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Screening and characterization of antagonistic potential of some rhizosphere fungi and PGPR against *Macrophomina phaseolina* in jute

A. K. BANDOPADHYAY, ANURADHA BANDOPADHYAY AND A. MAJUMDER
Biocontrol Laboratory, Division of Crop Protection, Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata 700 120, West Bengal

Antagonistic soil microorganisms were isolated and characterized for selection of potential biocontrol agent (BCA) effective against soil borne pathogen of jute. Pathogenic isolate R 9 was identified as highly virulent and RK 2 less virulent pathotypes of *Macrophomina phaseolina* isolated from diseased jute plant. Antagonist fungi and bacteria including *Trichoderma*, *Gliocladium*, *Penicillium*, *Aspergillus*, *Pseudomonas*, *Bacillus* and *Azotobacter* species isolated from rhizosphere and rhizoplane of jute plant were tested for antagonistic potential singly and in combination against the virulent pathotypes in dual culture. Isolates of *Trichoderma* JPT 1, JPT 9, JPT 14 and JPT 16; *Gliocladium* JPG 1, JPG 4 and JPG 9; *Aspergillus* A 7, AN 15, A 26 and AN 27 and *Penicillium* PN 12 showed promising antagonistic properties inhibiting R 9 and RK 2 to a great extent. PGPR isolates fluorescent *Pseudomonas* Psfl-1, Psfl-2, Phosphobacter *Pseudomonas striata* Pst-1, *Bacillus* BS-6, BS-14 and *Azotobacter* Azbc-2 and Azbc-5 were identified promising. JPT 9 and A 26, JPT 1 and Psfl-1, JPT 1 and Azbc-2, in combination, and Psfl-1 and Pst-1, Psfl-1 and BS-6, and Psfl-1 and Azbc-2 combined were most effective BCA.

Key word : Biocontrol agent (BCA), antagonism, PGPR, PGPF, *Trichoderma, Aspergillus, Penicillium, Gliocladium, Pseudomonas, Bacillus, Azotobacter Macrophomina phaseolina,* dual culture

INTRODUCTION

Macrophomina phaseolina (Tassi.) Goid. is an important pathogen of wide host range infecting nearly 500 plant species. Its destructive nature has been well documented (Beckman, 1987). In jute (*Corchorus olitorius* L. and *C. capsularis* L.) this necrotrophic fungus causes seedling blight, collar rot, stem rot and root rot disease complex throughout the growing stages from seedling till harvest (Bandopadhyay *et al.*, 2004). Infection of *M. phaseolina* on jute crop results loss of around 11-20% fibre yield and deteriorate its quality. Adoption of modern control measures to prevent *M. phaseolina* infection is inevitable for production and quality improvement of jute. Application of systemic and non systemic fungicide is common practice. But indiscriminate use of chemical pesticide contaminate the environment, cause hazardous effect on soil health, water resources, beneficial insect pollinators, predators and soil microflora. It also threats to the appearance of pesticide resistant strain of pathogen and population health. Thus safer, eco-friendly approach to biological management of the soil borne disease with antagonistic fungi and rhizobacteria seems to hold great promise to that effect (Bandopadhyay and Bandopadhyay, 2004).

Plant growth promoting rhizobacteria (PGPR) colonize the root zone and improve plant growth (Kloepper *et al.*, 1980; Weller, 1988). Fluorescent Pseudomonads and some *Bacillus* species are effective in biocontrol of soil borne plant pathogen and termed as PGPR because of their ability to improve plant growth by suppression of deleterious root colonizing microorganisms and production of plant growth regulators viz. IAA, gibberellins, cytokinins etc. (Suslow and Schroth, 1982). Nitrogen fixer *Azotobacter* and *Azospirillum* are

included in the PGPR (Subba Rao and Gaur, 2000). Antagonistic fungal species of *Trichoderma, Penicillium, Aspergillus* and *Phoma* have dual role of disease suppression and plant growth promotion and are recently termed PGPF (Nobuyo koike et al., 2001). Among these *Trichoderma* is widely known (Anandraj *et al.*, 2003).

Present study is concerning the isolation, characterization, screening, and selection of effective fungal antagonist and PGPR from rhizosphere and rhizoplane of jute plant for biocontrol potential against *Macrophomina phaseolina* singly and in compatible combinations.

MATERIALS AND METHODS

Isolation of pathogen

Twenty one isolates of *Macrophomina phaseolina* was collected from infected jute plant in diverse eco-geographical regions and screened for pathogenicity. The pathogens were isolated on potato dextrose agar (PDA) medium. After 7 days growth in BOD incubator at 27° ± 1° C these were purified by repeated subculturing and maintained in PDA slants. Two isolates were finally selected as virulent pathotypes for experimen-tations. Isolate R 9 was collected from diseased stem and root portion of jute (*C. olitorious* L.) grown at CRIJAF experimental farm and isolate RK 2 was collected from diseased plant part of (*C. capsularis* L.) at Kendrapara in Orissa..

Evaluation of pathogenicity

Pathogenicity of the two selected fungal isolates was tested for virulence on jute seedling (cv. JRO 524) in moist chamber on sterilized blotting paper circles in 150 mm size agar plates *in vitro*, in pot cultures and in field conditions (Bandopadhyay *et al.*, 2004). The isolates were grown in Czapeck Dox broth medium for 15 days. The metabolite produced in broth culture was then filtered through bacteriological grade G-5 sintered glass filter and collected aseptically. To study seed germination and seedling growth 50 jute seeds were surface sterilized with 0.1% $HgCl_2$, soaked in culture metabolites of each isolates for 24 h in sterilized Petri dishes and the seeds were allowed to germinate on sterilized blotting paper moistened with sterilized distilled water. Seeds soaked in sterilized distilled water alone served as control. Germination of seed and growth rate of seedling varied with the isolates. For pathogenicity test in pot culture, the isolates were grown in PDA medium. The inoculums were poured and mixed in sterilized pot soil prior to showing of seeds.

Isolation of biocontrol agents

Microbial biocontrol agents (BCA) were isolated from rhizosphere and rhizoplane of jute plant grown in experimental plot at CRIJAF as well as other jute fields. Soil dilution plate technique was followed to isolate the BCA in selective media. Out of total 451 microorganisms isolated from jute soil, 73 fungal isolates including 32 *Aspergillus*, 17 *Trichoderma*, 9 *Gliocladium*, and 15 *Penicillium* were purified by repeated subculturing and maintained in PDA slants at 27°C. 25 bacterial isolates including 4 *Pseudomonas*, 7 *Azotobacter*, 2 *Azospirillum*, 3 *Bradyrhizobium*, 8 *Bacillus* and 1 *Beijerinckia* were identified. Fluorescent Pseudomonads were isolated in *Pseudomonas* isolating medium with fluorescin, *Azotobacter* and *Beijerinckia* in Jenson nitrogen free agar (JNFA), *Azospirillum* in Dobereenier semi-solid medium, *Bradyrhizobium* in Yeast Extract Mannitol Agar and the *Bacillus* in Nutrient agar medium. The selected isolates were purified and maintained in King's B, JNF with glucose/ mannitol medium and nutrient agar slants respectively at 32° C.

Selection of BCA

In vitro screening of BCA against virulent fungal pathogen was conducted following dual culture technique (Dennis and Webstar, 1971; Bell *et al.* 1982). 20 ml of PDA medium was poured in sterilized 100 mm Petri plates. After solidification, 4 mm fungal discs cut out from the growing edge of 7 day old culture of both test antagonist and the pathogen were inoculated at a distance of 5 cm apart on the Petri plate. To study the combined inhibitory effect of two antagonists against the pathogen as also competition and antagonism among the antagonists if any, inoculums discs of two antagonists were placed at a distance of 5 cm in between the antagonist as well as pathogen disc.

Each treatment was replicated thrice and incubated in B.O.D incubator at 27°C. For screening bacterial antagonist *Azotobacter* and *Bacillus* Tryptone-glucose-yeast extract agar and for *Pseudomonas* modified Kings B medium supplemented with yeast extract powder @ of 3 g l^{-1} were used. 3 day old bacterial cultures were streaked on 20 ml medium in Petri plates at a distance of 5 cm from the 4 mm diameter disc of the fungal pathogen. The inoculated plates in triplicate were incubated in B.O.D incubator at 32°C. Plates without bacterial or fungal antagonist served as control. Per cent inhibition of pathogen, zone of inhibition and overlapped area of pathogen colony by the antagonist BCA colony was calculated after 7 days. Results were expressed in per cent increase/ decrease over control with the formula A-B/AX100 (Bandopadhyay, 2002). Compatibility for synergistic association among selected fungal and bacterial bioagents was also studied by dual culture method. For evaluating efficacy of compatibility after 7 days growth a 0-3 scale has been followed on the basis of difference of covered area between two bioagents where difference of covered area has been considered 0-20 cm^2 = Compatible (C), 21-40 cm^2 = Moderately Compatible (MC), and 41 cm^2 and above = Not Compatible (NC).

RESULTS

Pathogenicity test with *M. phaseolina* isolates revealed R 9 highly virulent and RK 2 less virulent pathotypes (Bandopadhyay *et al.*, 2004). Out of total 98 isolates of microbioagent from rhizosphere of jute plants screened for potential biocontrol agents, 15 *Trichoderma,* 8 *Gliocladium*, 10 *Penicillium,* and 15 *Aspergillus* isolates were found promising in antagonistic properties against the virulent pathogenic isolate R 9 of *M. phaseolina*. Among 15 *Trichoderma* isolates, *T. viride* JPT 1 showed maximum inhibition of the pathotype up to 53% and JPT 9 and JPT 14 52% each while less virulent pathotype RK 2 was inhibited by 72-74% over control (Table 1). *Trichoderma* JPT 16 formed larger inhibition zone of 0.9 cm and JPT 9 formed maximum overlapped colony area of 9.67 cm^2 against the virulent pathotype R 9. *Gliocladium* JPG 1 and JPG 9 revealed better inhibition up to 39% - 34% and 59% - 65% against R 9 and RK 2 respectively (Table 2). JPG 9 produced overlapping area of 8.55 cm^2 on R 9 colony. Among 15 isolates of *Aspergillus* A 26 produced maximum inhibition of 73.90% and 94.35% to R 9 and RK 2, followed by AN 15 showing 54.66% and 91.33% inhibition, AN 27 achieved 73.64%, 74.22% and A 7 showing 71.32% and 76.21% inhibition of R9 and RK 2 respectively (Table 3). *Penicillium* did not show promising result except PN 12 inhibiting R 9 by 72.0% and RK 2 by 77.0 % over control (Table 4). The PGPR fluorescent *Pseudomonas* Psfl-1 and Psfl-2 inhibited R 9 pathotype up to 64% -78% and RK 2 pathotype up to 70%-84% respectively (Table 5). Among 6 *Azotobacter* isolates Azbc-2 revealed highest inhibition of R 9 up to 69% with 0.3 cm inhibition zone and 1.15 cm^2 overlapped pathogen colony area followed by the highly efficient nitrogen fixer isolate Azbc-5 inhibiting R 9 up to 30% with 0.6 cm inhibition zone and 1.0 cm^2 overlapped pathogen colony area (Table 6). Out of 6 isolates of *Bacillus* species, BS-6 and BS-14 inhibited the virulent pathotype R 9 by 84% and 80% respectively. The bacterial isolates did not form inhibition zone or overlapped the pathogen colony (Table 7).

Compatibility for synergistic association was studied among the selected fungal antagonist. Out of 21 fungal antagonist combinations 8 were compatible the best being *Gliocladium* JPG 1 and JPG 9 with 6.2 cm^2 difference in area covered by both. Next in order were *Trichoderma viride* JPT 1 and *T. harzianum* JPT 9 with difference in area 10.95 cm^2, *T. viride* JPT 1 and *Aspergillus niger* AN27, and *T. harzianum* JPT 9 and *A. niger* AN 27 with 15.7 cm^2 difference in both, *T. viride* JPT 1 and *Gliocladium* JPG 1 (15.97 cm^2 difference), and *Gliocladium* JPG 1 and *A. niger* AN 27 (18.46 cm^2 difference). *Gliocladium* JPG 4 and *A. niger* AN 15 with 31.5 cm^2 difference was moderately compatible. Rests with more than 41 cm^2 difference in area covered were non compatible (Table 8).

Among 15 bacterial antagonists combinations 10 were compatible, of which fluorescent *Pseudomonas* Psfl-1 and phosphobactor *Pseudomonas striata* Pst-1 appeared best being completely merged with each other. Next in order were, N fixer *Azotobacter* sp. Azbc-2 and *Bacillus sp.* BS-6, *Bacillus* BS-6 and

BS-14, fluorescent *Pseudomonas* Psfl-2 and phosphobactor Pst-1, Pst-1 and Azbc-2, Pst-1 and BS-14, Psfl-1 and Psfl-2, Psfl-1 and Azbc-2, Psfl-1 and BS-14, and Psfl-2 and BS-6 combinations with 1.1 cm^2 – 15.7 cm^2 difference in area covered in dual culture. Fluorescent *Pseudomonas* Psfl-1 and *Bacillus* BS-6 combination with 26.26 cm^2 difference in area covered was moderately compatible (Table 9).

Total 28 promising fungal and bacterial antagonists' combinations were tested for efficacy in compatibility. Six antagonist fungal – bacterial combinations appeared compatible. Of the 6 compatible combinations *Aspergillus niger* AN 15 and phosphobactor *Pseudomonas striata* Pst-1 was most compatible with no difference in area covered by both. It was followed by *Trichoderma viride* JPT 1 and *Azotobacter* Azbc-2, *Trichoderma* JPT 1 and *fluorescent Pseudomonas* Psfl-1, *Aspergillus niger* AN 27 and *Azotobacter* Azbc-2, *Aspergillus* AN 15 and *Pseudomonas* Psfl-1, and *Gliocladium* JPG 1 and *Phosphobactor* Pst-1 in order with 5.06 – 15.70 cm^2 difference in area covered in dual culture. 10 combinations with 21.97 – 37.70 cm^2 difference were moderately compatible (Table 10).

The selected BCAs were also inoculated in combination against virulent pathotype. Isolates of *Trichoderma* JPT 9 and *Aspergillus* A 26 exhibited maximum inhibition of R 9 up to 78.60%. *Aspergillus* isolates A 7 and A 26 also exerted better

Table 1 : Interaction of different antagonist fungal isolates of *Trichoderma* with virulent R 9 and least virulent RK 2 pathotypes of *Macrophomina phaseolina in vitro*

Antagonist fungal isolates	Inhibition of *Macrophomina phaseolina*					
	Inhibition zone (cm)		Inhibition (%)		Overlapped colony (cm^2)	
Trichoderma	R 9	RK 2	R 9	RK 2	R 9	RK 2
JPT-1	—	—	53.00	74.00	0.24	4.52
JPT-2	0.65	0.50	50.00	72.00	2.98	1.54
JPT-3	—	—	44.00	61.00	—	2.81
JPT-4	—	—	35.00	57.00	2.40	6.38
JPT-5	—	—	40.00	62.00	1.77	8.04
JPT-6	—	—	46.00	58.00	0.38	3.44
JPT-7	—	—	43.00	64.00	0.07	7.54
JPT-8	—	—	49.00	68.00	0.44	2.01
JPT-9	—	—	52.00	73.00	9.67	5.37
JPT-12	—	—	40.00	63.00	0.05	7.80
JPT-14	—	—	52.00	72.00	0.38	7.80
JPT-15	—	—	40.00	68.00	0.78	9.09
JPT-16	0.9	0.55	51.00	71.00	4.34	1.04
Control	0.1	0.1	0.1	0.1	0.1	0.1
L.S.D (0.05)			13.00	17.17		

Table 2 : Interaction of antagonist fungal isolates of *Gliocladium* with *Macrophomina phaseolina in vitro*

Antagonist fungal isolates	Inhibition of *Macrophomina phaseolina*					
	Inhibition zone (cm)		Inhibition (%)		Overlapped colony (cm^2)	
Gliocladium	R 9	RK 2	R 9	RK 2	R 9	RK 2
JPG-1	0.15	—	39.00	59.00	4.91	3.30
JPG-2	4.5	—	33.00	33.00	5.30	3.46
JPG-3	—	0.2	18.00	32.00	—	—
JPG-4	—	—	24.00	36.00	5.30	1.54
JPG-5	—	—	30.00	54.00	—	2.41
JPG-6	0.5	—	26.00	40.00	3.63	0.50
JPG-7	—	0.25	21.00	41.00	0.10	—
JPG-9	—	—	34.00	65.00	8.55	2.41
Control	0.1	0.1	0.1	0.1	0.1	0.1
L.S.D (0.05)			9.16	15.93		

Table 3 : Interaction of fungal antagonist *Aspergillus* with *Macrophomina phaseolina in vitro*

Bioagent *Aspergillus*	Initial distance from pathogen (5.0 cm)	Inhibition (%) of *Macrophomina phaseolina*	
		R 9	RK 2
A-7	,,	71.30	76.21
A-12	,,	45.73	60.03
A-14	,,	42.75	71.08
AN-15	,,	54.66	91.33
A-18	,,	46.55	79.85
A-20	,,	42.41	55.25
A-23	,,	34.00	60.00
A-24	,,	53.76	69.14
A-26	,,	73.88	94.35
AN-27	,,	73.64	74.22
A-28	,,	52.55	76.10
A-29	,,	41.73	71.44
A-30	,,	42.41	59.18
A-31	,,	52.08	83.19
A-32	,,	37.59	22.27
Control	—	0.00	0.00
L.S.D. (0.05)		12.45	12.08

Table 8 : Compatibility between selected fungal antagonists

Bioagents (A: B)	Area covered 'A' (cm^2)	Area covered 'B' (cm^2)	Difference	Inference
JPT-1: JPG-4	50.24	3.14	47.10	NC
JPT-1: JPT-9	32.15	21.20	10.95	C
JPT-1: AN-27	12.56	28.26	15.70	C
JPT-1: A-26	0.50	55.38	54.88	NC
JPT-9: JPG-4	78.50	5.30	73.20	NC
JPT-9: A-26	78.50	12.56	65.94	NC
JPT-9: AN-27	12.56	28.26	15.70	C
JPG-1: JPT-1	8.03	24.60	15.97	C
JPG-1: JPG-9	16.60	22.80	6.20	C
JPG-1: JPG-4	28.26	78.50	50.24	NC
JPG-1: A-26	69.30	20.50	49.20	NC
JPG-1: AN-27	38.46	10.00	18.46	C
JPG-4: AN-27	3.14	78.50	75.36	NC
JPG-4:A-26	7.07	38.46	31.40	MC
AN-15:JPT-1	10.55	52.01	42.46	NC
AN-15:JPT-9	14.32	33.68	19.36	C
AN-15:JPG-1	32.84	52.22	19.38	C
AN-15:JPG-4	64.52	14.87	49.65	NC
AN-15:A-7	25.63	60.47	34.84	MC
AN-15:A-26	15.85	72.39	56.54	NC
AN-15:AN-27	27.56	51.62	24.06	MC

Table 4 : Interaction of fungal antagonist *Penicillium* with *Macrophomina phaseolina in vitro*

Bioagent *Penicillium*	Inhibition of *Macrophomina phaseolina*			
	Inhibition zone (cm)		Inhibition (%)	
	R 9	RK 2	R 9	RK 2
PN-4	0.5	0.6	14.00	46.00
PN-5	—	—	44.75	56.01
PN-7	—	—	26.61	32.40
PN-8	0.6	0.8	36.00	46.00
PN-10	0.6	1.1	38.00	54.00
PN-11	—	—	39.85	61.97
PN-12	—	—	72.00	77.00
PN-13	—	—	57.90	77.28
PN-14	—	—	6.90	55.85
PN-16	—	—	10.90	25.54
Control	0.1	0.1	0.1	0.1
L.S.D. (0.05)			13.06	18.07

Table10 : Compatibility between promising fungal and bacterial antagonists

Bioagents (A: B)	Area covered 'A' (cm^2)	Area covered 'B' (cm^2)	Difference	Inference
JPT-1: Psfl-1	26.42	31.5	5.08	C
JPT-1: Psfl-2	78.55	11.63	66.92	NC
JPT-1: Pst-1	35.23	7.54	27.69	MC
JPT-1:Azbc-2	26.42	31.5	5.08	C
JPT-9: Psfl-1	40.72	18.75	21.97	MC
JPT-9: Psfl-2	78.55	9.00	69.55	NC
JPT-9: Pst-1	36.30	5.30	31.00	MC
JPT-9: Azbc-2	40.72	18.75	21.97	MC
JPG-1: Psfl-1	33.16	9.6	23.56	MC
JPG-1: Psfl-2	78.5	2.54	75.96	NC
JPG-1: Pst-1	28.26	12.56	15.70	C
JPG-1:Azbc-2	35.30	7.00	28.30	MC
AN-15: Psfl-1	22.90	9.07	13.83	C
AN-15: Psfl-2	43.00	5.30	37.70	MC
AN-15: Pst-1	19.60	19.60	0.00	C
AN-15: Azbc-2	50.24	3.14	47.10	NC
A-26: Psfl-1	38.50	0.78	37.72	MC
A-26: Psfl-2	43.00	5.30	37.70	MC
A-26: Pst-1	50.24	3.14	47.10	NC
A-26: Azbc-2	78.55	9.0	69.55	NC
A-7: Psfl-1	45.34	1.53	44.04	NC
A-7: Psfl-2	52.78	2.54	50.24	NC
A-7: Pst-1	50.24	0.78	49.46	NC
A-7: Azbc-2	78.55	11.63	66.92	NC
AN-27: Psfl-1	42.90	5.30	37.60	MC
AN-27:Psfl2	58.00	1.53	56.47	NC
AN-27:Pst-1	60.00	15.00	45.00	NC
AN-27:Azbc-2	32.17	25.5	6.67	C

Difference of area covered between two bioagents : 0-20 cm^2 = Compatible (C) : 21-40 cm^2 = Moderately compatible (MC) 41cm^2 and above = Non compatible (NC)

Table 5 : Interaction of bacterial antagonist *Pseudomonas* with *Macrophomina phaseolina in vitro*

Bioagent *Pseudomonas*	Initial distance from pathogen (5.0 cm)	Inhibition of *Macrophomina phaseolina*			
		Inhibition (%)		Overlapped colony (cm^2)	
		R 9	RK 2	R 9	RK 2
Psfl -1	,,	65	70	2.4	1.77
Psfl- 2	,,	78	84	0.95	0.50
Psgl-M85	,,	52	70	3.8	1.77
Pst-1	,,	64	80	2.54	0.78
Control	—	0.1	0.1	100	100
L.S.D (1%)	—	13.17	7.74	0.89	0.22

Table 6 : Interaction of bacterial antagonist *Azotobacter* with *Macrophomina phaseolina in vitro*

Bioagent *Azotobacter*	Initial distance from pathogen (5.0 cm)	Inhibition of *Macrophomina phaseolina*					
		Inhibition (%)		Inhibition zone (cm)		Overlapped colony (cm^2)	
		R 9	RK 2	R 9	RK 2	R 9	RK 2
Azbc- 2	,,	69	80	0.3	2.6	1.15	1.0
Azbc- 3	,,	30	50	0.6	2.2	1.50	2.0
Azbc -4	,,	10	52	0.0	1.4	1.0	1.0
Azbc -5	,,	30	72	0.6	2.0	1.0	2.0
Azbc- 6	,,	46	68	1.8	2.6	0.8	1.2
Azbc -7	,,	54	68	1.6	3.0	1.6	3.0
Control	—	0.1	0.1	0.1	0.1	0.1	0.1
L.S.D (1%)		10.53	14.14	0.42	0.69	NS	0.63

Table 7 : Interaction of bacterial antagonist *Bacillus* with *Macrophomina phaseolina in vitro*

Bioagent *Bacillus*	Initial distance from pathogen (5.0 cm)	Inhibition of *Macrophomina phaseolina*					
		Inhibition (%)		Inhibition zone (cm)		Overlapped colony(cm^2)	
		R 9	RK 2	R 9	RK 2	R 9	RK 2
BS-2	,,	74	85	2.85	1.25	—	—
BS-3	,,	24	67	0.9	2.75	—	—
BS-6	,,	84	96	—	—	0.50	—
BS-7	,,	70	88	—	—	0.20	—
BS-14	,,	80	96	—	—	0.19	—
BS-16	,,	51	74	1.85	1.9	—	—
Control	—	0.1	0.1	0.1	0.1	100	—
L.S.D (1%)		12.17	10.60	0.37	0.375	0.1013	

inhibition up to 74.85%. Combined effect of *Trichoderma viride* JPT 1 and *Aspergillus* isolate A 26 was worth mentioning with 59.40% inhibition of the pathogen. Bacterial antagonist Psfl-1 and *Bacillus* sp. BS-6 in combination inhibited R 9 by 79.7%, 64.6%, followed by Psfl-1 and *Pseudomonas striata* Pst-1 up to 64.6%. Psfl-1 and *Azotobacter* Azbc-2 association inhibited R 9 by 54.5%. Fungal antagonist JPT 1 and *Azotobacter* Azbc-2 in association inhibited R 9 up to 61%. JPT 1 combined with PGPR fluorescent *Pseudomonas* Psfl-1inhibited R 9 up to 59%. *Gliocladium* isolate JPG 1 combined with Pst-1 also retarded growth of R 9 up to 51.5% (Fig. 1).

DISCUSSION

Plant growth promoting rhizobacteria (PGPR) of fluorescent *Pseudomonas* group repress the pathogen while stimulate plant growth producing siderophore and plant hormone in the rhizosphere (Kloepper *et al.*, 1980). *Azotobacter* and *Bacillii* also influence deleterious effect on many soil borne plant pathogens through production of siderophore and antibiotic compounds. *Trichoderma viride* has been effective as fungal antagonist against soil borne fungal pathogens associated with various crops (Elad *et.al.*, 1986; Elad, 2000). *Trichoderma* and *Gliocladium* suppress and antagonize fungal

Table 9 : Compatibility between selected bacterial antagonists

Bioagents (A: B)	Area covered 'A' (cm^2)	Area covered 'B' (cm^2)	Difference	Inference
Psfl-1: Pst-1	7.07	7.07	0.00	C
Psfl-1: Psfl-2	12.56	1.80	10.80	C
Psfl-1: Azbc-2	12.56	1.57	11.03	C
Psfl-1: BS-6	2.00	28.26	26.26	MC
Psfl-1: BS-14	4.90	19.60	14.70	C
Psfl-2:Pst-1	2.54	4.52	1.98	C
Psfl-2: Azbc-2	63.58	1.8	62.00	NC
Psfl-2: BS-6	12.56	28.56	15.70	C
Psfl-2: BS-14	3.14	50.24	47.10	NC
Pst-1: Azbc-2	4.90	0.50	4.40	C
Pst-1: BS-6	0.19	60.79	60.60	NC
Pst-1: BS-14	7.07	12.56	5.5	C
Azbc-2: BS-6	1.76	0.63	1.13	C
Azbc-2: BS-14	1.57	50.24	48.67	NC
BS-6 : BS-14	3.14	1.76	1.38	C

catecholate, hydroxamate and pyoverdin type of siderophores in antagonistic activities, rhizosphere competence, and plant growth promotion have been documented (Loper, 1988). Lytic enzymes pectinase, cellulase, chitinase, protease and urease and volatile compounds detected from fungal and bacterial antagonists tested are also responsible for inhibitory potential against the jute pathogen. Antagonistic property of *Trichoderma*, *Gliocladium* and *Aspergillus,* and fluorescent *Pseudomonas, Bacillii* and *Azotobacter* isolates when inoculated singly and in combination against *M. phaseolina* is well illustrated in the present study. *Penicillium* isolates do not show promising antagonistic property against the test pathogen. Therefore *Penicillium* isolates are not included in the studies

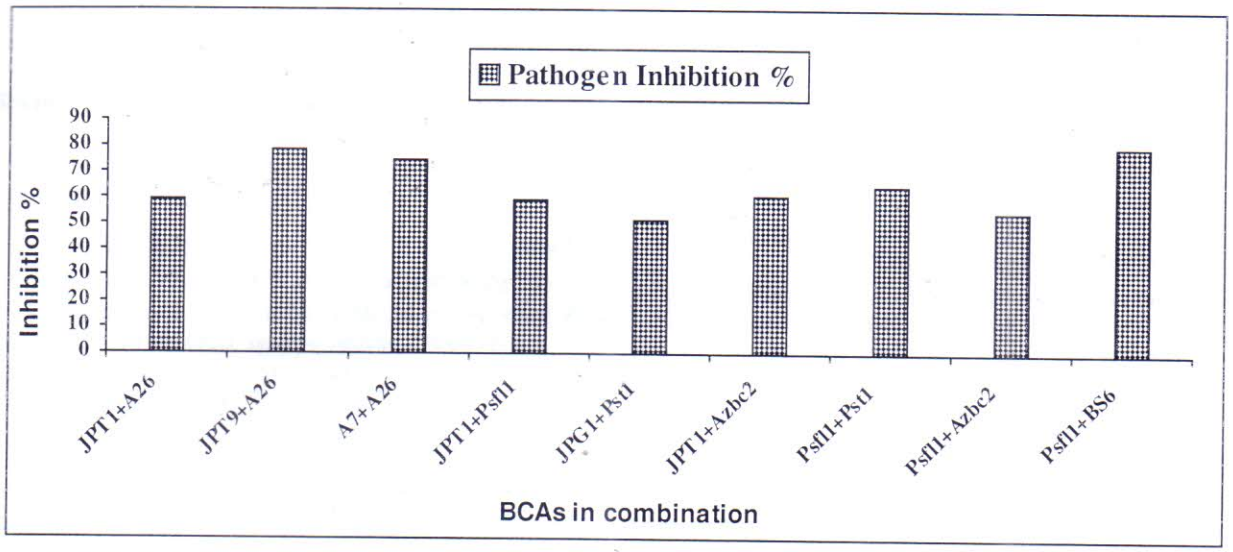

Fig. 1 : Interaction of BCAs in combination with *Macrophomina phaseolina in vitro*

pathogen either in competition for nutrients or mycoparasitism through mechanism of direct penetration and hyphal coiling or antibiosis by liberation of antibiotic compounds (Mukhopadhyay, 1987). Gliotoxin have effective role in inhibition of pathogen. Trichodermin, viridin and possibly other antibiotic like phenazine substances produced by the antagonists tested might have played major role in inhibiting the pathogen. Antifungal compound butanolide from *Trichoderma viride* and harzianolide from *T. harzianum* have been isolated (Claydon *et al.*, 1991). *Aspergillus niger* strain AN 27 produced butanolide with other compounds and both hydroxamate and catecholate groups of siderophores (Mondal and Sen, 1999). Role of for combined effect. Further studies on characterization of the active principles with the promising antagonists are in progress.

CONCLUSION

Isolation, conservation and exploitation of antagonist fungi viz. *Trichoderma, Gliocladium, Aspergillus,* and plant growth promoting rhizobacteria viz. fluorescent *Pseudomonas, Bacillus,* nitrogen fixer bacterial biofertilizer *Azotobacter* and phosphorus solubilizer *Pseudomonas striata* in compatible combinations will be practicable and ideal in the management of jute diseases. In this context *Trichoderma viride*

isolate JPT 1 alone or combination of JPT 1 with *Azotobacter* Azbc-2, *Gliocladium* JPG 1 with *Aspergillus* A 26, and PGPR fluorescent *Pseudomonas* Psfl-1 alone or with *Azotobacter* Azbc-2 in consortium have been proved to be highly effective and likely to be acceptable as eco-friendly technology for formulation of biopesticide.

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