J. Mycopathol. Res. **41**(2) : 147-151 (2003)

Management of gram root rot caused by *Macrophomina phaseolina* Tossi (Goid) with antagonistic bacteria (*Bacillus* spp.)

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Macrophomina phaseolina Tossi (Goid), a soil borne plant pathogen that damages a wide range of agricultural crops causing seeding blight, root rot, charcoal rot diseases. Possibilities of controlling root rot disease with antagonistic bacteria were studied in this investigation. The experiment were conducted on gram (*Cicer arietinum*) as a test crop and in sick soil inoculated with *Macrophomina phaseolina*. The pathogen was grown on PDA medium and Sand maize medium was also used for mass culture. Cultures of two bacterial isolates (S_{12} & S_{17}) of *Bacillus* sp. when applied as both seed and soil drench reduced root rot or *Macrophomina*-rot of gram. *Bacillus* sp. (S_{12}) at seed disease was lower by (-) 6.78% and (-) 15.20% from control but higher by 13.45% and 11.33% compared to *Macrophomina* sp. treated plants as regards the root and shoot length. Dry and fresh weight of *Bacillus* sp. (S_{12} & S_{17}) treated plant showed higher root and shoot length compared to only *Macrophomina phaseolina* treated plant. The yield increase of uninoculated control plants was higher by 86.58% compared to plants threated with *Macrophomina phaseolina* at soil. Incidence of diseases was reduced the plants treated with *Bacillus* sp. (S_{12}) both at seed and seedlings and subsequently produced significantly higher yield i.e. 67.13% compared to only *Macrophomina phaseolina* treated plants. In our present findings, the gram positive bacteria (*Bacillus* sp.) is not only the appealing candidate for biological control of plant disease but also play an important role in promotion of plant growth and yields.

Key words : Gram root rot, *Macrophomina phaseolina, Bacillus* sp. biocontrol

INTRODUCTION

Biological control of soil borne pathogens was not considered commercially feasible two decades back. Currently this view has been changed. The change is perhaps due to among other reasons, growth of public opinion regarding the hazard of chemical pesticides. In recent years there has been much success in obtaining biological control of plant pathogens using bacterization techniques (Colyer and Mount, 1984 ; Weller and Cook, 1983). The best Example of bacterial bio-control agent is *Agrobactrium radiobacter* strain K-84 which controls crown gall caused by A *tumifaciens* (Kerr, 1980 ; Verma and Dutta Majumder, 1986). This is the first bacterium used commercially all over the world for biocontrol. *Bacillus species* have great potentialities as biocontrol agents, because they produce endospores, which are tolerant to heat and desication. *B subtilis* strain A-13 has been isolated from lysed *Sclerotium rolfsii* mycelium (Broadbent *et al.,* 1971) and it has been found inhibitory to large number of pathogens.

Macrophomina phaseolina Tossi (Goid) is soil borne plant pathogen that damage a wide range of agricultural corps. *Macrophomina phaseolina* is known to cause seedling blight, root rot, charcoal rot and leaf blight of a variety of agricultural crops. Several potential antagonists for *Macrophomina phaseolina* have been identified and some appear to be of sufficient potential to be exploited commercially.

MATERIALS AND METHODS

Experiments were conducted with two bacterial isolates namely S_{12}, S_{17} and plant pathogenic fungal culture namely *Macrophomina phaseolina* Tassi (Goid) were obtained from the Department of Plant Pathology, BCKV, for the present study. Seeds of

Bengal gram (*Cicer arietinum*) were purchased from local market and were applied both in pots and fields to study the effect of antagonistic microorganism namely *Bacillus* sp. against the soil borne pathogen *Macrophomina phaseolina*. For subculturing of two bacterial isolates S_{12} and S_{17} and plant pathogenic fungus *Macrophomina phaseolina* potato dextrose agar and nutrient agar medium were used. Nutrient Agar medium composed of Pepton 5.0 g, Beef extract 3.0 g and Agar agar 20.0 g per 1000 ml of distilled water. In case of PDA 200 g peeled potato, 20 g dextrose and 20 g Agar for 1000 ml of distilled water. PD broth were prepared expecting the addition of Agar agar. For mass culture of the fungus *Macrophomina phaseolina* the pathogen was grown on sand maize medium containing maize meal 30 g and sand 70 g and water also required to moisten the mixture.
Statistical analysis of the data on disease intensity and yield were made by analysis of variance of different characters in field.

RESULT AND DISCUSSION

Effects on shoot and root length

From the data in Table 1 it was observed that the uninoculated control plants recorded highest shoot length compared to other treatments. *Macrophomina*-treated plants showed minimum shoot length. However, shoot length of *Bacillus* sp. (S_{12} and S_{17})-treated plants, both at seed and at seedling stages along with the *Macrophomina* sp. showed lower shoot length compared to control but higher compared to *Macrophomina*-treated plants.

Table 1 : Influence of *Bacillus* sp. on shoot length of gram in *Macrophomina*-treated soil

Treatments	Average shoot length (cm) 30 days	60 days
Unicoculation control	147.4	174.96
Macrophomina at soil, before sowing	121.1	133:2
Macrophomina at soil + *Bacillus* (S_{12}) at seed	137.4	148.38
Macrophomina at soil + *Bacillus* (S_{17}) at seed	129.1	135.63
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) at seed	133.9	140.66
Macrophomina at soil + *Bacillus* (S_{12}) both at seed and soil drenching after 15 DAS	140.5	157.4
Macrophomina at soil + *Bacillus* (S_{17}) both at seed and soil drenching after 15 DAS	131.3	141.0
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) both at seed and soil drenching after 15 DAS	136.6	144.2
CD at 5%	1.548	1.847
SEm ±	0.509	0.607

From the Table 2 it was found that uninoculated control plants recorded highest root length compared to other treatments. *Macrophomina phaseolina*-treated plants showed minimum root length. However, this was recorded to be lower by (-) 28.76% and (-) 30.06% compared to control at 30 DAS and 60 DAS respectively. Root length of *Bacillus* sp. (S_{12} and S_{17}) treated plants both at seed and at seedling stages along with *Macrophomina phaseolina* showed lower root length compared to control but higher compared to *Macrophomina phaseolina*-treated plants.

Table 2 : Influence of *Bacillus* sp. on root length of gram in *Macrophomina*-treated soil

Treatments	Average shoot length (cm) 30 days	60 days
Unicoculation control	102.9	105.1
Macrophomina at soil, before sowing	73.3	73.5
Macrophomina at soil + *Bacillus* (S_{12}) at seed	100.3	101.6
Macrophomina at soil + *Bacillus* (S_{17}) at seed	74.9	75.3
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) at seed	85.0	85.7
Macrophomina at soil + *Bacillus* (S_{12}) both at seed and soil drenching after 15 DAS	91.23	95.1
Macrophomina at soil + *Bacillus* (S_{17}) both at seed and soil drenching after 15 DAS	81.3	82.0
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) both at seed and soil drenching after 15 DAS	89.2	90.8
CD at 5%	1.968	2.852
SEm ±	0.647	0.938

Effects of fresh and dry weight

From the data in Table 3 it was observed that the uninoculated control plants recorded highest fresh weight compared to other treatments. *Macrophomina phaseolina*-treated plants showed minimum fresh weight. This was recorded to be lower by (-) 37.56% compared to control. Fresh weight of *Bacillus* sp. (S_{12} and S_{17})-treated plants both the seed and seedling stages along with *Macrophomina phaseolina* showed lower fresh weight compared to control but higher compared to *Macrophomina phaseolina*-treated plants. Plants treated with *Macrophomina phaseolina* at soil and *Bacillus* sp. (S_{12}) treated at seed stage was lower by (-) 2.26% from control but higher by 55.48% compared to *Macrophomina phaseolina*-treated plants. Plants treated with *Macrophomina phaseolina* at soil and *Bacillus* sp. (S_{12} + S_{17}) at seed stage was lower by (-) 16.66% from control but higher by 32.56% compared to *Macrophomina phaseolina*-treated plants.

Table 3 : Influence of *Bacillus* sp. on fresh weight of gram in *Macrophomina*-treated soil

Treatments	Average shoot length (cm) 30 days	60 days
Unicoculation control	29.16	28.83
Macrophomina at soil, before sowing	18.33	17.50
Macrophomina at soil + *Bacillus* (S_{12}) at seed	28.5	27.66
Macrophomina at soil + *Bacillus* (S_{17}) at seed	20.5	21.33
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) at seed	24.33	23.50
Macrophomina at soil + *Bacillus* (S_{12}) both at seed and soil drenching after 15 DAS	26.33	28.33
Macrophomina at soil + *Bacillus* (S_{17}) both at seed and soil drenching after 15 DAS	22.0	23.50
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) both at seed and soil drenching after 15 DAS	24.66	25.16
CD at 5%	1.603	4.077
SEm ±	0.527	1.340

It was also found that uninoculated control plants recorded highest dry weight campared to other treatments. *Macrophomina phaseolina*-treated plants showed minimum dry weight. However, this was recorded to be lower by (-) 39.29% compared to control. Dry weight of *Bacillus* sp. (S_{12} and S_{17}) treated plants both at seed and seedling stages drenching along with *Macrophomina phaseolina* showed lower dry weight compared to control but higher dry weight compared to *Macrophomina phaseolina*-treated plants. Plants treated with *Macrophomina phaseolina* at soil and *Bacillus* sp. (S_{12} + S_{17}) at seed stage was lower by (-) 18.48% from control but higher by 34.28% compared to *Macrophomina phaseolina*-treated plants.

Effects on disease intensity

As expected *Macrophomina phaseolina*-treated plants showed (Table 4) higher incidence of diseases compared to control, and was recorded to be higher by 75% from control plants. Incidence of foot rot was reduced to some extent where the plants were treated with *Bacillus* sp. (S_{12}) was applied at seed and seedling stage at 15 DAS along with *Macrophomina phaseolina* at soil. It was lower by (-) 34.92% from *Macrophomina phaseolina*-treated plant. In case of *Bacillus* sp. (S_{17}) it was lower by (-) 23.80% from *M. phaseolina*-treated plant. It has been appeared from the present study that few uninoculated control plant become infected, this might be due to the presence of soil borne pathogen in natural soil. However, this require further investigation.

Table 4 : Effect of both seed and soil treatment of *Bacillus* sp. on root rot of gram.

Treatments	Infection rate (%)
Unicoculation control	2.52
Macrophomina at soil, before sowing	4.41
Macrophomina at soil + *Bacillus* (S_{12}) at seed	2.92
Macrophomina at soil + *Bacillus* (S_{17}) at seed	3.36
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) at seed	3.10
Macrophomina at soil + *Bacillus* (S_{12}) both at seed and soil drenching after 15 DAS	2.87
Macrophomina at soil + *Bacillus* (S_{17}) both at seed and soil drenching after 15 DAS	3.33
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) both at seed and soil drenching after 15 DAS	3.08
CD at 5%	0.120
SEm ±	0.037

Effects on nitrogen fixation

Per cent N_2 fixation was recorded to be maximum in the uninoculated control plants (Table 5). Similar results were achieved in the plants treated with *Bacillus* sp. (S_{12}) both at seed and at seedling stages. Both the above mentioned treaments were higher by 61.9% as regards N_2 fixation compared to *Macrophomina*-treated plants only. The plants treated with *Bacillus* sp. (S_{12}) at seed along with *Macrophomina* at soil also showed significant increase in N_2, fixation and was recorded to be higher by 43.8% as compared to only *Macrophomina*-trreated plants. Increased N_2 fixation i.e., 34.28% was achieved by the plants treated with *Macrophomina* at soil and both the strains of *Bacillus* (S_{12} + S_{17}) at seed.

Table 5 : Influence of *Bacillus* sp. on nitrogen fixation of gram in *Macrophomina* treated soil

Treatments	Average nitrogen fixation (mg)
Unicoculation control	119.0
Macrophomina at soil, before sowing	73.5
Macrophomina at soil + *Bacillus* (S_{12}) at seed	105.7
Macrophomina at soil + *Bacillus* (S_{17}) at seed	79.8
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) at seed	98.7
Macrophomina at soil + *Bacillus* (S_{12}) both at seed and soil drenching after 15 DAS	119.0
Macrophomina at soil + *Bacillus* (S_{17}) both at seed and soil drenching after 15 DAS	89.6
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) both at seed and soil drenching after 15 DAS	98.7
CD at 5%	6.283
SEm ±	2.06

Effects of grain yields

As expected, the plants treated with *Macrophomina*

sp. at soil recorded minimum grain yield (Table 6). There was a co-relationship between pre cent infection and yield potentiality. Higher yields were recorded to be produced by uninoculated control plants, this plants actually recorded less disease intensity. The yield increase of uninoculated control plants was higher by 86.58% compared toplants treated with *Macrophomina* sp. at soil. The plants treated with *Macrophomina* sp. at soil and *Bacillus* sp. (S_{12}) both at seed and seedlings stages subsequently produced significantly higher yield (67.13%) compared to only *Macrophomina*-treated plants. As expected the remaining treatments also showed lower yields compared to uninoculated control plants.

Table 6 : Influence of *Bacillus* sp. on yield of gram in *Macrophomina*-treated soil

Treatments	Average yield Q/ha
Unicoculation control	4.633
Macrophomina at soil, before sowing	2.483
Macrophomina at soil + *Bacillus* (S_{12}) at seed	4.033
Macrophomina at soil + *Bacillus* (S_{17}) at seed	3.183
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) at seed	3.466
Macrophomina at soil + *Bacillus* (S_{12}) both at seed and soil drenching after 15 DAS	4.150
Macrophomina at soil + *Bacillus* (S_{17}) both at seed and soil drenching after 15 DAS	3.366
Macrophomina at soil + *Bacillus* (S_{12}+ S_{17}) both at seed and soil drenching after 15 DAS	3.983
CD at 5%	12.77
SEm ±	4.202

The data in Table 7 showed that plant growth characters, dry weight and N_2 fixation of all the treatment were positively corelated with yield potential. However, it was established from the corelation study (Table 7) that the pre cent intensity of disease had the ability to increase/decrease the yield.

Gram positive bacteria (*Bacillus)* has the potential to control various disease of agricultural crops caused by plant pathogenic fungi, bacteria and viruses. This bacteria is not only the appealing candidate for biological control of plant diseases but also plays an important role in promotion of plant growth, root and shoot dry weight and yield. Being environmentally safe and because of hazardous effects of agro-chemicals the interest in biocontrol of plant diseases has increased in the recent years.

Table 7 : Correlation study of average shoot length, root length, infectin percentage, dry weight and nitrogen fixation with yield.

Treatments	Yield	Shoot length		Root length		Infection	Dry	N_2
		30 DAS	60 DAS	30 DAS	60 DAS	(%)	weight (g)	(mg)
T_1	4.633	147.4	174.6	102.9	105.1	2.52	28.83	119.0
T_2	2.483	121.1	133.2	73.3	73.3	4.41	17.50	73.5
T_3	4.033	137.4	148.33	100.3	100.6	2.92	27.33	105.7
T_4	3.183	129.1	135.63	74.9	75.3	3.36	21.33	79.8
T_5	3.466	133.9	140.66	85.0	85.7	3.10	23.50	98.7
T_6	4.150	140.5	157.4	91.23	95.1	2.87	28.33	116.9
T_7	3.366	131.3	141.0	81.3	82.0	3.33	23.50	89.6
T_8	3.983	136.6	144.2	89.2	90.8	3.08	25.16	98.7

** = Significant at 1% level

As a suppressor bacteria or biocontrol agent *Bacillus* sp. have been tested on wide variety of corps for their ability to control plant diseases. The resistant endospores that *Bacillus* species produces, provide tolerance to heat and cold as well as to pH extreme, pesticides fertilizers and storage.

Seed bacterization with *Bacillus subtilis* significantly increased root and shoot dry weight of onion seedlings over uninoculated checks.

As regard yields of gram much better yield were obtained in case of seed treatment only and in case of both seed treatment and soil drenching with S_{12} (4.152 q/ha). But less yield were obtained in case of only seed treatment with *Bacillus* sp. (Strain S_{17}). Minimum yield was obtained where the plant were treated with the pathogen (*Macrophomina* sp.), only it produced more infection and eventually decreased the yield component (Bhattacharyya and Mukherjee, 1990).

Reduction of different soil borne diseases and increased corp yield through seed or soil treatments with *Bacillus* spp. was reported earlier by many workers on other corps like, zinger (Sharma and Jain, 1979) and rape (Luth *et al.,* 1993) when *Bacillus* sp. was used as seed tratment, in increased the yields of carrot by 48% (Merriman *et al.*, 1875), Oats by 33% (Merriman *et al.*, 1975) and pea nuts upto 37% (Turner *et al.*, 1986) According to Farzana and Gaffer (1991) the disease incidence by root infection fungi in soybean was reduced following seed treatments with *B. subtilis.*

ACKNOWLEDGEMENT

The authors express their deep sense of gratitude and sincere indebtedness to Prof. N. Mukherjee, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, for his valuable guidance.

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(Accepted for publication May 07 2003)