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Biological control of root rot of green gram caused by *Macrophomina phaseolina* by antagonistic microorganisms

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Macrophomina phaseolina (Tassi) Goid, a soil borne plant pathogen which has a wide host range causing seedling blight, collar rot, stem rot and root rot diseases of various crops. In the present investigation, attempts were made to control root rot of green gram [*Vigna radiata* (L.) Wilczek] (variety SML – 264) caused by *M. phaseolina* by suppressive bacteria following *in vitro, in vivo* and field studies. Three isolates of *Bacillus subtilis* namely BS – 12, BS – 17and BK – 1; two isolates of *Rhizobium* (AKR – 1 and M – 10) and *Pseudomonas fluorescens* were used in these experiments. Both *Rhizobium* M-10 and *Pseudomonas fluorescens* showed 76.65% inhibition of the growth of *Macrophomina phaseolina* in dual culture technique. Under *in vivo* condition, *Bacillus subtilis* (isolate BK – 1) treated crops showed 37.02% mortality compared to 67.28% mortality in inoculated control crops when applied as seed soaking along with soil drenching. Efficient 74.96% disease control was obtained when *Pseudomonas fluorescens* applied as seed soaking in field condition. Nitrogen fixation of green gram is maximum in case of *Rhizobium* treated plants. Inoculated control plants showed minimum grain yield which was recorded to the lower by (-) 41.60% as compared to uninoculated control and (-) 45.87% lower than *Rhizobium* (isolate AKR – 1) treated plants.Antagonists treated plants showed good average root length, shoot length, dry weight, nitrogen fixation and yield.

Key words: Green gram, root rot, *M. phaseolina*, antagonistic microorganisms

INTRODUCTION

Green gram or Mung bean (*Vigna radiata* (L) Wilczek) in one of the most important pulse crops. Mungbean belongs to family Leguminosae and sub family Papilionaseae. Pests and diseases which cause reduction in yield appear in the prevailing weather condition from seedling stage to pod forming stage. The fungi causing the important diseases of mung bean are *Macrophomina phaseolina Cercospora canescens; Glomerella lindemuthiana*; *Erysiphe polygoni*. Among these fungi, *Macrophomina phaseolina* causes dry root rot of mungbean. Since the fungus is soil borne it is difficult to control the disease. With the consideration of global importance of the fungus, *M. phaseolina*, causes seedling blight, root rot, charcoal rot, stem rot of a variety of economically important agricultural crops and also the awareness of the use of toxic fungicides, several potential antagonists against this pathogen have been identified in recent years. With this view, in the present context, an investigation has been carried out to control root rot of green gram caused by *M. phaseolina* by three isolates of *Bacillus* sp., *Pseudomonas fluorescens* and *Trichoderma harzianum* under laboratory, pot and field conditions.

MATERIALS AND METHODS

Experiments were conducted with three isolate of *Bacillus subtilis*, collected from Department of Plant Pathology, BCKV namely BS-12, BS-17 and BK-1; two isolates of *Rhizobium* sp. collected from NRL-BCKV, namely M-10 and AKR-1. In addition to that, two antagonists were also used such as *Pseudomonas fluorescens* and *Trichoderma harzianum*. *Macrophomina phaseolina* the test pathogen was isolated from naturally infected jute

seedling Green gram ev. – SML-264 was taken as test plant.

The bacterial and fungal cultures were maintained on NA (*Bacillus* sp); Kings B (*Ps. fluorescens*); PDA (*M. phaseolina* and *Trichoderma*) and Yeast Manital Agar (*Rhizobium* spp). For mass culture of the fungus *M. phaseolina* were first grown in Petriplates on PDA medium. Sand maize meal medium were prepared in polypropylene packets and sterilized. Then 15 mm diameters of fungal disc from the growing PDA medium were inoculated. The inoculated medium was incubated at 30± 1°C for 15 days. Antagonism of the selected bacterial isolates against *M. phaseolina* was tested *in vitro* by dual culture technique (Dhingra and Sinclair 1985). Inoculation of plants with the bacteria was done by I) Seed treatment and II) Soil application.

Field trial conducted with different treatments in a randomized block design. The plot size was 1 m^2. The soil was sandy loam, well drained having pH 6.7. Urea @ 25 kg/ha were also applied. *M. phaseolina* grown on sand maize meal medium were thoroughly mixed with the soil @ 200 g/m^2. Antagonists treated seeds were sown in the plots. Per cent mortality of seedlings of mung bean in the pot and in field condition was estimated as number of infected plants / total no. of plants x 100. Statistical analysis was carried out following the methods described by Chandel (1978). Total N content of the dried plant samples were estimated following the methods as described by Jackson (1967).

RESULTS AND DISCUSSION

Study of antagonism in vitro

The antagonistic study against *Macrophomina phaseolina* was carried out by direct antagonism i.e. dual culture technique. The dual culture study revealed that all the antagonists i.e. three isolates of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Rhizobium* sp. had the ability to inhibit the pathogen growth significantly. The inhibition ranged from 67.76% to 76.65%. The maximum inhibition i.e. 76.65% was shown by both *Rhizobium* M-10 and *Ps. fluorescens* and 67.76% inhibition was shown by *Bacillus subtilis* – BK – 1 (Table 1).

The population of bacterial suspension was estimated as 12 x 10^8 cells/ml by using Mc Farland Scale before sowing. The mung bean seeds were soaked separately in each antagonist suspension for one and half hour. Three replications were maintained for each treatments. Percent mortality

Table 1 : Inhibition of mycelial growth of *M. phaseolina* by antagonists (Dual culture technique) in vitro.

Antagonist	Colony diameter (mm)		Inhibition zone (mm)	Inhibition of pathogen (%)
	Pathogen	Antagonists		
Rh – M – 10	21 (1.322)*	65 (1.812)	4 (0.602)	76.65
Rh- AKR – 1	24 (1.380)	64 (1.806)	2 (0.301)	73.33
Ps. fluorescens	21 (1.322)	60 (1.778)	9 (0.954)	76.65
T. harzianum	22 (1.342)	62 (1.792)	6 (0.778)	75.56
BS – 12	23 (1.361)	64 (1.806)	3 (0.477)	74.44
BS – 17	24 (1.380)	62 (1.792)	4 (0.602)	73.33
BK – 1	29 (1.462)	58 (1.763)	3 (0.477)	67.76
Contorl	90 (1.954)	—	—	—
S. Em ±	0.051	0.014	0.017	
CD at 5%	0.109	0.030	0.037	
CD at 1%	0.151	0.042	0.051	

** The figures in parenthesis are log transformed values.*

Study of antagonism in vivo

Effect of Seed soaking of antagonists on per cent mortality of root rot

was recorded upto 30 days after sowing (Table 2).

Minimum percent mortality was recorded in uninoculated control plants i.e. 12.88% and as expected maximum mortality was shown by inoculated control plants which appeared as 65.74%. *T. harzianum* and bacterial antagonists had shown suppressive effect against mortality.

Table 2 : Effect of seed soaking of antagonists on per cent mortality of root rot of green gram in pot trial.

Treatments	Replications			Mean
	R_1	R_2	R_3	
Uninoculated control	9.00 (17.46)*	2.00 (8.13)	5.13 (13.05)	12.88
Inoculated control	85.00 (67.21)	82.20 (65.05)	82.14 (64.97)	65.74
Rh – M – 10	45.26 (42.25)	47.30 (43.45)	50.12 (45.06)	43.58
Rh – AKR – 1	46.16 (42.76)	47.25 (43.39)	49.20 (44.54)	43.56
Ps. fluorescens	51.10 (45.63)	53.50 (47.01)	52.00 (46.15)	46.26
Trichoderma harzianum	50.00 (45.00)	46.70 (43.11)	47.10 (43.34)	43.81
BS – 12	60.40 (51.00)	56.40 (48.68)	57.30 (49.30)	49.66
BS – 17	50.20 (45.11)	48.69 (44.20)	52.60 (46.49)	45.26
BK – 1	62.42 (52.18)	65.00 (53.73)	61.14 (51.41)	52.44
S. Em±				1.512
CD at 5%				3.205
CD at 1%				4.416

** Figures in parenthesis are angular transformed values.*

Table 3 : Effect of seed soaking and soil drenching of antagonists on per cent mortality of root rot of green gram in pot trial.

Treatments	Replications			Mean
	R_1	R_2	R_3	
Uninoculated control	6.00 (14.18)*	2.67 (9.28)	5.63 (13.69)	12.38
Inoculated control	85.87 (67.86)	83.41 (65.96)	86.00 (68.03)	67.28
Rh – M – 10	30.12 (33.27)	29.80 (33.09)	26.31 (30.85)	32.40
Rh – AKR – 1	28.70 (32.39)	29.52 (32.90)	(29.00 (32.58)	32.62
Ps. fluorescens	35.11 (36.33)	34.12 (35.73)	33.45 (35.30)	35.78
Trichoderma harzianum	28.21 (32.08)	31.20 (33.96)	30.00 (33.21)	33.08
BS – 12	33.43 (35.30)	35.15 (36.33)	35.00 (36.27)	35.96
BS – 17	31.55 (34.14)	30.19 (33.27)	31.87 (34.33)	33.91
BK – 1	35.98 (36.81)	33.58 (35.37)	39.40 (38.88)	37.02
S. Em±				1.066
CD at 5%				2.259
CD at 1%				3.113

** The figures in parenthesis are in angular transformed values*

Study of antagonism in field

Effect of seed soaking and soil drenching of antagonists on per cent mortality of root rot

Mung bean seeds were soaked initially by the suspension of bacterial and fungal antagonists. After 5 days of sowing, the suspension of antagonists of about 200 ml were applied in each sick pot. Mortality of seedlings were recorded from 10 days and continued upto 30 days after sowing. The data in Table 3 indicate that antagonists showed significant suppressiveness against *M. phaseolina*.

Influence of antagonists on plant growth characters were studied in *M. phaseolina* treated soil at field. Uninoculated control plants recorded highest root length compared to treated plants at 45 DAS followed by *Rhizobium* treated plants. Root length of inoculated control plants was recorded to be lower by (-) 48.60% compared to uninoculated control plants. Similar trends were also observed in case of shoot length where *M. phaseolina* treated plants were lower by (-) 43.87% as compared to un-inoculated control at 45 DAS. Similar trends were also followed by plant dry wt (Table 4). Percent N_2 fixation were recorded to be maximum in *Rhizobium* treated plants.

As regards grain yield, the plants treated with *M. phaseolina* recorded minimum grainyield. The highest yield was recorded in case of Rh-AKR-1 treated plants (16 q/ha) followed by Rh-M-10 treated plants, which appeared to be 15.50 q/ha. Grain yield of *M. phaseolina* treated plants were recorded to be lower by 41.60% as compared to uninoculated control and by 45.87% over Rh-AKR-1 treated plants (Table 5).

Umechuruba (2004) studying the antagonistic effect of *Bacillus subtilis* strain *in vitro* against *M. phaseolina*, causal agent of root rot of mung bean, indicated that the gram (+) bacteria inhibited the growth of the soil borne pathogen *in vitro*. This

Table 4 : Influence of antagonists on plant growth characters of 45 days old seedling of green gram in Macrophomina treated soil at field.

Treatments	Root length (cm)	Shoot length (cm)	Dry weight (g)	N_2 fixation (%)
Uninoculated control	11.09	16.25	182.05	8.07
Inoculated control	5.70	9.12	111.12	4.38
Rh – M – 10	10.71	15.88	182.62	9.44
Rh – AKR – 1	11.11	15.78	181.38	9.55
Ps. fluorescens	9.94	14.61	174.86	6.39
T. harzianum	10.16	15.06	176.38	5.27
BS – 12	10.68	15.61	177.07	7.00
BS – 17	9.68	14.65	177.92	7.09
BK – 1	9.24	14.86	181.56	6.53
S. Em±	0.857	0.764	3.902	0.552
CD at 5%	1.816	1.619	8.272	1.170
CD at 1%	2.503	2.231	11.397	1.612

Table 5 : Influence of antagonists on yield of green gram in Macrophomina treated soil

Treatments	Replications			Average Yield (q/ha)
	R_1	R_2	R_3	
Uninoculated control	15.0	14.0	15.5	14.83
Inoculated control	6.0	11.0	9.0	8.66
Rh – M – 10	15.5	15.0	16.0	15.50
Rh – AKR – 1	18.0	14.0	16.0	16.00
Ps. fluorescens	15.0	16.5	12.0	14.50
T. harzianum	13.5	14.0	14.5	14.00
BS – 12	15.0	14.5	13.0	14.16
BS – 17	15.0	12.0	14.5	13.83
BK – 1	13.5	16.5	14.0	14.66
S. Em±				1.878
CD at 5%				3.981
CD at 1%				5.485

has been confirmed in the present work. Biological control of *M. phaseolina* by *Bacillus subtilis* on chickpea was studied by Shaid *et al.*. (2000). This has been confirmed with the present work. In the present studies, the maximum per cent of disease control, recorded as 66.09% where *Bacillus* strain namely BS-17 applied as both seed soaking and soil drenching.

Inhibition of *M. phaseolina* by *Ps. fluorescens* was already known and indicated by several workers. Management of *M. phaseolina* causal agent of root rot disease of ground nut by *Ps. fluorescens* was reported by Vimala *et al.*. (2000). This has been confirmed with the present work. In another work bio-control of *Pythium aphanedermatum* in chilli by the treatment of *Ps. fluorescens* in seed reported by Manoranjitham *et al.*. (2000) also supported our present work. In our present work *Ps. fluorescens* showed up to 76.65% inhibition of pathogen *in vitro*. The per cent disease control by *Ps. fluorescens* in soil application method were recorded upto 53.74% in the seed treatment and 64.22% in seed followed by soil drenching respectively.

Soil application and seed treatment of *T. harzianum, T. viride* and *T. virens* showed biocontrol of *M phaseolina* on mung bean, reported by Rajeswari *et al.*. (1999). This has been confirmed with the present work. Under *in vitro* condition inhibition of pathogen by *T. harzianum* was recorded upto 75.56%. Under *in vivo* condition, *T. harzianum* reduced the root rot of green gram up to 66.92% where the fungus was applied as seed treatment followed by soil application.

Rhizobia harbouring the rhizosphere and root tissue of legumes are expected to interact with the soil borne pathogens. They fix atmospheric nitrogen in the symbiotic stage within root nodule thereby increasing crop yield. The effect of *Rhizobium* sp in reducing the disease intensity and thereby maintaining a level of nodulation was indicated by several workers. Suppressive effect of *Rhizobium* sp. on *M. phaseolina* was reported by Siddiqui *et al.*. (2000) and Shahnaz *et al.* (2005) on mung bean and okra, which also support our present work.

A challenge to the pathogen by antagonists has been expressed. The possibility of using a *Bacillus / Rhizobium* inoculation for lowering the pressure of root rot of green gram caused by *M. phaseolina* is clearly expressed from the present work. This has opened up an area of possible use of *Bacillus* sp., *Rhizobium* sp. and *Ps. fluorescens* not only as plant growth promoting rhizobacteria but also as suppressor in the soil against the plant pathogen to lower the disease pressure.

REFERENCES

Chandel, R. S. 1978. *A Handbook of Agricultural Statistics.*

Dhingra, O. D. and Sinclair, J. B. 1985. *Basic Plant Pathology Methods*. CRC Press Inc. Boca Raton, Florida. pp. 355.

Jackson, M. L. 1967. *Soil Chemical Analysis*, Prentice Hall, Inc. New Jersy, U.S.A.

Manoranjitham, S. K.; Prakasam, V.; Rajappan, K. and Amutha, G. 2000. Control of chilli damping-off using bioagents. *Journal of Mycology and Plant Pathology*. **30** (2) : 225 – 228.

Rajeswari, B. ; Rao, K. C. and Kumar, C. P. C. 1999. Efficacy of antagonists and carbendazim against dry root rot of mungbean [*Vigna radiata* (L.) Wilczek] incited by *Macrophomina phaseolina* (Tassi.) Goid under glasshouse conditions. *Journal of Biological control*. **13** (1 – 2) : 93 – 99.

Shahnaz, D.; Perveen, F. and Atif, D. 2005. Effect of different strains of *Rhizobium* spp. in the control of root infecting fungi and growth of crop plants. *International Journal of Biology and Biotechnology*. **2** (2) : 415 – 418.

Shaid, A. ; Mukesh, S.; Ahamad, S. and Srivastava, M. (2000). Biological control of dry root rots of chickpea with plant products and antagonistic microorganisms. *Annals of Agricultural Research*. **21** (3) : 450 – 451.

Siddiqui, I. A.; Ehteshamul – Haque, S.; Zaki, M.J. and Ghaffar, A. 2000. Greenhouse evaluation of rhizobia as biocontrol agent of root-infecting fungi in okra. *Acta Agrobotanica* **53** (1) : 13 – 22.

Umechuruba, C. I. 2004. Inhibition of growth of some seed-borne fungal pathogens by extract from *Bacillus subtilis*. *Global Journal of Pure and Applied Sciences*. **10** (1) : 17 – 21.

Vimala, R.; Sheela, J. and Packiaraj, D. 2000. Management of root rot disease of groundnut by bioagents. *Madras Agricultural Journal* . **87** (4-6) : 352 – 354.

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