Effect of Biocontrol Agent Consortia for Ecofriendly Management of Stem and Root Rot in Olitorius Jute caused by *Macrophomina phaseolina*

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ABSTRACT

Stem and root rot in jute caused by *Macrophomina phaseolina* hold a great problem in managing the diseases since the pathogen is seed, soil and air borne. Biological control of the diseases can be successfully utilized especially within the framework of integrated disease management system. Three biocontrol agents namely *Trichoderma viride*, *Aspergillus niger* (AN 27) and *Pseudomonas fluorescens* were evaluated for their growth promoting ability in jute under in vitro condition. Significantly higher vigour index was observed when *T. viride* and *P. fluorescens* were applied in consortia, whereas, *A. niger* had low plant growth promoting ability irrespective of consortia used. Field experiment was conducted in two consecutive years to assess the integrated management of *M. phaseolina* induced stem and root rot by application of three bioagents through seed treatment or soil application either single or in consortium with seed treating fungicide carbendazim. The biocontrol agents applied in consortium through seed treatment and soil application was significantly superior management option to reduce stem and root rot although at par with the treatment where the bioagents were applied through seed treatment and soil application integrated with fungicide seed treatment. Fibre yield increased significantly when bioagents particularly T. *viride* and P. *fluorescens* applied individually or in consortia integrated with carbendazim resulting 60-83% increase in fibre yield in comparison to control.

Keywords: Jute, *macrophomina phaseolina*, biocontrol agent consortia, growth promotion, disease management.

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Jute (*Corchorus* sp. L.) is one of the most important bast fibre crops grown in extensive areas of terai region of West Bengal during pre kharif season. The major constrains of jute fibre production is *Macrophomina phaseolina* incited disease complex comprising of damping off, seedling blight, stem rot, root rot and collar rot among which stem and root rot are most prevalent in this region. Primarily the pathogen is soil borne, however, it can also survive in

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seed and during growing season the pycnidia of the fungus spreads through wind and makes it difficult to manage. The disease is endemic in all jute growing areas which results in considerable yield loss and deterioration of fibre quality as well. Approximately 40-50% crop losses have been reported due to stem rot which appear late in the season when the condition is hot and humid (Mandal 1990). Late infection is also responsible for contamination of seeds which in turn act as primary source of inoculum during next season. The extent of stem rot epidemic can be gauged from primary infection as secondary infection is usually four times the primary infection (Anonymous 2001-2006). Seed treatment or spraying with carbendazim has been recommended for effective chemical management of the disease. However, considering the growing concern for health and environmental hazards, resistance problem, resurgence of new biotypes, lethal effect on beneficial microorganisms, alternative control strategies such as biological control as one of the major components in integrated disease management is desirable. Antagonistic fungal isolates of *Trichoderma, Gliocladium, Aspergillus*, *Penicillium* and PGPR isolates like fluorescent *Pseudomonas*, *P. striata*, *Bacillus* and *Azotobacter* showed promise by inhibiting virulent isolates (R 9) of *M. phaseolina* in addition to substantial plant growth promotion under in vitro condition (Bandyopadhyay *et al.,* 2004). Moreover, application of multiple antagonists provides better disease control over individual antagonist for exhibiting broad spectrum of activities (Bandopadhyay and Bandopadhyay 2004). Several reports have been documented on potential of the bioagents against *M. phaseolina* in different crops under field condition but information is very meager in jute-*M. phaseolina* interaction. Hence, the present investigation was aimed to develop suitable integrated management strategy for control of stem and root rot in jute by using three popular biocontrol agents namely *T. viride*, *A. niger* and *P. fluorescens* in consortium mode under field condition. Since the biocontrol agents beside acting as antagonistic microorganisms are also known for their plant growth promoting ability, therefore, in the present study plant growth promotion in jute by the influence of the bioagents was also evaluated.

MATERIALS AND METHODS

Plant growth promoting ability of bioagents. Three biocontrol agents namely *T. viride, A. niger* and *P. fluorescens* in talc based formulation were obtained from Central Research Institute of Jute and Allied Fibres, Barrackpore, West Bengal, India containing viable propagules of 1×10^9 colony forming units per g of formulation. The plant growth promoting ability of the bioagents was measured by rolled paper towel method and calculating the vigour index. The seeds of olitorius jute variety JRO 524 (Nabin) were surface sterilized with 0.1% mercuric chloride solution and then treated with talc based formulation either single, double or triple combination of three bioagents in equal proportion @ 10g/kg of seed in total. This treated seeds were then placed on wet paper towel followed by covering with another paper towel. This paper towels were rolled and finally placed in plastic bags after putting rubber band at both ends. The seeds for control were soaked with sterile water and placed on wet paper in same arrangement. These rolls were placed in seed germinator at 25°C with 12 hrs light exposure. After 10 days of incubation the root length, shoot length, germination percentage were recorded for calculating vigour index by the equation VI = (Root length + shoot length) × germination % (Abdul Baki and Anderson 1973).

Field trials with consortium of bioagents. Field experiment was conducted at Instructional farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar in randomized block design during pre-kharif season of two consecutive years (2008 and 2009). Three biocontrol agents namely T. viride, P. fluorescens and A. niger were applied through seed treatment and or soil application either alone or in consortium mode of two or three bioagents whereas, seed treatment with carbendazim @2g/kg of seed was considered as farmer's practice. The biocontrol agents were applied as seed treatment either individually or in consortium mode @10g/kg of seed whereas, for soil application they were mixed with the soil at root zone @12kg/ha at 15 days after sowing. For untreated check, neither biocontrol agents nor carbendazim was applied. Seeds of olitorius variety JRO 524 (Nabin) after seed treatment with bioagent(s) and/or carbendazim were sown in 3m×4m plot with 25cm×6cm spacing and normal agronomic practices were followed during crop period. The stem rot and root rot incidence were recorded at harvesting stage. Stem rot incidence expressed in percent disease index (PDI) and was worked out on the basis of actual damage that may cause loss in fibre yield. For this purpose numerical rating was done on actual damage in individual plant, where some specific factors were taken under consideration. The factors were as follows and numerical rating for each factor is given accordingly within bracket. (I) Size of lesion where, 1= minor dots or lesion <0.5 cm^2, 2= lesion size 0.6-1.0 cm^2, 3= lesion size 1.1-2.0 cm^2 and 4= lesion size more than 2.0 cm^2, (II) position of the lesion on the stem, where 1= 1st quarter at the top, 2= 2nd quarter from top, 3= 3rd quarter from top and 4= last quarter at the bottom, (III) lesion type where 1 = lesion covering less than 10% of the stem diameter, 2 = lesion covering 10.1-25% of stem diameter, 4 = lesion covering 25.1-40% of the stem diameter and 8 = lesion covering >40% of the stem diameter. So the maximum and minimum score value of an affected plant are 16 and 3, respectively and the unaffected plant is assigned as 0. PDI was calculated by the formula, PDI= (sum of total numerical rating × 100) ÷ (Number of plants observed × highest value).

Root rot incidence was calculated as percentage value of affected plant in population of specified area. The fibre yields was recorded and mean percent increase in yield over two years was calculated to sort out the most effective management strategy for Macrophomina disease complex.

The replicated data generated from different experiments were analyzed statistically using statistical package of MSTATC and the ANOVA determined the probability for significant variation among the treatments.

RESULTS

The plant growth promoting ability of different biocontrol agents either singly or in consortia revealed that significantly higher root and shoot length could be achieved when *T. viride* and *P. fluorescens* were applied in consortia. The single application with *T. viride* and *P. fluorescens* exhibited shoot length statistically at par with consorted use of the two bioagents (Table 1). Further significantly higher germination percentage was also recorded where *T. viride* and *P. fluorescens* applied in consortium resulting in significantly higher vigour index, whereas, use of *A. niger* showed poor plant growth promoting ability irrespective of individual or consorted combination.

Table 1. Plant growth promotion ability of biocontrol agents

Treatments	Root length (mm)	Shoot length (mm)	Germination (%)	Vigour Index
T. viride	13.87	26.43	95.67	3856.07
P. fluorescens	13.33	25.73	95.33	3724.60
A. niger	12.50	23.50	93.67	3372.47
T. viride + *P. fluorescens*	16.60	27.50	98.33	4337.17
P. fluorescens + *A. niger*	13.20	24.97	94.33	3600.00
T. viride + *A. niger*	12.83	24.30	92.33	3428.67
T. viride + *P. fluorescens* + *A. niger*	13.23	25.23	92.33	3549.53
Control	10.27	20.63	89.67	2768.90
SEm(±)	0.81	0.65	0.86	93.17
CD(P=0.05)	2.45	1.96	2.60	283.41

The field experiment over two years on ability of different bioagents and carbendazim as seed treating fungicide in integrated manner (Table 2) revealed that during first year experiment stem rot severity and root rot incidence was quite high as the selected experimental plot was moderately sick with *M. phaseolina* and further introduction of bioagents during second year made them fit with the ecosystem for better activity as reflected through greater reduction in stem rot severity and root rot incidence. Beside that, environmental factors particularly lower rainfall distribution throughout the growing period during 2009 may also be responsible for comparatively low occurrence of diseases as shown in control plots. Lowest stem rot severity was observed where three bioagents viz., *T. viride*, *P. fluorescens* and *A. niger* were applied in consortium through seed treatment and soil application which was significantly at par with the treatment where *T. viride, P. fluorescens* and *A. niger* were applied through seed treatment and soil application integrated with carbendazim seed treatment. *T. viride* and *A. niger* as individual bioagents

Table 2. Integrated management of stem rot and root rot of olitorius jute by use of consortium of bioagents

Treatment	Stem rot (PDI)			Root rot (%)			Fibre yield (q/ha)		
	2008	2009	% reduction over control on mean of 2008 & 2009	2008	2009	% reduction over control on mean of 2008 & 2009	2008	2009	% increase over control on mean of 2008 & 2009
T1: ST with T. viride + SA of T. viride	19.73 (26.37)*	6.61 (14.89)	46.55	1.20 (6.29)	0.88 (5.38)	87.25	23.77	30.56	49.53
T2: ST with A. niger + SA of A. niger	19.84 (26.45)	10.42 (18.82)	38.55	1.97 (8.07)	1.55 (7.15)	78.43	23.20	29.93	46.18
T3: ST with P. fluorescens + SA of P. fluorescens	20.01 (26.57)	8.37 (16.73)	42.36	2.13 (8.39)	3.70 (11.09)	64.34	23.34	30.82	49.09
T4: ST with T. viride + Carbendazim	21.29 (27.48)	11.32 (19.65)	33.75	2.21 (8.55)	1.52 (7.08)	77.08	28.15	34.26	71.82
T5: ST with A. niger + Carbendazim	21.93 (27.92)	13.80 (21.78)	27.42	3.39 (10.61)	2.63 (9.33)	63.11	22.63	28.35	40.29
T6: ST with P. fluorecens + Carbendazim	28.66 (32.37)	14.11 (22.05)	13.12	2.73 (9.51)	4.86 (12.74)	53.43	27.47	33.02	66.43
T7: ST with T. viride + Carbendazim + SA of T. viride	10.26 (18.68)	5.96 (14.12)	67.06	0.00 (0.00)	0.55 (4.25)	96.69	29.13	35.89	78.92
T8: ST with A. niger + Carbendazim + SA of A. niger	13.22 (21.32)	6.25 (14.47)	60.44	0.40 (3.63)	1.28 (6.50)	89.71	29.07	35.64	78.04
T9: ST with P. fluorescens + +SA of P. fluorescens + Carbendazim	18.12 (25.19)	7.50 (15.88)	47.97	1.00 (5.74)	1.48 (6.99)	84.80	28.66	34.44	73.58
T10: T1+T2+T3	9.23 (17.69)	5.59 (13.63)	69.90	0.00 (0.00)	0.00 (0.00)	100.00	29.17	37.57	83.65
T11: T4+T5+T6	18.67 (25.60)	9.69 (18.13)	42.40	2.10 (8.33)	1.19 (6.26)	79.78	28.70	35.39	76.33
T12: T7+T8+T9	15.63 (23.29)	6.65 (14.90)	54.75	2.12 (8.37)	0.74 (4.93)	82.48	26.86	31.54	59.71
T13: ST with carbendazim	25.55 (30.36)	8.64 (17.01)	30.54	3.90 (11.39)	2.60 (9.28)	60.17	22.21	27.78	37.53
T14: Control	30.76 (33.28)	18.47 (25.44)	-	9.20 (17.66)	7.11 (15.46)	-	16.19	20.15	-
CD (P=0.05)	6.85	2.07		2.70	3.09		4.43	6.04	

Figures in parentheses are angular transformed values; ST- seed treatment; SA- soil application applied through seed and soil treatment along with carbendazim were also effective in reducing the disease. Among the bioagents *T. viride* irrespective of combination and method of application provided considerable reduction in stem rot severity. Similar trend was observed in case of root rot, where all the integrated treatments were found to be significantly effective in reducing the incidence, however, considering the potentiality the biocontrol agents *T. viride* was found superior irrespective of combination with other two bioagents and carbendazim. Fibre yield increased significantly when bioagents particularly *T. viride* and *P. fluorescens* applied individually or in consortia integrated with carbendazim resulting 60-83% increase in fibre yield in comparison to control. *A. niger* applied alone through seed treatment and soil application had significantly less promise in relation to fibre yield as a consequence of reduced plant growth promotion compared to *P. fluorescens* and *T. viride*.

DISCUSSION

Biological control of *Macrophomina* diseases complex using antagonistic fungi and bacteria can be successfully utilized especially within the frame work integrated disease management was documented by (Sanker and Jayarajan 1996, Bandyopadhyay, 2002). *A. versicolor* multiplied in compost medium for 10 days controlled *M. phaseolina* induced root rot in jute by 56 percent in pot culture (Bhattacharya *et al.,* 1985). Seed treatment with *T. viride* was reported as effective protection against M. phaseolina through precolonization of infection site by the antagonists and it also helped to increase plant vigour (Suriachandraselvan *et al.,* 2004). Combination of T. harzianum and A. versicolor resulted in 31% reduction of dry root rot in mung bean caused by M. phaseolina (Choudhary *et al.,* 2010). Different combinations of Trichoderma, Azotobacter, Aspergillus and fluorescent Pseudomonas controlled effectively stem and root diseases in jute and increased plant biomass with fibre yield (Roy *et al.,* 2008). The efficacy of Trichoderma and plant growth promoting rhizobacteria in reducing several soil borne diseases along with increased plant vigour has been attributed to the ability of the strains to produce siderophores, lytic enzymes, volatile and non volatile antibiotics or growth regulators (Bandyopadhyay *et al.,* 2007). Carbendazim although is the most effective fungicide for control of *M. phaseolina* induced diseases in several crop (Jaiman *et al.,* 2009) and may particularly be integrated with *P. fluorescens* (Khan and Gangopadhyay 2007), however, carbendazim is sensitive to *Trichoderma* spp. at 0.5 ppm (Papavizas, 1985). Integration of bio-control microflora with carbendazim at sub lethal doses certainly has some advantage in controlling the *M. phaseolina* incited diseases in jute. In the present study, seed treatment and soil application of biocontrol agents especially *T. viride* and *P. fluorescens* in consortium mode proved to be an effective integrated management system for stem and root rot in jute.

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