T_{13} - RI-2 + RI-3; T_{14} - RI-2 + RI-3 + *T. harzianum*.

Moisture level of the soil was maintained by adding sterilised water. After 75 days of plant growth, percentage of disease control, shoot and root length, and shoot and root dry weight were analysed. The ANOVA approach was used to evaluate the efficacy of biocontrol agent application in pot experiments. A comparison among treatment mean and means of appropriate control treatments was made with Duncan's multiple range test (DMRT), (P = 1% and 5% level) (Snedecor and Cochran, 1987).

Results and Discussion

In the dual culture method, *Trichoderma harzianum* showed around 57% of inhibition against *Sclerotium rolfsii* (Table 1). Control of *Sclerotium rolfsii* using *Trichoderma harzianum* was reported by several researchers (Muthamilan and Jeyarajan, 1996; Ganesan at al, 2003; Ganesan, 2004; Ganesan and Sekar, 2004a).

When *Rhizobium* is applied as a biofertiliser and *Trichoderma harzianum* as a biocontrol agent, the nature of interaction among them is important for better growth of the groundnut plants. In the dual culture method among the 6 isolates of *Rhizobium* the isolates RI-2 and RI-3 alone showed slight inhibition of the growth of *Trichoderma harzianum* and the remaining isolates, RI-1, RI-4, RI-5 and RI-6, did not inhibit the growth of *Trichoderma harzianum*.

Jayaraj and Ramabadran (1999) reported that culture filtrates of *Trichoderma harzianum* inhibited the growth of *Rhizobium.* Against *S. rolfsii*, among the 6 isolates only 2 (RI-2 and RI-3) showed above 60% inhibition. Therefore, for further studies *Rhizobium* isolates 2 and 3 were used (Table 1). When culture filtrate of *Rhizobium* (RI-2 and RI-3) was incorporated into the medium, growth of *Sclerotium rolfsii* was significantly inhibited. Inhibition varies depending upon the concentration of the filtrate (Table 2).

Table 1. Antagonistic activity (dual culture method) of *Rhizobium* and *Trichoderma harzianum* against *Sclerotium rolfsii.*

S. No.	Treatments	Radial growth of Sclerotium rolfsii (mm)*	% of inhibition
1	Control	80 ± 0.50	-
2	Trichoderma harzianum	31.6 ± 0.92	60.50
3	Rhizobium Isolate (RI)-1	75 ± 0.44	6.25
4	RI-2	32 ± 0.60	60.00
5	RI-3	30 ± 0.30	62.50
6	RI-4	74 ± 0.50	7.50
7	RI-5	48 ± 0.10	40.00
8	RI-6	38 ± 0.36	52.50

* = Each value is mean of triplicate ± = Standard deviation

Table 2. Effect of non-volatile compound produced by *Rhizobium* on *Sclerotium rolfsii.*

Treatment	C	Radial growth of S. rolfsii (mm)*				Growth reduction (%)			
		25%	50%	75%	100%	25%	50%	75%	100%
RI-2	36 ± 0.52	34 ± 0.36	10 ± 0.45	11 ± 0.30	-	5.5	72.2	69.0	100
RI-3	36 ± 0.52	13 ± 0.20	12 ± 0.56	12 ± 0.50	11± 0.20	63.8	67	67	69

C = Control (without culture filtrate) * = Each value is mean of triplicate
% = Concentration of culture filtrate in percentage ± = Standard deviation

In volatile activity a slight inhibition of the growth of the pathogen was noted against both *Rhizobial* isolates. Isolate RI-2 showed 8% and isolate 3 showed 11% inhibition of the pathogen (Table 3). Culture filtrates of *Bradyrhizobial* strains significantly inhibited the growth of *Macrophomina phaseolina* as reported by Chakraborty and Purkayastha (1984) and Deshwal et al. (2003). Inhibition of the pathogen by *Rhizobium* may be due to the production of siderophore and Rhizobiotoxin (Chakraborty and Purkayastha, 1984). Deshwal et al. (2003) reported the absence of HCN production by *Bradyrhizobium* strain to inhibit *Macrophomina phaseolina*. Inhibition of *Phytopththora megasperma* by *Rhizobium* was reported by Tu (1978).

Table 3. Effect of volatile compound produced by *Rhizobium* on *Sclerotium rolfsii*.

S. No.	Isolate number	Radial growth of *S. rolfsii* (mm)*	Growth reduction (%)
1.	Control	36 ± 0.36	-
2.	RI-2	33 ± 0.40	8
3	RI-3	32 ± 0.70	11

* = Each value is mean of triplicate ± = Standard deviation

In the pot culture experiment the groundnut plants treated with *Trichoderma harzianum, Rhizobium* isolates and pathogen showed significant increases in shoot length and root length when compared with the control. Treatments T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12} showed higher shoot and root length compared with plants treated with *Sclerotium rolfsii* alone (T_2) (Table 4).

In biomass accumulation pathogen alone treated plants (T_2) showed lower shoot and root dry weight compared with *Trichoderma, Rhizobium* and pathogen treated plants (T_6-T_{12}) (Table 5). In the same way, plants treated with *Trichoderma harzianum* and *Rhizobium* alone showed higher shoot and root length and dry weight compared with plants without any treatment (T_1) (Table 5). These results showed that application of *T. harzianum* and *Rhizobium* not only controls the pathogen but also improves the plant growth.

Limited work has been carried out to determine the interaction among rhizobacteria, biocontrol agents and pathogen. *Rhizobium* are known to increase nodulation and nodule weight in legumes along with increases in host

Table 4. Effect of antagonist micro-organisms on plant growth (shoot and root length).

S. No	Treatment*	Shoot length** (cm)	Root length** (cm)	Shoot/root ratio
1.	T_1	15.667^{b} ± 0.568	9.800^{b} ± 0.360	1.7
2.	T_2	11.667^{a} ± 0.305	7.167^{a} ± 0.251	1.7
3.	T_3	20.800^{d} ± 0.655	15.733^{f} ± 0.351	1.4
4.	T_4	20.533^{d} ± 0.850	19.167^{h} ± 0.450	1.1
5.	T_5	23.867^{f} ± 0.568	13.867^{e} ± 0.378	1.7
6.	T_6	21.833^{e} ± 0.152	11.600^{c} ± 0.556	1.8
7.	T_7	18.900^{c} ± 0.141	14.367^{e} ± 0.305	1.3
8.	T_8	18.600^{c} ± 0.360	12.133^{cd} ± 0.568	1.5
9.	T_9	19.100^{c} ± 0.264	12.500^{d} ± 0.458	1.6
10.	T_{10}	23.700^{f} ± 0.400	12.667^{d} ± 0.450	1.7
11.	T_{11}	23.933^{f} ± 0.251	11.967^{cd} ± 0.305	1.7
12.	T_{12}	23.733^{f} ± 0.351	18.100^{g} ± 0.264	1.3
13.	T_{13}	26.067^{g} ± 0.251	17.833^{g} ± 0.251	1.3
14.	T_{14}	27.267^{h} ± 0.351	17.700^{g} ± 0.360	1.3

* For the treatments see Materials and Methods.

** Significant at 1% level, Means followed by a common letter within a column are not significantly different at 5% level by DMR test, Each value is mean of triplicate, ± = Standard deviation.

Table 5. Effect of antagonist micro-organisms on shoot and root dry weight of treated plants.

S. No	Treatment*	Shoot length** (cm)	Root length** (cm)	Shoot/root ratio
1.	T_1	$207.667^{b} \pm 4.5$	$37.667^{a} \pm 3.05$	5.5
2.	T_2	$93.000^{a} \pm 7.21$	$31.333^{a} \pm 2.51$	3.0
3.	T_3	$326.000^{e} \pm 13.22$	$91.000^{g} \pm 4.00$	3.5
4.	T_4	$274.000^{cd} \pm 9.64$	$78.000^{de} \pm 3.60$	3.5
5.	T_5	$361.333^{c} \pm 25.5$	$71.333^{d} \pm 2.51$	5.0
6.	T_6	$370.000^{f} \pm 7.93$	$51.333^{b} \pm 2.50$	7.2
7.	T_7	$259.333^{c} \pm 4.72$	$86.333^{fg} \pm 6.42$	3.0
8.	T_8	$288.667^{d} \pm 6.8$	$60.667^{c} \pm 2.51$	4.8
9.	T_9	$371.333^{f} \pm 2.51$	$63.000^{c} \pm 4.00$	6.0
10.	T_{10}	$406.667^{g} \pm 4.5$	$72.000^{d} \pm 3.60$	5.6
11.	T_{11}	$313.333^{e} \pm 4.04$	$61.000^{c} \pm 2.00$	5.1
12.	T_{12}	$371.000^{f} \pm 2.00$	$52.333^{b} \pm 4.16$	7.1
13.	T_{13}	$426.333^{g} \pm 6.42$	$82.000^{ef} \pm 3.60$	5.2
14.	T_{14}	$555.000^{h} \pm 32.4$	$85.667^{fg} \pm 6.11$	6.5

* For the treatments see Materials and Methods.

** Significant at 1% level, Means followed by a common letter within a column are not significantly different at 5% level by DMR test, Each value is mean of triplicate, ± = Standard deviation.

plant growth and development besides protecting roots from the attack of pathogen (Tu, 1978; Chakraborty and Purkavastha, 1984; Chakraborty and Chakraborty, 1989; Muthamilan and Jayarajan, 1996; Tilak et al., 2006; Huang and Erickson, 2007). In the same way, *Trichoderma* sp. controls the pathogen but also improves the overall health of the host (Windham et al., 1986; Ganesan, 2004). A significant reduction in the incidence of root rot caused by *Rhizoctonia* in *Rhizobium* and *Trichoderma* treatment was reported by Jayaraj and Ramabadran (1999).

Chakraborty et al. (2003) reported that combined application of *Bradyrhizobium japonicum* and *Trichoderma harzianum* significantly reduced root rot disease in soya bean. They also showed that activity of PAL and peroxidase enzymes and Phytoalexin (Glyceollin) activity increased in treated plants. In the present study also overall growth of the groundnut plant increased in *Rhizobium* and *Trichoderma* combined treatments.

Conclusion

The results of the present study indicate that combined application of selected symbiotic N_2 fixing and antagonistic *Rhizobium* isolates and biocontrol agent *Trichoderma harzianum* controls the pathogen significantly and increases the plant growth. It may be due to the competition for space and nutrient, parasitism, production of enzymes, volatile and non-volatile metabolites or combined action of these mechanisms of biocontrol organisms against the pathogen. The growth-promoting activity may be due to the solubilisation and sequestration of inorganic plant nutrient, production of unidentified growth promoting chemicals or by the N_2 fixing activity of the *Rhizobium* isolates. Hence these *Rhizobium* and *T. harzianum* can be used as biocontrol agents and biofertiliser for the management of groundnut crop. Further research has also been undertaken to find out the mechanism of action and efficacy of the combined application of these microbes at field level.

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