INT. J. BIOL. BIOTECH., 2 (2): 415-418, 2005.

EFFECT OF DIFFERENT STRAINS OF *RHIZOBIUM* SPP. IN THE CONTROL OF ROOT INFECTING FUNGI AND GROWTH OF CROP PLANTS

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ABSRACT

Biocontrol potential of *Rhizobium* and *Bradyrhizobium* species against soil borne root infecting fungi was tested. All strains showed infectivity and produced nodules on mungbean. Rhizobia used as seed dressing and soil drenching showed a significant increase in shoot length, root length, shoot weight and root weight in mungbean and okra plants. The number of nodules were highest in mungbean roots where Rhizobia were used as seed dressing and soil drenching. Seed dressing and soil drenching with Rhizobia were found effective methods for the control of soil borne root infecting fungi like *Fusarium* spp, *Macrophomina phaseolina* and *Rhizoctonia solani* on mungbean and okra.

Key words: Rhizobia, strains, root rot fungi, okra and mungbean.

INTRODUCTION

The soil borne fungal pathogens play a major role in the development of root rot disease complex on many important field and horticultural crops which often results in the death of plant. Since soil applied pesticides are costly and produce environmental hazards, several fungi and bacteria have received considerable attention in the control of soil borne root infecting fungi (Papavizas and Lumsden, 1980).

Bacteria of the genus *Rhizobium* and *Bradyrhizobiuym* are of considerable scientific and economic interest because of their ability to fix nitrogen in leguminous plants (Hynes and O'Connel, 1990) and in the control of soil borne root infecting fungi in leguminous and non leguminous plants (Ehteshamul-Haque and Ghaffar, 1993; Siddique *et al*., 1998a, 1998b). *M. phaseolina* produces charcoal rot over 500 species of plants (Siclair, 1982). Similarly *Fusarium* spp. are known to attack a wide range of host plants (Booth, 1971). *R. solani* exists as active mycelium in the soil, attacks over 2000 species of plants (Parmeter, 1970). The ability of Rhizobia to inhibit certain soil borne plant pathogens has increased the importance of Rhizobia in addition to their use in nitrogen fixation (Chakraborty and Purkayastha, 1984; Zaki and Ghaffar, 1987). Experiments were therefore carried out to study the effect of different strains of *Rhizobium* spp. in the control of root infecting fungi and growth of crop plant of mungbean and okra.

MATERILAS AND METHODS

Nine strains of *Rhizobium* spp. were isolated from roots of different plant species, strain 1 (*Bradyrhizobium* sp.) isolated from *Sesbania sesbans*, strain 2 (*Bradyrhizobium* sp.) isolated from *Vigna radiata*, strain 3 (*Rhizobium* sp.) from *Melilotus alba*, strain 4 (*Bradyrhizobium* sp.) from *Vigna radiata*, strain 5 (*B. japonicum*) from *Cicer arietinum*, strain 6 (*Rhizobium* sp.) from *Phaseolus vulgaris*, strain 7 (*B. japonicum*) from *Glycine max*, strain 8 (*Rhizobium* sp.) from *Cymopsis tetragonata* and strain 9 *(Rhizobium* sp.) isolated from *Senna oxidentalis*. Soil used for experiments was obtained from the experimental plot of Department of Botany, University of Karachi. The soil was sandy loam with pH 7.4, and water holding capacity was 39%. The soil had natural infestation of 3 sclerotia of *M. phaseolina* as found by wet sieving technique (Sheikh and Ghaffar, 1975), 5-10% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu / g of soil *Fusarium* spp. as assessed by soil dilution technique (Nash and Snyder, 1962). Mungbean and okra were used as test plants.

Nine strains of *Rhizobium* spp. and *Bradyrhizobium* spp. were used as seed dressing and soil drenching. For seed dressing experiment, seeds of mungbean and okra were treated with 48 h old culture of different Rhizobial strains in 1% gum arabic solution as sticker and non treated seeds served as control. Population of baceria on seeds of mungbean and okra were determined for each strains. For mungbean population was strain 1 (11.3×10^2 cfu/seed), strain 2 (58.5×10^2 cfu/seed), strain 3 (39×10^2 cfu/seed), strain 4 (10.4×10^3 cfu/seed), strain 5 (41.5×10^2 cfu/seed), strain 6 (76×10^2 cfu/seed), strain 7 (80×10^2 cfu/seed), strain 8 (16.4×10^3 cfu/seed), strain 9 (62×10^2 cfu/seed) and for okra population was strain 1 (7.95×10^3 cfu/seed), strain 2 (7.6×10^3 cfu/seed), strain 3 (9×10^3 cfu/seed), strain 4

($8.7x10^3$ cfu/seed), strain 5 ($4.9.5x10^3$ cfu/seed), strain 6 ($1.2x10^4$ cfu/seed), strain 7 ($5.3x10^3$ cfu/seed), strain 8 ($0.9x10^3$ cfu/seed), strain 9 ($1.3x10^2$ cfu/seed). Five seeds were sown in each pot. Each pot containing 250 g soil and each treatment was replicated 4 times.

In soil drenching experiment, seeds of mungbean and okra were sown in plastic pots. Each pot containing 250 g soil. After sowing the seed, 20 ml suspension of actively growing culture of each strain of *Rhizobium* spp was drenched in each pot with the population of strain 1 ($6.5x10^9$ cfu/ml), strain 2 ($16.4x10^9$ cfu/ml), strain 3 ($9.65x10^9$ cfu/ml), strain 4 ($7.6x10^8$ cfu/ml), strain 5 ($8.85x10^9$ cfu/ml), strain 6 ($7.9x10^9$ cfu/ml), strain 7 ($4.7x10^9$ cfu/ml), strain 8 ($4.9x10^9$ cfu/seed), strain 9 ($7.7x10^8$ cfu/ml). Non-treated soil serves as control. Each treatment was replicated 4 times. The pots were kept in randomized block design in screen house. After 30 days of growth shoot length, root length, root weight, shoot weight and number of nodules were counted. Roots were washed with running tap water, surface sterilized with 1% $Ca(OCl)_2$ and five 1 cm long root pieces were transferred on PDA containing penicillin (100,000 units/l) and streptomycin (0.2 g/l). Dishes were incubated for 5 days at 28ºC to observe the infection of roots by soil borne root infecting fungi. Data were analyzed statistically by Gomez and Gomez (1984).

RESULTS AND DISCUSSIONS

Seed treatment:

The shoot and root length of mungbean significantly increased ($P<0.05$) in seed treatment of all strains of *Rhizobium* spp.as compared to control. The shoot weight was highest in strain 5 followed by strain 2, 6, 7, 8 and strain 9 respectively. Whereas root weight was highest in all strains of *Rhizobium* spp. (Table 1). Infection of *Fusarium* spp was significantly reduced in strain1 followed by strain.4, 6, 3, 7 and strain.9 respectively (Table 2). Infection of *M. phaseolina* was completely controlled by strain 2, 3, 6, 7 where as strain 8 significantly reduced the infection of *M. phaseolina* (Table 2). No significant reduction in *R. solani* infection was observed where seeds were treated with different rhizobial strains.

The shoot length of okra was significantly increased ($p<0.05$) in all the strains of Rhizobia except strain 1. The root length was increased in all the strains of Rhizobia compared to controls. Shoot weight was increased in case of strain 3, 5, 6, 7, 8 and strain 9. The root weight was increased in strain 5, 6, 7 and strain 8. No significant reduction of root weight was observed in any strain of Rhizobia. Infection of *Fusarium* spp. was completely controlled by strain 4, 5, 6 and strain 8. No significant reduction in *R. solani* infection was observed in any strain. Infection of *M. phaseolina* was completely controlled from strain 2, 3, 4, 8 and strain 9 (Table 2).

Soil drenching:

A 20 ml suspension of 5 days old culture of different *Rhizobium* spp. strains were used. Mungbean and okra were used as test plants. The shoot length of mungbean was increased in strain 2, 6, 7, 8 and strain 9. In okra plant, shoot length was significantly increased and maximum shoot length and root length was observed in strain 5 (Table 1). In mungbean plants, root weight was greater in strain 6 and strain 8 as compared to control. Highest no. of nodules were observed in strain 4. Root weight of okra was highest in strain 5 , the root weight was significantly increased when soil treated with different strains of Rhizobia (Table 1) Infection of *Fusarium* spp. was significantly controlled in strain 5 followed by strain 4, 8, .9, 2, 3, 6 and strain 7 respectively in mungbean root. Whereas in okra plants infection of *Fusarium* spp. was significantly controlled by strain 5 and strain 9 followed by strain 1, 2, 4, 7 and strain 8. Infection of *M. phaseolina* was significantly controlled in strain 3, 2, 4, 5 and strain 7 in mungbean and okra plants. Infection of *R. solani* was significantly control in strain 1, 3, 4 and strain 7 in mungbean and okra plants (Table 2).

Present results showed that growth of mungbean and okra plants was significantly increased where seeds were coated and soil was drenched with different strains of Rhizobia. In mungbean plant the production of nodules was highest in each treatment because Rhizobia fix atmospheric nitrogen in leguminous plants and enhance the production of growth hormones such as auxin, cytokinin and gibberallin. These hormones directly act on plant and affect growth so its results increase in plant growth like shoot length, root length, shoot weight and root weight. When we introduced the Rhizobia in the soil as seed dressing or soil drench may have the opportunity to be the first colonizer of roots. The ability of Rhizobia to inhibit certain soil borne plant pathogens (Chakraborty and Purkayastha, 1984; Zaki and Ghaffar, 1987) has increased the importance of *Rhozobia* in addition to their use in nitrogen fixation.

In the present study, different strains of Rhizobia significantly control the infection of *Fusarium* sp., *M. phaseolina* and *R. solani* whereas in strain 3, 2, 4, 5 and strain 7 were more effective and significantly control the *M. phaseolina* infection in mungbean and okra roots. Similarly, Chao (1990) reported tremendous variability among the strains of Rhizobia in antagonistic ability against fungi. Some strains of *B. japonicum* are known to produce a wide

Table 1. Effect of different strains of *Rhizobium* species as seed dressing and soil drenching on growth of mungbean and okra plants.

Treatments	Mungbean										Okra							
	Soil drenching					Seed dressing					Soil drenching				Seed dressing			
	Shoot Length	Shoot Weight	Root Length	Root Weight	No. of Nodules	Shoot Length	Shoot Weight	Root Length	Root Weight	No. of Nodules	Shoot Length	Shoot Weight	Root Length	Root Weight	Shoot Length	Shoot Weight	Root Length	Root Weight
Control	22.8	2.1	15.3	0.4	16.7	20.6	13.6	1.1	0.2	6	20	12.2	2.05	1.2	16.25	7.5	1.88	0.27
Bradyrhizobium sp. (Starin 1)	17.7	1.9	14.5	0.4	12.7	24.7	16.2	2.5	0.4	17	21.2	13	3.5	1.5	15.8	9.5	0.67	0.11
Bradyrhizobium sp. (Starin 2)	24.2	2.5	14.7	6.4	21.5	22.8	19.5	1.6	0.27	11	19.2	12.2	2	0.6	23.12	12.1	11.55	0.12
Rhizobium sp. (Starin 3)	17.5	1.6	14.7	0.5	14	22.5	13	1.3	0.31	11	20.3	8.75	2.45	0.32	28.6	14.5	2.17	0.27
Bradyrhizoium sp. (Starin 4)	22.2	2.1	12.8	0.3	26.21	21.5	15.5	1.2	0.3	20	24.5	13.3	2.3	0.37	26.12	12.8	1.8	0.27
B. japonicum (Starin 5)	20	2.1	18.7	0.4	23	18.7	15.6	2.72	0.5	4	26.7	16.5	3.5	0.55	18.6	15.8	2.27	0.35
Rhizobium sp. (Starin 6)	23	3	15.3	0.3	17	23.1	10.6	2.0	0.5	18	24.3	14	2.6	0.57	26.12	16	3.15	0.5
B. japonicum (Starin 7)	23.2	2.9	17	0.4	16.2	22.8	13.6	1.45	0.35	9.25	25.1	13.5	3.9	0.5	24.12	12.2	2.5	0.45
Rhizobium sp. (Starin 8)	24.6	2.5	21.6	0.4	21.7	21.6	17.5	1.42	0.35	7	25.8	14.2	3.8	0.5	26.5	19.75	3.1	0.5
Rhizobium sp. (Starin 9)	25.2	2.5	13	0.2	14.2	24.5	15	1.9	0.22	6.5	22.8	11.75	3.5	0.36	23	10.12	2.07	0.2
LSD 0.05	1.568	1.836	0.327	0.64	4.628	1.032	2.124	0.308	0.057	2.901	1.408	1.108	0.463	0.108	1.942	2.195	1.32	1.208

Table 2. Effect of different starins of Rhizobia as seed dressing and soil drenching in the control of *Fusarium* spp. *Macrophomina phaseolina* and *Rhizoctonia solani* on mungbean and okra.

Treatment	Soil drenching			Seed dressing			Soil drenching			Seed dressing		
	Fusarium spp.	*M. phaseolina*	*R. solani*	*Fusarium* spp.	*M. phaseolina*	*R. solani*	*Fusarium* spp.	*M. phaseolina*	*R. solani*	*Fusarium* spp.	*M. phaseolina*	*R. solani*
Control	100	50	25	100	50	50	75	25	50	100	25	75
Bradyrhizobium sp. (Starin 1)	100	50	0	0	75	75	25	0	0	25	25	75
Bradyrhizobium sp. (Starin 2)	75	25	25	75	0	75	25	50	25	25	0	0
Rhizobium sp. (Starin 3)	75	0	0	50	0	100	50	0	0	75	0	75
Bradyrhizoium sp. (Starin 4)	50	25	0	25	50	50	25	0	100	0	0	75
B. japonicum(Starin 5)	25	25	25	75	50	75	0	0	75	0	75	100
Rhizobium sp. (Starin 6)	75	75	25	25	0	75	50	25	100	0	25	75
B. japonicum(Starin 7)	75	25	0	50	0	75	25	0	50	50	25	100
Rhizobium sp. (Starin 8)	50	50	25	75	25	100	25	25	25	0	0	75
Rhizobium sp. (Starin 9)	50	75	25	50	50	75	0	100	75	100	0	100
LSD 0.05	21.637	23.555	18.151	21.637	21.233	19.531	21.232	15.013	15.013	15.013	16.127	9.805

range of biocide and are reported to secrete rhizobiotoxin, which was found to protect soybean roots from the infection by *M. phaseolina*. Some strains of Rhizobia which fix atmospheric nitrogen in association with leguminous plants and non leguminous plants. Rhizobia which are good rhizosphere organisms for leguminous or non leguminous plants, presumably prevent the contact of pathogenic fungi on roots by covering the hyphal tip of the fungus and parasitizing it (Tu, 1978). There is need to use the effective strains on commercial basis in the control of soil borne root infecting fungi for obtaining high yield of crops.

ACKNOWLEDGEMENTS

We are grateful to Dean, Faculty of Science, University of Karachi for provision of facilities through a University grant.

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(*Accepted for publication March 2005*)

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	Shoot Length	Shoot Weight	Root Length	Root Weight	No. of Nodules	Shoot Length	Shoot Weight	Root Length	Root Weight	No. of Nodules	Shoot Length	Shoot Weight	Root Length	Root Weight	Shoot Length	Shoot Weight	Root Length	Root Weight
Control.	22.8	2.1	15.3	0.4	16.7	20.6	13.6	1.1	0.2	6	20	12.2	2.05	1.2	16.25	7.5	1.88	0.27
Bradyrhizobium sp. (Starin 1)	17.7	1.9	14.5	0.4	12.7	24.7	16.2	2.5	0.4	17	21.2	13	3.5	1.5	15.8	9.5	0.67	0.11
Bradyrhizobium sp. (Starin 2)	24.2	2.5	14.7	6.4	21.5	22.8	19.5	1.6	0.27	11	19.2	12.2	2	0.6	23.12	12.1	11.55	0.12
Rhizobium sp. (Starin 3)	17.5	1.6	14.7	0.5	14	22.5	13	1.3	0.31	11	20.3	8.75	2.45	0.32	28.6	14.5	2.17	0.27
Bradyrhizoium sp. (Starin 4)	22.2	2.1	12.8	0.3	26.21	21.5	15.5	1.2	0.3	20	24.5	13.3	2.3	0.37	26.12	12.8	1.8	0.27
B. japonicum (Starin 5)	20	2.1	18.7	0.4	23	18.7	15.6	2.72	0.5	4	26.7	16.5	3.5	0.55	18.6	15.8	2.27	0.35
Rhizobium sp. (Starin 6)	23	3	15.3	0.3	17	23.1	10.6	2.0	0.5	18	24.3	14	2.6	0.57	26.12	16	3.15	0.5
B. japonicum (Starin 7)	23.2	2.9	17	0.4	16.2	22.8	13.6	1.45	0.35	9.25	25.1	13.5	3.9	0.5	24.12	12.2	2.5	0.45
Rhizobium sp. (Starin 8)	24.6	2.5	21.6	0.4	21.7	21.6	17.5	1.42	0.35	7	25.8	14.2	3.8	0.5	26.5	19.75	3.1	0.5
Rhizobium sp. (Starin 9)	25.2	2.5	13	0.2	14.2	24.5	15	1.9	0.22	6.5	22.8	11.75	3.5	0.36	23	10.12	2.07	0.2
LSD 0.05	1.568	1.836	0.327	0.64	4.628	1.032	2.124	0.308	0.057	2.901	1.408	1.108	0.463	0.108	1.942	2.195	1.32	1.208

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	Soil drenching			**Seed dressing**			**Soil drenching**			**Seed dressing**		
Treatment	***Fusarium* spp.**	***M. phaseolina***	***R. solani***	***Fusarium* spp.**	***M. phaseolina***	***R. solani***	***Fusarium* spp.**	***M. phaseolina***	***R. solani***	***Fusarium* spp.**	***M. phaseolina***	***R. solani***
Control	100	50	25	100	50	50	75	25	50	100	25	75
Bradyrhizobium sp. (Starin 1)	100	50	0	0	75	75	25	0	0	25	25	75
Bradyrhizobium sp. (Starin 2)	75	25	25	75	0	75	25	50	25	25	0	0
Rhizobium sp. (Starin 3)	75	0	0	50	0	100	50	0	0	75	0	75
Bradyrhizoium sp. (Starin 4)	50	25	0	25	50	50	25	0	100	0	0	75
B. japonicum(Starin 5)	25	25	25	75	50	75	0	0	75	0	75	100
Rhizobium sp. (Starin 6)	75	75	25	25	0	75	50	25	100	0	25	75
B. japonicum(Starin 7)	75	25	0	50	0	75	25	0	50	50	25	100
Rhizobium sp. (Starin 8)	50	50	25	75	25	100	25	25	25	0	0	75
Rhizobium sp. (Starin 9)	50	75	25	50	50	75	0	100	75	100	0	100
LSD 0.05	21.637	23.555	18.151	21..637	21..233	19.531	21.232	15.013	15.013	15.013	16.127	9.805