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BIOLOGICAL CONTROL OF ROOT ROT DISEASE OF MUSTARD

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Abstract

Infection of *Macrophomina phaseolina* on 30 day old mustard (*Brassica juncea*) seedling was inhibited by *Bradyrhizobium japonicum, Trichoderma harzianum, Gliocladium virens* and *Paecilomyces lilacinus* when used as seed dressing or as soil drench, while on 60 day old plants *B. japonicum* combined with *T. harzianum* and *P. lilacinus* were effective. Infection of *Rhizoctonia solani* on 30 day old seedling was completely inhibited by *B. japonicum, T. harzianum, T. viride, G. virens, P. lilacinus* and *Stachybotrysatra* and on 60 day old plant by *B. japonicum, T. viride, G. virens, P. lilacinus* and *S. atra* when used as seed dressing and or as soil drench. *Fusarium* infection was completely controlled by *B. japonicum* and *T. viride* and by combined use of *B. japonicum* with *T. harzianum, T. viride. G. virens, P. lilacinus* and *S. atra* in 30 day old seedlings and by *S. atra* in 60 day old plants when used as seed dressing and or as soil drench.

Introduction

Mustard (*Brassica juncea* (L.) Czern & Goss), an important oil seed crop is cultivated over 0.3 million hectares in Pakistan (Anon., 1990). The plant is attacked by soilborne root infecting fungi viz., *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Kühn and *Fusarium* spp., resulting in the development of root rot disease (Kolte, 1985). Since most of the plants have little or no resistance to certain plant pathogen, use of microorganisms in the biological control of plant pathogen is an alternate method for disease control (Lumsden & Lewis, 1989). Experiments were, therefore, carried out to study the effect of some selected biocontrol agents on control of root-rot disease of mustard caused by *M. phaseolina, R. solani* and *Fusarium* spp., in pots.

Materials and Methods

Bradyrhizobium japonicum (TAL 102) from Nitrogen Fixation in Tropical legumes, Hawaii. (NifTAL) and *Trichoderma harzianum* (KUMH 115), *T. viride* (KUMH 656); *Gliocladium virens* (KUMH 464), *Paecilomyces lilacinus* (KUMH 244) and *Stachybotrys atra* (KUMH 668) obtained from Karachi University culture collection were used. *B. japonicum* were used alone or mixed with *T. harzianum, T. viride, G. virens, P. lilacinus* and *S. atra*. Five day old culture of microbial antagonists grown on Potato Dextrose Agar, were used for seed dressing or soil drench. For seed dressing 1% gum arabic was used as sticker. For soil drench 25 ml suspension of each biocontrol agents were drenched in pots containing 250 gm of soil. Population

Table 1. Population of biocontrol agents used as seed dressing or soil drench.

No.	Treatment	Fungus	Rhizobia
	SEED TREATMENT	**Conidia per seed**	**Cells per seed**
A.	Control	---	---
B.	*Bradyrhizobium japonicum*	--	1.6×10^7
C.	*Trichoderma harzianum*	5×10^6	---
D.	*T. viride*	7×10^6	---
E.	*Gliocladium virens*	3.1×10^6	---
F.	*Paecilomyces lilacinus*	1.2×10^7	---
G.	*Stachybotrys atra*	3.2×10^6	---
H.	*B. japonicum* + *T. harzianum*	1.3×10^6	1.4×10^6
I.	*B. japonicum* + *T. viride*	0.9×10^7	4×10^6
J.	*B. japonicum* + *G. virens*	3.6×10^6	2.5×10^6
K.	*B. japonicum* + *P. lilacinus*	3.3×10^8	1×10^8
L.	*B. japonicum* + *S. atra*	1.5×10^5	2.2×10^6
	SOIL DRENCH	**Conidia per ml**	**Cells per ml**
A.	Control	---	---
B.	*B. japonicum*	--	2.3×10^9
C.	*T. harzianum*	8×10^7	---
D.	*T. viride*	6.1×10^7	---
E.	*G. virens*	3.6×10^7	---
F.	*P. lilacinus*	1.8×10^7	---
G.	*S. atra*	1.6×10^7	---
H.	*B. japonicum* + *T. harzianum*	4×10^7	1.5×10^9
I.	*B. japonicum* + *T. viride*	3×10^7	1.5×10^9
J.	*B. japonicum* + *G. virens*	1.8×10^7	1.5×10^9
K.	*B. japonicum* + *P. lilacinus*	1.9×10^7	1.5×10^9
L.	*B. japonicum* + *S. atra*	0.8×10^7	1.5×10^9

of biocontrol agents used in seed dressing and soil drench are shown in Table 1. Soil used in this experiment had a natural infestation of 3-5 sclerotia of *M. phaseolina* g^{-1} of soil as found by using wet sieving and dilution technique (Sheikh & Ghaffar, 1975), 5% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu g^{-1} of soil of *Fusarium* spp., as assessed by soil dilution plate method (Waksman & Fred, 1922). Eight seeds were sown in each pot and each treatment was replicated three times and the pots were randomized on a greenhouse bench.

Plants were uprooted after 30 and 60 days of growth. Ten one cm long root pieces from each plant were cut randomly, surface sterilized with 1% $Ca(OCl)_2$ for 3 minutes and transferred onto PDA plates containing Penicillin (100000 units/litre) and Streptomycin (0.2 gm/litre). Plates were incubated for 5 days at 28°C and the incidence of root infecting fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., recorded.

Results

T. harzianum and *P. lilacinus* when used as seed dressing and *B. japonicum, T. harzianum, P. lilacinus* and *G. virens* when used as soil drench completely inhibited *M. phaseolina* infection on 30 day old seedlings. In 60 day old seedlings *B. japonicum* combined with *T. harzianum* used as seed dressing and with *P. lilacinus* as soil drench completely prevented *M. phaseolina* infection. Use of *B. japonicum* with *T. viride, G. virens* and *S. atra* in 30 day old plants, when used as seed dressing and with *T. viride, T. harzianum* and *P. lilacinus* in 60 day seedlings when used as seed dressing and or as soil drench were found more effective than their separate use (Fig.1).

Infection of *R. solani* was completely controlled by *B. japonicum, T. viride, G. virens, P. lilacinus* and *S. atra* both in 30 day and 60 day old seedlings when used as seed dressing, while in soil drench method *S. atra* in 30 day and 60 day, *T. harzianum* and *P. lilacinus* in 30 day and *T. viride* in 60 day old mustard seedlings completely inhibited *R. solani* infection. Use of *B. japonicum* with *T. viride* and *G. virens* showed better results in reducing *R. solani* infection after 30 days (Fig.1).

Fusarium infection was completely controlled by *B. japonicum, T. viride* and combined use of *B. japonicum* with *T. viride, T. harzianum, G. virens* and *S. atra* in 30 day old seedling and by *S. atra* in 60 day old plants when used as soil drench. *B. japonicum* when used with *G. virens, P. lilacinus* and *S. atra* as seed dressing completely inhibited the infection of *Fusarium* spp., on 30 day old mustard seedlings (Fig.1).

Discussion

There does not appear to be any report on the use of biocontrol agents against root rot pathogens attacking mustard crop whereas fungicides have been used in the control of *M. phaseolina* infection on mustard (Rai & Srivastava, 1977; Rana & Tripathi, 1983). In the present study use of *B. japonicum* with *T. harzianum, T. viride, P. lilacinus, G. virens* and *S. atra* against *M. phaseolina, T. viride* and *G. virens* against *R. solani* and *G. virens, P. lilacinus* and *S. atra* against *Fusarium* spp., showed better results in the control of root rot diseases of mustard. There are reports that rhizobia were stimulated by *T. viride* and *S. atra* (Butt & Ghaffar, 1972, Gangawane & Salve, 1987).

B. japonicum has been found to inhibit the growth of *M. phaseolina, R. solani* and *Fusarium* spp., (Chakraborty & Purkayastha, 1984; Tu, 1978; Ghaffar, 1990). Application of *T. harzianum, P. lilacinus* and *G. virens* as seed dressing and or as soil drench has been found to reduce infection of *M. phaseolina, R. solani* and *Fusarium* spp., on sunflower, soybean, mungbean and okra (Ehteshamul-Haque *et al.*, 1990, Hussain *et al.*, 1990). *In vitro S. atra* inhibited the growth of *M. phaseolina, R. solani* and *Fusarium* spp., (Ghaffar, 1988).

The present study would suggest that microbial antagonists has the potential to protect the mustard roots from mono-or multipathogenic attacks. In some cases use of *B. japonicum* with other biocontrol agents showed better results than their separate use. There is therefore, need to select potential biocontrol agents and use them

alone or in different combination for the control of root rot disease instead of using chemical pesticides which are hazardous to environment.

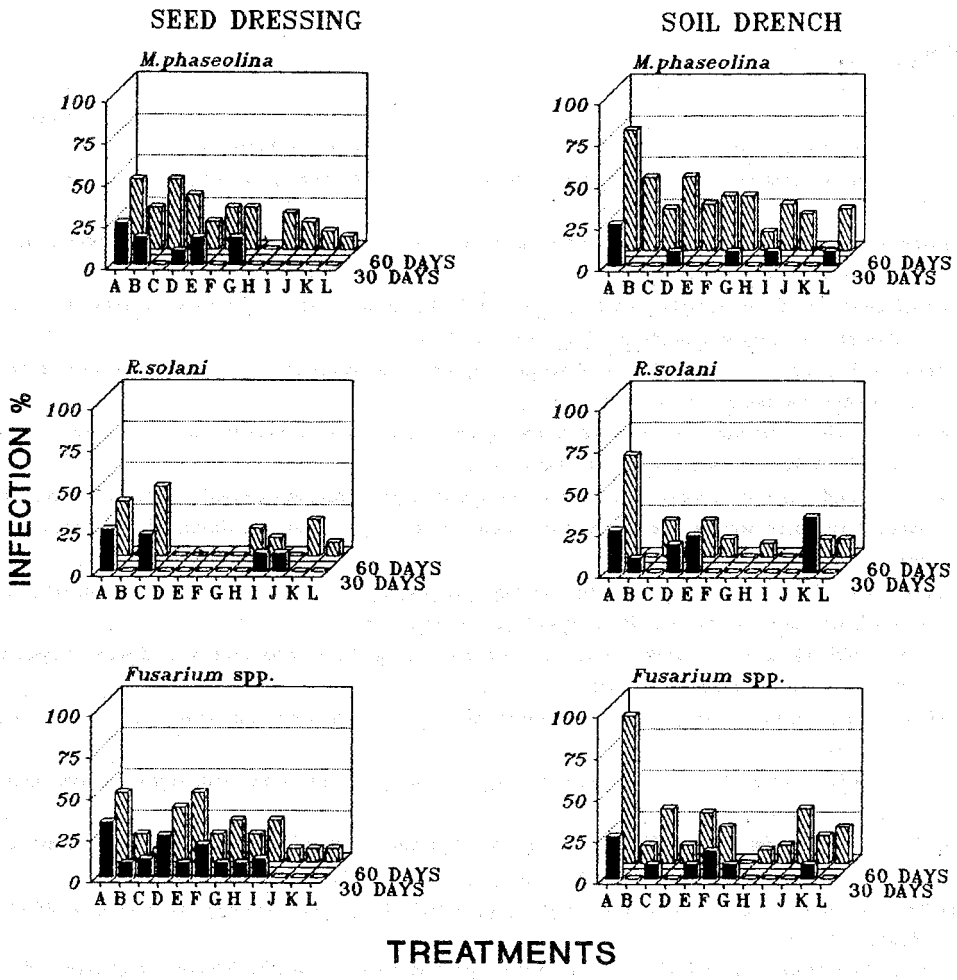

Fig. 1. Effect of microbial antagonists used as seed dressing or as soil drench on infection of roots of mustard by *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp.

A = Control, B = *Bradyrhizobium japonicum*, C = *Trichoderma harzianum*, D = *T. viride*
E = *Gliocladium virens*, F = *Paecilomyces lilacinus*, G = *Stachybotrys atra*, H = B+C,
I = B+D, J = B+E, K = B+F, L = B+G.

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