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Management of *Macrophomina phaseolina* (Tassi) Goid causing seedling blight of jute by bioagents *in vitro*

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Marcrophomina phaseolina (Tassi) Goid, a soil borne plant pathogen has a wide host range causing seedling blight, collar rot, stem rot and root rot diseases of various crops. In the present investigation, attempts have been made to control seedling blight disease of jute (cv JRO-524) caused by *M. phaseolina* by suppressive bacteria following *in vitro* studies. Five isolates of *Bacillus subtilis* namely BK-1, BM-1, BS-12, BS-14, BS-17 and *Pseudomonas fluorescens* have been used in the present studies.

In vitro study revealed that all the antagonistic bacteria inhibited the mycelial growth of *M. phaseolina* and highest mycelial inhibition (78.89%) was recorded in BS-17 in case of dual-culture technique, but in cell free bacterial suspension method BK-1 (79.56%) showed the highest inhibition.

Key words : Seedling blight of jute, *Macrophomina phaseolina*, bioagents

INTRODUCTION

The fungus *Macrophomina phaseolina* (Tassi) Goid is the most important and destructive fungus to the jute crop causing seedling blight, collar rot, stem rot and root rot diseases etc. It is primarily a soil borne plant pathogen causing economic loss of jute crops in terms of quality as well as quantity of jute fibre. Attempts have been made to control soil borne plant pathogen by the applications of some supressive bacteria in our present studies. Among the bacteria, species of *Bacillus* (*Merriman et al.* 1975) and *Pseudomonas* (Kloepper *et al.*, 1980) have been exploited extensively.

MATERIALS AND METHODS

The pathogen *M. phaseolina* was isolated from infected jute and maintained on potato destrose agar medium (PDA). The antagonists i.e. five isolates of *Bacillus subtilis* namely BK-1, BM-1, BS-12, BS-14 and BS-17 were obtained from the Department of Plant Pathology, Fuculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal and *Pseudomonas fluorescens* were isolated from the rhizosphere of jute and identified in the laboratory by following Bergey's Manual of Determinative Bacterialogy (9th Edition). 1995 and maintained on King's B agar medium enriched with cetrimide.

Two techniques were applied namely a) Dual culture technique (Dhingra and Sinclair, 1985) and b) Poison food technique (Lilly and Barnett, 1951) to study the antagonistic effects of supressive bacteria against *M. phaseolina in vitro*. In Dual culture technique, the bacterial antagonists were placed in front of the pathogen in sterilized petriplates. The bacterial antagonists were streaked 1 cm from periphery and 10 cm diameter disc of pathogen was inoculated aseptically in sterilized pertiplates. The plates were incubated at 28 ± 1°C and zone of inhibition of the pathogen by the antagonists were recorded upto 7 days.

Secondly, PD broth and King's B broth were inoculated with respective bacteria and incubated for 7 days at 30 ± 1°C . The cultures were shaked in

a shaker for 2 h continuously per day. The culture was centrifused at 10,000 rpm for 25 minutes and supernatants were collected. Then, the antagonism by this cell free culture suspensions was tested against *M. phaseolina* by following 'poisoned food technique' (Lilly and Barnett, 1951.) Ten millimeter of mycelial discs of the pathogen were placed at the center of the dishes and incubated at 28 ± 1°C. The difference in colony diameter between treated medium and control was used to calculate the pre cent mycelial inhibition. The observations were taken after 72 h of inoculation upto 7 days.

RESULTS AND DISCUSSION

The antagonistic study against *Macrophomina phaseolina* was carried out into two aspects : a) Direct antagonism i.e. Dual-culture technique, b) Indirect antagonism i.e. Cell free bacterial suspension method (Poison food technique)

a) Dual-culture technique : The dual-culture study revealed that all the antagonists, i.e. five isolates of *Bacillus subtilis* and *Pseudomonas fluorescens*, were able to inhibit the growth of pathogen (*M. phaseolina*) significantly. The inhibition ranged from 56.67% to 78.89%. The maxmimu inhibition (i.e. 78.89%) was shown by *Bacillus subtilis* isolate BS-17 followed by *P. fluorescens* (75.56%) (Table 1). Other isolates of *Bacillus subtilis* of BS-12 (73.33%), BS-14 (72.22%), BM-1 (68.89%) were also seemed to be promising antagonists. Selvarajan and Jeyaranjan (1996) studied the antagonistic effect of *Bacillus subtilis* strains against *M. phaseolina*, causal agent of root rot disease of chickpea and indicated that *B. subtilis* inhibited the growth of *M. phaseolina in vitro*, so our present studies are also confirmed with the earlier findings.

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

Antagonists	Colony diameter (mm)		Inhibition zone (mm)	Inhibition of pathogen growth (%)
	Pathogen	Antagonist		
B. subtilis				
BK-1	31(1.491)*	56(1.748)	3(0.477)	65.56
BM-1	28(1.447)	53(1.724)	9(0.954)	68.89
BS-12	24(1.380)	62(1.792)	4(0.602)	73.33
BS-14	25(1.398)	63(1.799)	2(0.301)	72.22
BS-17	19(1.279)	61(1.785)	10(1.000)	78.89
P. fluorescens	22(1.342)	61(1.785)	7(0.845)	75.56
Control	90(1.954)	—	—	—
S.Em ±	0.0075	0.003	0.039	
CD at 5%	0.023	0.009	0.118	
CD at 1%	0.032	0.012	0.164	

* Figures in parenthesis are log transformed values.

b) Cell free bacterial suspenstion method : The study on the application of cell free suspension of antagonists revealed that all the antagonists inhibited the growth of pathogen (*M. phaseolina*) significantly and the inhibition ranged from 50.67% to 79.56%. The isolate of *Bacillus subtilis*, BK-1 showed the maximum inhibition (79.56%) followed by BM-1 (78.67%) (Table 2) Other bactierial antagonists like BS-17 (75.22%), *P. fluorescens* (61%), BS-12 (67.67%) also showed their inbitory effect against *M. phaseolina*. Inhibition of *M. phaseolina* by cell free culture filtrate of *B. subtilis* was indicated by several workers. Cell free culture filtrate of *B. subtilis* inhibited the pathogen, *Sclerotium rolfsii* causing rot of lentil (Agwarl *et al.*, 1978). Podile and Laxmi (1998) also indicated that cell free culture filtrate of *B. subtilis* showed the inhibition of *Fusarium udum*, the incitent of pigeon pea wilt. In our present studies, maximum inhibition of *M. phaseolina* was recorded to be 79.56% by the cell free bacterial culture suspension of the isolate BK-1.

Table 2 : Efficacy of cell free bacterial suspension on mycelial growth of *M. phaseolina* (poison food technique) *in vitro*

Antagonists	Colony diameter (mm)	% Inhibition of Control
B. subtilis		
BK-1	18.4 (1.265)*	79.56
BM-1	19.2 (1.283)	78.67
BS-12	29.1 (1.464)	67.67
BS-14	44.4 (1.647)	50.67
BS-17	22.3 (1.348)	75.22
P. fluorescens	35.3 (1.548)	61.00
Control	90.0 (1.954)	—
S.Em ±	0.008	
CD at 5%	0.025	
CD at 1%	0.035	

* Figures in parenthesis are log transformed values.

A challenge of the pathogen by the antagonist has been expressed. The possibility of using a high *Bacillus* inoculum for lowering the pressure of seedling blight disease of jute due to *Macrophomina phaseolina* (Tassi) Goid is clearly expressed from

the present work. This has opened up area of possible use of *Bacillus* sp. and *P. fluorescens* not only as plant growth promoting rhizobacteria but also in competition in the soil aganist plant pathogen to lower the disease pressure.

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