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Fluorescent *Pseudomonas* as plant growth promoting Rhizobacteria and biocontrol agents in groundnut crop (*Arachis hypogaea* L.)

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Abstract

Microorganisms have been extensively used for the biological control of soil borne plant disease as well as for promoting plant growth. In the present work bio control activity of eleven *Pseudomonas* isolates was tested against *Sclerotium rolfsii* causing stem rot disease in Groundnut by dual culture method. Seven isolates showed above 68% of inhibition. Mechanisms of bio control activity of the *Pseudomonas* isolates were determined by studying production of Non-Volatile compound (Antibiosis), Volatile compound (HCN production) Siderophore (Microbial Iron Transport Agents) and Chitinase activity (Lysis). All the isolates showed varied level of these activities. Effect of *Pseudomonas* isolates as Plant Growth Promoting Rhizobacteria (PGPR) in Groundnut and as bio control agent against *S. rolfsii* was studied in pot culture experiment. Bio control agent treated plants showed vigorous growth and no disease incidence was noticed. Utilization of *Pseudomonas fluorescence* as PGPR and bio control agent in Groundnut crop is discussed.

Keywords: *Pseudomonas, Sclerotium rolfsii,* biocontrol, Mechanism, Groundnut.

Introduction

Stem rot caused by *Sclerotium rolfsii* Sacc. is a potential threat to groundnut production and is of considerable economic significance for groundnut grown under irrigated conditions. The disease causes severe damage to the crop at any stage of crop growth period and yield losses over 25% (Mayee and Datar, 1988). Chemical control of soil borne diseases is frequently ineffective because of the physical and chemical heterogeneity of the soil, which may prevent effective concentration of the chemical from reaching the pathogens. Microorganisms have been used extensively for the biological control of soil borne plant diseases as well as for promoting plant growth (Weller, 1988; Thomashow and Weller; 1990, Baker *et al.,* 1991). Successful control of disease caused by *S. rolfsii* by application of fungal and bacterial biological agents to the soil has been reported by several workers (Elad *et al*., 1979; Elad *et al*., 1980; Papavizas, 1985; Upadhayay and Mukhopadhyay, 1986; Ganesan and Gnanamanickam, 1987; Ordentilich *et al*., 1988; Padile and Dube, 1988b; Arghari and Mayee, 1990; Ristaino *et al*.,1991; Muthamilan and Jayarajan , 1996; Ganesan, 2004; Ganesan and Sekar, 2011). In the present work an attempt was made to find out the biocontrol efficiency of Fluoresence *Pseudomonas* against *Sclerotium rolfsii* Sacc, causing stem rot diseases in Groundnut (*Arachis hypogaea* L.).

Materials and Methods

Sclerotium rolfsii was isolated from Groundnut plants showing stem rot symptoms (Ansori and Agnittori, 2000) and pathogenicity of isolated pathogen was tested in pot culture experiment (Sinelair and Backman, 1989).

The biocontrol agent *Pseudomonas fluorescence* was isolated from different rhizospheric soil by standard dilution plate technique on *Pseudomonas* agar medium (Hi-media). Pure cultures of biocontrol agent and the pathogen were maintained on *Pseudomonas* agar and Potato dextrose agar medium respectively.

Antagonistic activity of isolated *Pseudomonas* was tested against *S.rolfsii* by dual culture method (Rangeswaran and Prasad,2000). Inhibition percentage was calculated using the following formula,

$$\text{Inhibition percentage} = \frac{A_1 - A_2}{A_1} \times 100$$

Where A_1 = Area covered by the pathogen in control.

A_2 = Area covered by the pathogen in dual culture.

To ascertain the effect of volatile and non-volatile compounds produced by *P. fluorescence* the following experiment was carried out. Culture filtrate of the biocontrol agent was prepared as described by Amer *et al*.,(1998) and effect of this filtrate on the pathogen was studied as described by Jariwala *et al*., (1991). Volatile activity of biocontrol agent was tested by paired petriplate technique (Lorck, 1948). Siderophore production was tested by $Fecl_3$ test and chrome azurol assay test (Dave and Dubey, 2000; Jalal and Dick Vander Helm, 1990). Chitinase activity was tested on Chitin amended medium as described by Roberts and Selitrennikoff, (1988). Biocontrol activity of *Pseudomonas* against *S. rolfsii* and its effect on Groundnut growth was studied in vivo by pot culture method (Wells *et al*.,1972; Dubey, 1998).

Results and Discussion

In dual culture, isolates of *Pseudomonas* showed different level of inhibition to the pathogen. (Table-1). Six isolates showed above 68% of inhibition and these isolates were used for further study. Culture filtrates of different isolates of *Pseudomonas* showed varying degree of inhibition to the pathogen (Table 2) it depends on the isolate and concentration of the filtrate. Isolate 2 showed maximum inhibition at all concentration of the filtrate. Rengashwaran and Prasad (2000) reported the inhibition of Sclerotial germination and mycelial growth of *S. rolfsii* causing rot in sunflower by the *Pseudomonas* culture filtrate.

Table- 1: Antagonistic activity of Fluorescent *Pseudomonas* against *Sclerotium rolfsii.*

Sl. No.	Isolated number	Radial growth of *S. rolfsii* *(mm)	% of inhibition
1	Control	67	-
2	PIS1	20	70.14
3	PIS2	18	73.13
4	PIS 3	31	53.73
5	PIS 4	31	53.73
6	PIS 5	21	68.65
7	PIS 6	18	73.13
8	PIS 7	26	61.19
9	PIS 8	26	61.19
10	PIS 9	15	77.61
11	PIS 10	27	59.70
12	PIS 11	19	71.64
13	PIS 12	24	64.17
14	PIS 13	21	68.65
15	PIS 14	25	62.68
16	PIS 15	25	62.68

*-Each value is mean of triplicates.

Table- 2: Effect of non-volatile compound produced by *P. fluorescence* on *S. rolfsii* Sacc.

S. No.	Radial Growth of *S. rolfsii* (mm)*					Growth Reduction (%)			
Isolate number	100%	75%	50%	25%	C	100%	75%	50%	25%
PIS 1	-	-	3.5	10.0	38	100	100	90.78	73.68
PIS 2	-	4.5	7.5	7.0	38	100	88.15	80.26	81.57
PIS 5	-	5.5	6.0	10.0	38	100	85.52	84.21	73.68
PIS 6	4.5	5.5	7.0	12.5	38	88.15	85.52	81.57	67.10
PIS 9	2.5	7.5	11.0	12.5	38	93.42	80.26	71.05	67.10
PIS 11	5.5	13.5	15.5	18.5	38	85.52	64.47	59.21	51.31
PIS 13	-	12.5	18.0	22.0	38	100	67.10	52.63	42.10

C = Control (without culture filtrate).
% = Concentration of culture filtrate in percentage;
*= Each value is mean of triplicates

Table 3. Effect of Volatile Activity of Fluorescent *Pseudomonas* on the growth of *S. rolfsii*

S. No	*Pseudomonas* Isolates	Radial growth of *S. rolfsii* (mm)	Growth Inhibition of *Sclerotium rolfsii*	
			At 3rd day (%)	At 5th day (%)
1	Control	38	-	-
2	PIS 1	-	100%	100%
3	PIS 2	-	100%	100%
4	PIS 5	-	100%	100%
5	PIS 6	-	100%	100%
6	PIS 9	-	100%	100%
7	PIS 11	-	100%	100%
8	PIS 13	-	100%	100%

*=Each value is mean of five replicates

Table - 4: Chitinase activity of *Pseudomonas* isolates

S.No.	Isolate Number	Chitinase Activity
1	PIS 1	+
2	PIS 2	+
3	PIS 5	-
4	PIS 6	-
5	PIS 9	-
6	PIS 11	+
7	PIS 13	-

"+" Clearing zone present;
"-" Clearing zone absent

Table - 4a: Siderophore production

S. No.	*Pseudomonas* Isolates (PIS)	Siderophore Production
1	PIS1	+
2	PIS 2	+
3	PIS 5	-
4	PIS 6	-
5	PIS 9	+
6	PIS 11	+
7	PIS 13	-

+ Formation of orange color
- Absence of color formation

Table- 5: Root and shoot length

S. No	Treatments	Root Length*(cm)	Shoot length* (cm)
1	A	19.2 ± 5.260	22.8 ± 1.78
2	B	09.6 ±7.371	13.8 ± 3.42
3	C	23.2 ±2.580	23.6 ± 3.64
4	D	19.8 ±2.580	25.8 ± 1.78
5	E	20.6 ±1.949	26.8 ± 2.58
6	F	22.0 ±1.580	25.6 ± 2.30
7	G	21.8 ±4.490	22.8 ± 1.64
8	H	22.6 ±3.640	25.6 ± 1.51
9	I	19.2 ±2.380	24.8 ± 4.14

* Each value is mean of 5 - replicates; ± = Standard deviation
Legends for Treatments: A = Control, B = Pathogen alone, C = PIS1, D = PIS 2, E = PIS 5, F = PIS 6, G = PIS 9, H = PIS 11, I = PIS 13

Table – 6: Root and Shoot fresh weight

Sl. No	Treatments	Root fresh weight * (gm)	Shoot fresh weight * (gm)
1	A	0.250 ± 0.125	1.109 ± 0.224
2	B	0.260 ± 0.078	0.439 ± 0.066
3	C	0.551 ± 0.144	1.236 ± 0.501
4	D	0.374 ± 0.133	1.615 ± 0.373
5	E	0.330 ± 0.112	1.814 ± 0.518
6	F	0.555 ± 0.253	1.782 ± 0.483
7	G	0.366 ± 0.104	1.732 ± 0.485
8	H	0.261 ± 0.116	2.102 ± 0.538
9	I	0.488 ± 0.296	1.909 ± 0.587

* Each value is mean of 5 replicates; ± = Standard deviation
Legends for Treatments: A = Control, B = Pathogen alone, C = PIS1, D = PIS 2, E = PIS 5, F = PIS 6, G = PIS 9, H = PIS 11, I = PIS 13

Table -7: Root and shoot dry weight

S. No	Treatments	Root Dry weight*(gm)	Shoot Dry weight * (gm)
1	A	0.097 ± 0.058	0.233 ± 0.125
2	B	0.076 ± 0.027	0.029 ± 0.180
3	C	0.106 ± 0.034	0.388 ± 0.205
4	D	0.159 ± 0.141	0.275 ± 0.178
5	E	0.118 ± 0.048	0.434 ± 0.093
6	F	0.109 ± 0.063	0.351 ± 0.106
7	G	0.086 ± 0.013	0.391 ± 0.242
8	H	0.153 ± 0.224	0.340 ± 0.116
9	I	0.060 ± 0.023	0.439 ± 0.155

*Each Value is mean of 5 replicates; ± = Standard deviation
Legends for Treatments: A = Control, B = Pathogen alone, C = PIS1, D = PIS 2, E = PIS 5, F = PIS 6, G = PIS 9, H = PIS 11, I = PIS 13

All the isolates showed 100% inhibition of the pathogen by volatile metabolite production in paired petriplate technique. Volatile cyanide production by rhizosphere Pseudomonas was reported by several workers (Bakker *et al.*, 1991; Haas *et al*., 1991; Laha *et al*.,1996; Rengashwaran and Prasad 2000; Ganesan, 2004). Microorganisms capable of lysing other organisms can serve as powerful tool for biological control (Chet *et al*., 1990). Chitinase is one such enzyme, which degrade chitin present on the wall of fungal pathogen.

In the present work isolates 1, 2 and 11 alone produced chitinolytic enzymes on the chitin-amended medium, (Table -4) which indicates the role of chitinase in lytic activity of the biocontrol agent. Fluorescent *Pseudomonas* produced extra cellular siderophore (Microbial Iron Transport Agents) which efficiently complex environmental iron, making it less available or unavailable to pathogen and native microflora (Kloepper *et al*., 1980a; Hamden *et al*., 1991; Loper, 1988; Duiffy *et al*., 1993; Dave, 2000). Among the 7 isolates of *Pseudomonas* only 4 isolates viz., 1, 2, 9 and 11 showed the production of siderophore.

In pot culture experiment, pathogen alone-inoculated plants showed typical stem rot symptom. Biocontrol agent *P. fluorescence* treated plants showed reduced disease incident, and showed vigorous growth and healthy looking compared with control plants. The length of the root and shoot, and fresh and dry weight of the shoot and root also increases at different level over control plant (Table 5 - 7).

Production of plant growth promoting substances by biocontrol agent *P. fluorescence* and which helps in the vigorous growth of the plant has been reported by several workers (Klopper and Scroth, 1978; Scroth and Hamcock, 1982; Klopper *et al*., 1988; Weller, 1988; Thomashow and Weller, 1990; Bakker *et al*.,1991; Vanpeer *et al*.,1991). Present work showed the *P. fluorescence* could be effectively used as biocontrol agent and also for improving plant growth in Groundnut crop (*Arachis hypogaea* L.).

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