Biological Sciences

*Pak J Sci Ind Res 2001 **44**(3) 156-158*

Effect of Benlate on the Efficacy of *Bradyrhizobium* sp., in the Control of Root Rot Diseases of Crop Plants

I A Siddiqui *, S Ehteshamul-Haque, M J Zaki and A Ghaffar

Department of Botany, University of Karachi, Karachi-75270, Pakistan

(Received 6 August 1999; accepted 5 August 2000)

Effects of Benlate fungicide on the efficacy of *Bradyrhizobium* sp., in the control of root infecting fungi viz., *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp. were evaluated under field conditions on mungbean, soybean, cotton and sunflower. *Bradyrhizobium* sp., used alone or mixed with Benlate showed good biocontrol and growth promoting effects. Benlate was found as the most effective treatment in the suppression of *F. solani* infection.

Key words: Benlate, *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani.*

Introduction

Seed treatment with fungicides affects the rhizobial survival on seeds and consequently nodulation and nitrogen fixation (Keeskes and Vincent 1973; Graham *et al* 1980). There are diverse opinions as to whether seed dressing materials adversely affect the *Rhizobium* spp and hence the nodulation. In pea, Ceresan adversely affects nodulation (Milthorape 1945) whereas Captan and Thiram have no adverse effect under field conditions (Nene *et al* 1969). In recent past, use of rhizobia as microbial antagonists enhanced the importance of rhizobia as seed dressing (Zaki and Ghaffar 1987; Siddiqui *et al* 1998). Little information is available on the use of rhizobia with fungicides in the control of root rot diseases caused by *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp. The present experiment aims to examine the effect of Benlate fungicide on the efficacy of *Bradyrhizobium* sp in the control of root infecting fungi on legumes like mungbean *Vigna radiata* (L.) Wilczek, and soybean *Glycine max* L. and other crops like cotton *Gossypium arboreum* L. and sunflower *Helianthus annuus* L.

Materials and Methods

The experiment was carried out at the Department of Botany, University of Karachi.. The soil had a natural infestation of *M. phaseolina* (3-11 sclerotia g^{-1} of soil) as estimated by wet sieving and dilution technique (Sheikh and Ghaffar 1975), 5-10% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm 1955) and 3500 cfu g^{-1} of soil of mixed population of *Fusarium* spp as assessed by soil dilution technique (Nash and Snyder 1962). *Bradyrhizobium* sp (KUCC-823 originally isolated from nodules of mungbean) multiplied on Yeast Extract Mannitol Agar medium was used in this study.

* Author of correspondence

Surface sterilized (1% $Ca(OCl)_2$) seeds of mungbean, soybean, cotton and sunflower were i) dipped in a suspension of *Bradyrhizobium* sp containing 1.3×10^9 cfu ml^{-1} in 1% gum arabic used as sticker, ii) treated with Benlate (2g kg^{-1}) in small container and iii) treated with rhizobia after treatment with fungicide. Untreated seeds were used as control. Thirty seeds were sown in 5 feet furrows. There were three replicates of each treatment and plots (2x1 meter) were randomized. Plots were watered as needed. Experiment was terminated after 30 days of seedling emergence and observations on plant height and fresh weight of shoot were recorded. Roots were washed in running tap water and the surface was disinfested with 1% Ca $(OCl)_2$. Root pieces (1-cm) from tap root were transferred on PDA petri dishes containing penicillin (100,000 units l^{-1}) and streptomycin (0.2g l^{-1}). Dishes were incubated for 5 days at 28°C to confirm infection and colonization by root-infecting fungi. Infection percentage was calculated as follows:

$$\text{Infection \%} = \frac{\text{Number of plants infected by a fungus}}{\text{Total number of plants}} \times 100$$

Data were subjected to Factorial ANOVA (FANOVA) followed by Least Significance Difference (LSD) according to Gomez and Gomez (1984).

Results and Discussion

More than 50% suppression in *M. phaseolina* infection was recorded in the treatment where *Bradyrhizobium* sp was used separately in mungbean and cotton (Table 1). Similarly, *Bradyrhizobium* sp combined with Benlate resulted in more than 75 and 50% suppression in *M. phaseolina* infection in soybean and sunflower, respectively. Benlate used alone

Table 1

Effect of Benlate on the efficacy of *Bradyrhizobium* sp. in the control of root infecting fungi in mungbean (Mb), soybean (Sb), cotton (Co) and sunflower (Sf)

Treatments	Infection%											
	Macrophomina phaseolina				*Fusarium solani*				*Rhizoctonia solani*			
	Mb	Sb	Co	Sf	Mb	Sb	Co	Sf	Mb	Sb	Co	Sf
Control	50	69	67	50	75	55	42	58	33	50	17	8
Bradyrhizobium sp.	25	36	33	42	67	72	33	42	17	41	25	25
Benlate	58	50	50	58	8	8	0	42	25	50	8	33
Bradyrhizobium sp. + Benlate	41	22	50	17	8	58	25	25	17	17	0	33
LSD < 0.05	Pathogen = 9.62				Treatment=11.11				Host=11.11			

Table 2

Effect of Benlate and *Bradyrhizobium* sp., on growth of mungbean (Mb), soybean (Sb), cotton (Co) and sunflower (Sf)

Treatments	Plant height (cm)				Shoot weight (g)			
	Mb	Sb	Co	Sf	Mb	Sb	Co	Sf
Control	13.2	24.3	12.2	55.0	4.1	5.7	2.7	33.4
Bradyrhizobium sp.	17.5	34.2	13.8	75.5	5.4	8.0	3.5	64.5
Benlate	15.1	36.2	15.1	59.7	5.5	4.8	3.0	36.0
Bradyrhizobium sp. + Benlate	16.9	34.4	16.5	86.5	6.4	5.6	4.2	85.4
LSD < 0.05	1.9	19.2	3.5	13.1	2.8	3.6	0.7	40.8

resulted in complete control of *F. solani* infection in cotton and 88% suppression in soybean. Similary *Bradyrhizobium* sp mixed with Benlate showed more than 80 and 50% suppression in *F.solani* infection on mungbean and sunflower, respectively. Benlate alone was found to be the most effective treatment in the suppression of *F. solani* infection. A complete suppression in *R. solani* infection was found in the treatment where *Bradyrhizobium* sp was used in combination with Benlate in cotton. In mungbean more than 50% and in soybean more than 75% suppression in *R. solani* infection was recorded where *Bradyrhizobium* sp was used with Benlate. *Bradyrhizobium* sp and Benlate either used separately or in combination resulted in an increase in *R. solani* infection. *Bradyrhizobium* sp used alone exhibited maximum plant height in mungbean and sunflower (Table 2). Maximum plant height in soybean and cotton were recorded with Benlate and combined use of *Rhizobium* and fungicide, respectively. *Bradyrhizobium* sp mixed with fungicide showed maximum fresh weight of shoot in mungbean, cotton and sunflower whereas in soybean alone use of *Bradyrhizobium* gave maximum fresh weight of shoot.

There are diverse reports on the effect of fungicides on rhizobia. Vitavax had no significant effect but Benlate greatly reduced rhizobial survival on bean seeds (Ramos and Ribeiro 1993). Simultaneous inoculation of seeds with rhizobia and fungicides affected the rhizobial survival on bean seeds (Lopes and Portugal 1986). In the present study, use of *Bradyrhizobium* sp with Benlate showed better biocontrol and growth promoting effects. Heneberg *et al* (1983) reported Benlate as the least and Radotiram as the most lethal to rhizobia. Similarly *Bradyrhizobium* sp with Benlate or Bavistin and *Rhizobium meliloti* isolates with Captan or Topsin-M showed better nodulation and control of *Fusarium solani* on chickpea roots than their separate use (Siddiqui *et al* 1998). There are also reports where seed treatment with fungicides can cause an increase (Lopes and Portugal 1986), a reduction (Jones and Gidden 1984) or no effect (Kucey and Bonetti 1988) on nodulation. Seed treatment with Bavistin and rhizobium showed highest number of nodules per plant as well as yield of chickpea (Gupta *et al* 1985). Presumably fungicides reduce competitive rhizospheric microorganisms and may provide easy chance for rhizobial activity. The use of rhizobial strains naturally tolerent to fungicides can increase nodulation and maintain the survival of inoculated strains on seeds (Lennox and Alexander 1981) which may also provide better protection of roots from invasion by root-infecting fungi thus resulting in healthy plant growth (Siddiqui *et al* 1998).

Acknowledgement

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

References

Gomez K A, Gomez A A 1984 *Statistical Procedures for Agriculture Research*. 2nd ed Wiley, New York pp 680.

Graham P H, Ocampo G, Ruiz L D, Dyque A 1980 Survival of *Rhizobium phaseoli* in contact with chemical seed protectants. *Agron J* **72** 625-627.

Gupta R P, Katiar R P, Singh D P 1985 Effect of seed treatment with Bavistin and *Rhizobium* on wilt incidence, nodulation and yield of chickpea. *Indian Phytopath* **38** 569 (Abstr.).

Heneberg R, Cvjetkovic B, Sertic D 1983 Influence of the fungicides on formation of nodules on soybean roots. *Polvo priverdea Znanstvenor smotra* **61** 309-317.

Jones R, Giddens J 1984 Interaction of effective N_2 fixing rhizobia strains into the soybean plants by use of fungicide resistance. *Agron J* **76** 599-603.

Keeskes M, Vincent J M 1973 Compatibility of fungicide treatment and *Rhizobium* inoculation of vetch seed. *Acta Agron Acad Sci Hung* **22** 1-2.

Kucey R M N, Bonetti R 1988 Effect of vesicular arbiscular mycorhizal fungi and captan on growth and N_2 fixation by *Rhizobium* inoculated field beans. *Can Soil Sci* **68** 143-149.

Lennox L B, Alexander M 1981 Fungicide enhancement of nitrogen fixation and colonization of *Phaseolus vulgaris* by *Rhizobium phaseoli. Appl Environ Microbiol* **41** 404-411.

Lopes E B, Portugal E B 1986 Compatibilidade entre o trataentpo de sementes d amendoim confungicidas, sobrevivencia de Rhizobium e nodulacao. *Bragantia* **45** 293-302.

Milthorape F L 1945 The compatibility of protectant seed dusts with root nodule bacteria. *J Aust Inst Agric Sci* **11** 89-92.

Nash S M, Snyder W C 1962 Quantitative estimations by plate count of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology* **52** 567-572.

Nene Y L, Agarwal K, Srivastava S S L 1969 Influence of fungicidal seed treatment on the emergence and nodulation of soybean. *Pesticides* **3** 26-27.

Ramos M L G, Ribeiro W Q 1993 Effect of fungicides on survival of rhizobium on seed and nodulation of bean (*Phaseolus vulgaris L.*). *Plant and Soil* **152** 145-150.

Sheikh A H, Ghaffar A 1975 Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak J Bot* **7** 13-17.

Siddiqui I A, Ehteshamul-Haque S, Ghaffar A 1998 Effect of fungicides on the efficacy of *Rhizobium meliloti* and *Bradyrhizobium* sp in the control of root infecting fungi on chickpea. *Pak J Bot* **30** 69-74.

Wilhelm S 1955 Longivity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathology* **71** 929-933.

Zaki M J, Ghaffar A 1987 Effect of *Rhizobium* spp on *Macrophomina phaseolina. Pak J Sci Ind Res* **30** 305-306.