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Suppression of seedling blight of jute with bacterial antagonists

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Macrophomina phaseolina, a soil borne plant pathogen, has a wide host range causing root-rot, stem rot, collar rot and seedling blight diseases of various crop plants. Jute plant were used as test crop in sick soil inoculated with *M. phaseolina* in our present investigation. *M. phaseolina* were grown in sand maize meal medium and introduced in earthen pots of 30 cm diameter in glasshouse. All the five bacterial isolates, namely BS-12, BS-14, BS-17, BK-1 and BM-1 identified as *Basillus* sp., inhibited growth of *M. phaseolina* causing seedling blight of jute. All the six fungicides namely bavistin, kavach, dithane M-45, saff, krilaxyl and blitox reduced seedling blight. Bavistin and krilaxyl performed better as compared to other fungicides in sick soil. *Trichoderma* sp. and *Pseudomonas fluorescencs* reduced the disease intensity significantly by 47.57% and 47.91% respectively.

Key words : Jute, *Macrophomina phaseolina*, *Bacillus* sp. *P. fluorescens* ; *Trichoderma* sp.

INTRODUCTION

Jute is a bast fibre obtained from the stem of the plant. The two species of *Corchorous* i.e. *Corchorus capsularis* and *C. olitorius* are grown extensively in many parts of North India and most extensively in West Bengal, Bihar and U.P. The seedling blight of jute is caused by *Macrophomina phaseolina* (Maubl) Ashby. The disease is soil as well as seed borne. The primary inoculum is received from infected seed and soil in the form of sclerotia. In recent years there has been much success in obtaining biological control of plant pathogen using bacterization techniques (Weller, 1988). *Bacillus* spp. have great potentialities as biocontrol agent. In our present investigation 5 isolates of *Bacillus* sp., *Trichoderma* sp. and *Pseudomonas fluorescens* were used as biocontrol agent against seedling blight of jute caused by *Macrophomina phaseolina.*

MATERIALS AND METHODS

The experiment was conducted in the glass house to study the antagonistic effect of the bacterial isolates against seedling blight of jute. Jute seedling of JRO-524 (Nabin) was used as test plants. Bacterial isolates of *Bacillus* sp. namely BS-12, BS-14, BS-17, BK-1 and BM1, which were taken from Bacteriology laboratory, Department of Plant Pathology, BCKV. *Pseudomonas fluorescens* and *Trichoderma*, sp., a commercial product were taken from ''Krishi Rashayan'' which were also used for our present studies. *Macrophomina phaseolina,* the test pathogen was isolated from naturally infected jute seedlings. Few fungicides namely bavistin (0.1%), dithane M-45 (0.2%), kavach (0.2%), saff (0.1%), krilaxyl MZ (0.1%) and blitox (0.1%) were also used in our studies. Bacterial isolates were grown in Nurient agar medium (NA) and in Nutrient both. *Macrophomina phaseolina* was grown in Potato Dextrose Agar (PDA) medium for maintenance. Mass culture of the fungus was necessary for conducting the pot experiment for assaying the antagonistic activity of the bacteria towards the disease. For this purpose *M. phaseolina* was first incubated in the conical flask containing potato dextrose broth along with some broken glass

pieces. The flask were shaken at intervals for breaking of mycelium mat into smaller fragments and thus a mycelial suspension was prepared. Sand maize meal (sand 70 g ; crushed maize meal 30 g and required amount of water) medium was prepared in polypropylene packets and inoculation of this medium was done by injecting the fungal suspension (10 ml) by sterilized syringe. All the inoculated media i.e., both bacterial isolates and fungus were incubated at 30°C ± 1°C for 15 days for mass culture.

Garden soil and farmyard manure in 5 : 1 ratio were mixed and filled in flat eathen pots of 30 cm diameter. Fifteen days old culture of *Macrophomina phaseolina* grown on sand maize meal medium was thoroughly mixed with soil of the pot at the rate of 200 g/pot to prepare sick pot.

The disease symptoms were generally observed after 4 days of sowing. Death of seedling were recorded upto 9th day of sowing. During that period moist condition of soil were maintained.

RESULTS AND DISCUSSION

All the test bacterial isolates were gram positive, spore forming and rod shaped, belonged to size range of 1.5-4.0 × 0.5-1.5 µm. Comparing the characters described in Bergey's Manual of Determinative Bacteriology (9th Edition, 1984) the test bacteria were primarily identified as *Bacillus* sp.

Soil application of liquid culture of *Bacillus* sp. before / after sowing

Five isolates of *Bacillus* sp. were grown for seventy two hours in PD broth medium. The bacterial suspensions of about 200 ml were separately added to each sick pot. Commercial product of *Trichoderma* sp. *and P. fluorescens* @ 10 g/L were also applied. The population of the bacterial suspension was estimated using Mc. Farland Scale. i.e. 9×10^8 cells / ml. Jute seeds were sown in rows just before 24 h and after 24 h of application of antagonists. The pots were kept in glass house and sterile water was added regularly. Four replications were maintained for each treatments and control plants also. Mortality of seedlings was recorded from 4th day of sowing and continued upto 8th day of sowing. Results were presented in Table 1.

As regards present disease control, the treatment

Table 1 : Effect of soil application of antagonist before and after seed sowing on percent disease control of seedling blight of jute.

Bacterial Antagonist	Replications								Mean	
	R_1		R_2		R_3		R_4			
	X	Y	X	Y	X	Y	X	Y	X	Y
B. subtilis										
BS-12	40.00	31.37	36.74	34.12	31.19	24.80	37.86	30.50	37.09	33.28
	(39.23)	(34.02)	(37.29)	(35.73)	(33.90)	(29.87)	(37.94)	(33.52)		
BS-14	50.88	45.00	42.72	36.85	46.02	44.75	46.34	40.15	42.96	40.18
	(45.46)	(42.13)	(40.08)	(37.35)	(42.71)	(41.96)	(42.88)	(39.29)		
BS-17	41.00	36.00	36.18	30.18	30.79	26.20	31.00	31.00	25.00	32.73
	(39.82)	(36.87)	(36.93)	(33.27)	(33.65)	(30.79)	(33.83)	(33.83)	(30.00)	
BK-1	55.00	48.75	60.79	54.80	50.80	49.80	52.00	44.37	47.66	44.65
	(47.87)	(44.26)	(51.18)	(47.75)	(45.46)	(44.89)	(46.15)	(41.73)		
BM-1	51.80	46.69	46.78	41.82	44.65	38.43	42.34	32.41		
	(46.03)	(43.05)	(43.11)	(40.28)	(41.90)	(38.29)	(40.57)	(34.70)	42.90	39.08
Trichoderma sp.	49.00	39.82	56.37	42.75	54.52	48.12	58.12	50.80		
	(44.43)	(39.11)	(48.62)	(40.80)	(47.58)	(43.91)	(49.66)	(45.46)	47.57	42.32
P. fluorescens	54.67	48.15	59.00	53.00	51.79	44.15	55.00	46.02		
	(47.64)	(43.91)	(50.18)	(46.72)	(45.97)	(41.61)	(47.87)	(42.82)	47.91	43.76
Control (Ino)	20.35	14.50	14.79	6.79	10.34	8.67	12.45	10.34		
S.Em ±									0.73	1.34
CD at 5%									1.53	2.81
CD at 1%									2.09	3.84
Percent survival in control									14.48	10.07

Angular transformed values are in parenthesis

X = Before ; Y = After

showed remarkable variations. *Trichoderma* sp. and *P. fluorescens* reduced the disease intensity significantly by 47.57% and 47.91% respectively. Strains of *Bacillus* namely BK-18, and BS-14 showed their ability in reducing disease intensity significantly by 47.66% and 42.96% respectively.

Similar trends were observed where liquid culture of *Bacillus* sp. were applied on soil on day after sowing. Here the population of the bacteria was estimated using Mc Farland Scale as 12×10^8 cells/ml. (Table 1).

Soil application of fungicides before / after seed sowing

The fungicides namely bavistin (0.1%), blitox (0.1%) krylaxyl (0.1%), kavach (0.2%), dithane M-45 (0.2%) and saff (0.1%) were added to each sick pot separately before 24 h of seed sowing @ 200 ml/pot. Reduction in disease intensity was recorded in all the treatments. Significant control was observed where plants were treated with blitox (61.69%) followed by the fungicide saff (58.72%)

Soil application of fungicides after 24 h of sowing maintaining the same concentrations and doses and also following the similar methods of applications as before. Disease control was recorded maximum in case of bavistin followed by krylaxyl. It was also clear from the above findings that soil application of fungicides one day after seed sowing was better in recducing disease incidence (Table 2).

Zhu *et al.* (1992) reported that soil application of carbendazim (50%) one day after sowing gave 90% control in reducing damping off disease csused by *R. solani.* In this finding bavistin (0.1%) one day after sowing gave 73.67% control in reducing seedling blight.

Gram positive bacteria (*Bacillus*) has the potential to control various diseases of agricultural crops caused by pathogenic fungi, bacteria and viruses. This bacteria is not only the appealing candidate for biological control of plant diseases but also this plays an important role in promotion of plant growth, root and shoot dry weights and yield. That the inoculation with *Bacillus* sp. decreased disease intensity of seedling blight of jute was already known. Biological control of *M. phaseolina* by *Bacillus subtilis* on chickpea was studied by Siddiqui and Mohmood (1993). Gabr *et al.* (1998) indicated that three isolates of *Bacillus subtilis* were able to suppress root rot disease (*M. phaseolina*) of sesame when applied artificially in infested soil.

Table 2 : Effect of soil application of Fungicides before and after seed sowing on percent disease control of seedling blight of jute.

Fungicides	Replications								Mean	
	R_1		R_2		R_3		R_4			
	X	Y	X	Y	X	Y	X	Y	X	Y
Bavistin	66.42	95.21	65.62	93.24	64.48	90.48	71.44	88.93	54.92	73.67
	(54.57)	(77.34)	(54.09)	(74.88)	(53.37)	(71.95)	(57.67)	(70.54)		
Krylaxyl	71.28	81.63	76.44	87.66	67.21	87.25	65.00	83.41	56.81	67.24
	(57.54)	(64.60)	(60.94)	(69.38)	(55.06)	(69.04)	(53.73)	(65.96)		
Kavach	68.80	72.42	72.41	68.24	67.60	64.81	63.81	67.18	55.67	55.64
	(56.07)	(58.31)	(58.31)	(55.67)	(55.30)	(53.61)	(55.01)	(55.00)		
Dithane M-45	64.81	59.21	65.20	64.52	69.21	62.19	69.88	57.28	55.10	51.21
	(53.61)	(50.30)	(53.85)	(53.43)	(56.29)	(52.00)	(56.66)	(49.14)		
Blitox	70.40	73.62	76.00	74.66	80.88	80.40	82.22	71.28	61.69	60.02
	(57.04)	(59.08)	(60.67)	(59.74)	(64.01)	(63.72)	(65.05)	(57.54)		
Saff	70.40	76.41	70.48	79.68	74.80	75.41	76.43	69.58	58.72	60.21
	(57.04)	(60.94)	(57.04)	(63.15)	(59.87)	(60.27)	(60.94)	(56.48)		
Control (Ino)	71.6	94	57.7	86	63.6	82	68.9	89		
S.Em ±									0.53	1.05
CD at 5%									1.12	2.23
CD at 1%									1.55	3.08
Percent survival in control									65.4	87.75

Angular transformed values are in parenthesis

X = Before ; Y = After

Inhibition of *M. phaseolina* by *Trichoderma*, and *Ps. fluorescens* was already known and indicated by several workers. Ushamalani *et al.* (1997) indicated that *T. harzianum* and *T. viride* and *Ps. fluorescens* were the most effective and inhibited *M. phaseolina* significantly. Reguchander *et al.* (1998) indicated that *T. viride* greatly reduced the sclerotial number of *Macrophomina* and root rot incidence of soybean. A challenge of the pathogen by the antagonists has been expressed. The possibility of using a high *Bacillus* inoculum for lowering the pressure of stem rot disease of jute due to *Macrophomina phaseolina* (Maubl) Ashby is clearly expressed from the present work. This has opened up a area of possible use of *Bacillus* sp. not only as plant growth promoting rhizobacteria but also in a competition in the soil against the plant pathogen to lower the disease pressure.

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