Pak.J.Bot., 30(2): 279-286, 1998.

EFFECT OF RHIZOBIA AND FUNGAL ANTAGONISTS IN THE CONTROL OF ROOT INFECTING FUNGI ON SUNFLOWER AND CHICKPEA

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Abstract

Use of *Bradyrhizobium* spp., (chickpea isolate and mungbean isolate) and *Rhizobium trifolii* (berseem isolate) with or without fungal antagonists viz., *Paecilomyces lilacinus, Memnoniella echinata, Trichoderma harzianum* and *Stachybotrys atra* significantly ($p<0.05$) controlled *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* infection on sunflower and chickpea. Use of rhizobia with fungal antagonists showed better results in the control of root infecting fungi with enhancement in plant growth.

Introduction

The ability of rhizobia to inhibit certain soilborne plant pathogens (Chakraborty & Purkayastha, 1984 ; Ehteshamul-Haque & Ghaffar, 1992) has increased the importance of rhizobia besides their use in nitrogen fixation. Since rhizobia have shown promising results in the control of root infecting fungi viz., *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Kühn and *Fusarium* spp., on both leguminous and non leguminous plants (Ehteshamul-Haque & Ghaffar, 1993), an experiment was therefore carried out to study the effect of 2 isolates of *Bradyrhizobium* spp., and one isolate of *Rhizobium trifolii* with or without fungal antagonists viz., *Trichoderma harzianum* Rifai, *Paecilomyces lilacinus* (Thom) Samson, *Memnoniella echinata* (Rivolte) Galloway and *Stachybotrys atra* Corda in the control of root infecting fungi on chickpea (*Cicer arietinum* L.), a leguminous plant and sunflower (*Helianthus annuus* L.) used as a non-leguminous plant.

Materials and Methods

Soil obtained from the experimental field of the Department of Botany, University of Karachi was screened through 2mm mesh sieve to discard non soil particles. The soil had a natural infestation of 6-9 sclerotia of *M. phaseolina* g^{-1} of soil as found by wet sieving and dilution technique (Sheikh & Ghaffar, 1975), 6.5% colonization of *R. solani* on sorghum seeds used as bait (Wilhelm, 1955) and 2500 cfu g^{-1} of soil of a mixed population of *Fusarium* spp., as assessed by soil dilution technique (Nash & Snyder, 1962). Five day old cultures of *Bradyrhizobium* spp., chickpea isolate (Karachi University Culture Collection - 811), *Bradyrhizobium* sp., mungbean isolate (KUCC - 823) and *Rhizobium trifolii* berseem isolate (KUCC-842) multiplied on Yeast Extract Mannitol Agar and *P. lilacinus* (KUCC-244), *T. harzianum* (KUCC-801), *S. atra*

(KUCC-812) and *M. echinata* (KUCC-848) maintained on Potato Dextrose Agar medium were used. Seeds of chickpea and sunflower treated with cell suspension of rhizobia and/or conidial suspension of antagonistic fungi using 1% gum arabic as sticker were sown in 8 cm diam., plastic pots each containing 250g soil. There were three replicates of each treatment and pots were randomized on a screen house bench where soil was kept at 50% WHC (Keen & Raczkowski, 1921). Plants were uprooted after 6 weeks growth and 5 one cm long root pieces from each plant after surface disinfection with 1% $Ca(OCl)_2$ for 3 minutes were transferred onto PDA plates containing penicillin (100,000 units/L) and streptomycin (0.2g/L). After incubation at 28°C for 5 days, incidence of root infecting fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., were recorded. Data were analysed and subjected to Factorial ANOVA (FANOVA) followed by Least Significant Difference (LSD) according to Gomez & Gomez (1984).

Results

Effect on chickpea: Use of rhizobia with or without fungal antagonists significantly ($p < 0.05$) reduced *M. phaseolina, R. solani* and *F. solani* infection. A complete control of *R. solani* infection was observed in treatment where chickpea isolate of *Bradyrhizobium* sp., and *P. lilacinus* were used alone or where chickpea isolate was used with either *M. echinata* or *T. harzianum*, mungbean isolate of *Bradyrhizobium* sp., used with *M. echinata* and berseem isolate of *Rhizobium trifolii* used with either *P. lilacinus, T. harzianum* or *S. atra*. Berseem isolate used alone, mungbean isolate used with *P. lilacinus*, chickpea isolate used with *M. echinata*, mungbean isolate used with *M. echinata* and berseem isolate used with *T. harzianum* reduced more than 50% infection caused by *M. phaseolina*. *T. harzianum* used alone, berseem isolate of *Rhizobium trifolii* used with *S. atra*, chickpea isolate used with *P. lilacinus* and berseem isolate used with *M. echinata* reduced *F. solani* infection by more than 50% (Table 1).

Use of rhizobia with or without fungal antagonists showed an increase in plant height especially in treatment where chickpea isolate was used with *T. harzianum* followed by mungbean isolate used with *P. lilacinus*. Highest fresh weight of shoot was found in the treatment where mungbean isolate was used alone followed by chickpea isolate used alone. Greater number of nodules per plant were produced in treatment where chickpea isolate was used alone or mixed with *M. echinata* or *P. lilacinus* (Table 2).

Effect on Sunflower: Complete control of *R. solani* infection was observed where *M. echinata* was used alone, chickpea isolate used with *T. harzianum* or *S. atra*, mungbean isolate used with *M. echinata* or *S. atra* and berseem isolate used with *S. atra*. Similarly mungbean isolate, berseem isolate and *M. echinata* used alone, chickpea isolate used with *P. lilacinus*, chickpea isolate used with *S. atra*, mungbean isolate used with *M. echinata* or berseem isolate used with *S. atra* reduced *R. solani* infection by more then 50%. *S. atra* alone or in combination with chickpea isolate reduced *M. phaseolina* infection by more than 50%. A complete control of *F. solani* infection was observed in the treatment where chickpea isolate was used with *P. lilacinus* and mungbean isolate was used with *M. echinata*. Similarly berseem isolate used with *S. atra* showed more than 50% control in *F. solani* infection (Table 1).

Table 1. Use of rhizobia with other microbial antagonists in the control of root infecting fungi on sunflower and chickpea.

Treatments	INFECTION %					
	M. phaseolina		*F. solani*		*R. solani*	
	sunflower	chickpea	sunflower	chickpea	sunflower	chickpea
Control	41.66	66.66	41.66	100.00	58.33	25.00
Bradyrhizobium sp. KUCC (811) (chickpea isolate)	55.33	33.33	27.66	66.66	63.66	0.00
Bradyrhizobium sp. KUCC (823) (mungbean isolate)	63.66	33.33	25.00	75.00	25.00	16.66
Rhizobium trifolii KUCC (843) (*Trifolium alexandrinum* isolate)	27.66	16.66	47.00	58.33	11.00	8.33
Paecilomyces lilacinus	52.66	41.66	36.00	58.33	0.00	0.00
Memnoniella echinata	44.33	41.66	60.66	66.66	16.66	8.33
Trichoderma harzianum	25.00	41.66	55.33	41.66	30.33	8.33
Stachybotrys atra	16.66	58.33	0.00	66.66	33.33	8.33
Bradyrhizobium sp. KUCC 811 + *P. lilacinus*	52.66	58.33	66.66	50.00	19.33	8.33
Bradyrhizobium sp. KUCC 811 + *M. echinata*	50.00	27.77	41.66	72.22	0.00	0.00
Bradyrhizobium sp. KUCC 811 + *T. harzianum*	27.66	50.00	72.00	83.33	0.00	0.00
Bradyrhizobium sp. KUCC 811 + *S. atra*	16.66	50.00	33.33	66.66	25.00	25.00
Bradyrhizobium sp. KUCC 823 + *P. lilacinus*	66.66	8.33	0.00	58.33	0.00	16.66
Bradyrhizobium sp. KUCC 823 + *M. echinata*	38.66	25.00	50.00	58.33	16.66	0.00
Bradyrhizobium sp. KUCC 823 + *T. harzianum*	25.00	41.66	58.33	58.33	0.00	8.33
Bradyrhizobium sp. KUCC 823 + *S. atra*	33.33	58.33	27.66	83.33	0.00	16.66
Rhizobium trifolii KUCC 843 + *P. lilacinus*	66.66	75.00	33.33	66.66	50.00	0.00
Rhizobium trifolii KUCC 843 + *M. echinata*	41.66	50.00	25.00	50.00	33.33	50.00
Rhizobium trifolii KUCC 843 + *T. harzianum*	33.33	33.33	41.66	66.66	0.00	0.00
Rhizobium trifolii KUCC 843 + *S. atra*	38.66	41.66	19.33	16.66	8.33	0.00
L.S.D. < 0.05	Treatment	35.79	Pathogen = 13.86		Host =	11.31

Table 2. Use of rhizobia with microbial antagonists on growth of chickpea (*Cicer arietinum* L.). [Values in parenthesis represent a percentage (+ve) increase or (-ve) decrease over control]

Treatments	Population/seed		Plant height (cm)	Shoot weight (gm)	No. of nodules per plant
	Fungus	Bacterium			
Control	-	-	17.75	2.55	0.00
Bradyrhizobium sp. (811)	-	$4.5x10^6$	23.83 (+34.25)	3.00 (+17.64)	12.30
Bradyrhizobium sp. (823)	-	$6.3x10^6$	21.00 (+18.30)	3.29 (+29.01)	0.16
Rhizobium trifolii (843)	-	$3.2x10^6$	24.41 (+37.52)	2.84 (+11.37)	0.25
Paecilomyces lilacinus	$2.6x10^6$	-	23.41 (+31.88)	2.76 (+8.23)	0.00
Memnoniella echinata	$1.4x10^6$	-	22.66 (+27.66)	2.76 (+8.23)	0.00
Trichoderma harzianum	$3.0x10^6$	-	23.33 (+31.43)	2.95 (+15.68)	0.00
Stachybotrys atra	$4.4x10^6$	-	22.50 (+26.76)	2.61 (+2.35)	0.00
Bradyrhizobium sp. 811 + *P. lilacinus*	$2.1x10^6$	$3.7x10^6$	24.16 (+36.11)	2.64 (+3.52)	13.25
Bradyrhizobium sp. 811 + *M. echinata*	$4.3x10^6$	$2.5x10^6$	22.22 (+25.18)	2.63 (+3.13)	15.30
Bradyrhizobium sp. 811 + *T. harzianum*	$3.8x10^6$	$4.9x10^6$	25.47 (+43.49)	1.97 (-22.74)	0.75
Bradyrhizobium sp. 811 + *S. atra*	$1.9x10^6$	$1.3x10^6$	24.91 (+40.33)	2.49 (-2.35)	4.16
Bradyrhizobium sp. 823 + *P. lilacinus*	$3.4x10^6$	$3.6x10^6$	25.33 (+42.70)	2.97 (+9.41)	2.41
Bradyrhizobium sp. 823 + *M. echinata*	$1.9x10^6$	$2.3x10^6$	24.66 (+38.92)	2.50 (-1.96)	4.83
Bradyrhizobium sp. 823 + *T. harzianum*	$2.6x10^6$	$3.8x10^6$	22.75 (+28.16)	2.62 (+2.74)	5.00
Bradyrhizobium sp. 823 + *S. atra*	$1.2x10^6$	$4.3x10^6$	23.83 (+34.25)	2.52 (+1.17)	0.83
Rhizobium trifolii 843 + *P. lilacinus*	$3.8x10^6$	$1.9x10^6$	21.00 (+18.30)	2.57 (+0.78)	0.00
Rhizobium trifolii 843 + *M. echinata*	$9.5x10^6$	$2.6x10^6$	22.33 (+25.80)	2.93 (+14.90)	0.00
Rhizobium trifolii 843 + *T. harzianum*	$2.5x10^6$	$3.6x10^6$	23.08 (+30.02)	2.78 (+9.01)	0.00
Rhizobium trifolii 843 + *S. atra*	$2.0x10^6$	$2.9x10^6$	20.75 (+16.90)	2.33 (-8.62)	0.00
L.S.D. $p<0.05$	-	-	3.22	0.57	4.44

Maximum increase in plant height was observed where *P. lilacinus* and *S. atra* were used alone. Highest increase in fresh shoot weight was recorded in treatment where mungbean isolate was used with *P. lilacinus* followed by mungbean isolate used alone (Table 3).

Discussion

In the present study, use of rhizobia on both leguminous (chickpea) and non-leguminous (sunflower) plants enhanced plant growth. Beside fixation of atmospheric nitrogen, the growth promotive effect may be due to the production of plant growth regulators such as auxins, cytokinins and gibberellin like substances (Triplett *et al.*, 1981; Evensen & Blavins, 1981; Sheng, 1993) and also the production of toxic metabolites (Chakraborty & Purkayastha, 1984) which have inhibitory effect on soilborne plant pathogens.

Rhizobia showed significant control of root rot pathogens on both leguminous plant like chickpea and non-leguminous plant like sunflower used in the present study. Use of rhizobia for the control of root infecting fungi on both leguminous and non-leguminous plants have been reported (Ehteshamul-Haque & Ghaffar, 1993). The rhizobia present in the rhizosphere of plants presumably prevent the contact of pathogenic fungi on roots by covering the hyphal tip of the fungus and by parasitizing it (Tu, 1978). Besides parasitizing the hyphae, rhizobia also produce antibiotics (Malajczuk, 1983) which resulted in lysis of the fungal hyphae (Malajczuk *et al.*, 1984).

There are reports where *P. lilacinus, T. harzianum* (Ehteshamul-Haque *et al.*, 1992), *S. atra* (Butt & Ghaffar, 1972) and *M. ehinata* (Dawar *et al.*, 1993) have shown promising results in the control of root infecting fungi. In the present study also *P. lilacinus, T. harzianum, M. echinata* and *S. atra* have shown promising results in the control of root infecting fungi on chickpea and sunflower. *S. atra* (Butt & Ghaffar, 1971), *T. viride* (Gangawane & Salve, 1987), *Paecilomyces* sp., (Subba Rao, 1977) have also been reported to stimulate the growth of rhizobia.

The biocontrol agents showed variable results in the control of root infecting fungi on chickpea and sunflower. Differences in the efficacy of rhizobia on different hosts may also be due to the quality of root exudates which influence the colonization by bacteria (Parke, 1991). Use of rhizobia with other microbial antagonists showed promising results in the control of root infecting fungi. Such similar observation were made by (Ehteshamul-Haque & Ghaffar, 1991) where use of rhizobia with other microbial antagonists showed better results than their separate use. There is therefore need to select potential biocontrol agents that could be effective against more than one pathogens on more than one host plant. Integration of two or more biocontrol agents could also provide better results than their separate use. There is also need to establish a corelation between the population of pathogen(s) in soil and inoculum dose of biocontrol agent(s) required for effective suppression of the disease as suggested by Dawar *et al.*, (1993).

Table 3. Use of rhizobia with microbial antagonists on growth of sunflower (*Halianthus annus* L.). [Values in parenthesis represent a percentage (+ve) increase or (-ve) decrease over control]

Treatments	Population/seed		Plant height (cm)	Shoot weight (gm)
	Fungus	Bacterium		
Control	-	-	19.30	2.15
Bradyrhizobium sp. (811)	-	$1.3x10^6$	22.22 (+15.12)	2.69 (+25.11)
Bradyrhizobium sp. (823)	-	$6.9x10^6$	23.69 (+22.74)	3.00 (+39.53)
Rhizobium trifolii (843)	-	$1.7x10^6$	20.38 (+5.59)	2.30 (+6.97)
Paecilomyces lilacinus	$2.8x10^6$	-	24.36 (+26.21)	2.15 (+0.00)
Memnoniella echinata	$1.9x10^6$	-	22.83 (+18.29)	2.56 (+19.06)
Trichoderma harzianum	$2.4x10^6$	-	21.69 (+12.38)	2.10 (-2.32)
Stachybotrys atra	$5.5x10^6$	-	23.91 (+23.88)	2.46 (+14.41)
Bradyrhizobium sp. 811 + *P. lilacinus*	$2.6x10^6$	$3.7x10^6$	20.66 (+7.04)	2.03 (-5.58)
Bradyrhizobium sp. 811 + *M. echinata*	$1.8x10^6$	$2.4x10^6$	21.41 (+10.93)	2.27 (+5.58)
Bradyrhizobium sp. 811 + *T. harzianum*	$3.6x10^6$	$4.8x10^6$	23.05 (+19.43)	2.27 (-5.58)
Bradyrhizobium sp. 811 + *S. atra*	$1.8x10^6$	$2.8x10^6$	23.75 (+23.05)	2.27 (-5.58)
Bradyrhizobium sp. 823 + *P. lilacinus*	$2.2x10^6$	$3.8x10^6$	23.16 (+20.00)	3.07 (+42.79)
Bradyrhizobium sp. 823 + *M. echinata*	$4.6x10^6$	$3.7x10^6$	21.91 (+13.52)	2.19 (+1.86)
Bradyrhizobium sp. 823 + *T. harzianum*	$2.5x10^6$	$3.2x10^6$	22.00 (+13.98)	2.25 (+4.65)
Bradyrhizobium sp. 823 + *S. atra*	$1.4x10^6$	$2.8x10^6$	20.05 (+3.88)	2.03 (-5.58)
Rhizobium trifolii 843 + *P. lilacinus*	$2.5x10^6$	$2.2x10^6$	21.50 (+11.39)	1.95 (-9.30)
Rhizobium trifolii 843 + *M. echinata*	$5.2x10^6$	$3.3x10^6$	20.66 (+7.04)	1.94 (-9.76)
Rhizobium trifolii 843 + *T. harzianum*	$1.7x10^6$	$2.4x10^6$	21.22 (+5.18)	2.41 (+12.09)
L.S.D. $p<0.05$	-	-	3.67	0.67

Acknowledgement

The work has been carried out under the research grant of Pakistan Science Foundation which is sincerely acknowledged.

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(Received for publication 5 August 1998)