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Growth Promotion of Transplanted Rice Plant by Fungal and Bacterial Bioagents Effective against Brown Leaf Spot Caused by *Drechslera oryzae*

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The experiment was conducted to evaluate growth promotion of transplanted rice by fungal and bacterial bioagents effective against brown leaf spot of rice. Bioagent formulations (*T. viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis*) were applied as seed treatment and foliar spray and compared with untreated check. All bioagent formulations were significantly effective in increasing number of tillers per hill, root length, fresh and dry root weight, fresh and dry shoot weight and grain yield over check. Present study revealed that potential fungal and bacterial bioagents effective against brown leaf spot disease of rice, exhibited plant growth promoting activities and resulted, 21.69 to 28.77 reducing the disease severity and 34.42 to 40.99 increase in grain yield over untreated check, even if there is no disease.

Keywords: Brown leaf spot, bioagents and rice.

Brown leaf spot of rice is one of the major fungal disease of rice which occurs in almost all the rice growing areas (R.S. Singh, 2005). The disease is of great importance in several countries and has been reported to cause considerable losses. In India, disease occurs sporadically. The disease is currently managed through application of chemical fungicides, which is highly recalcitrant, toxic and non - (Dath, 1990). The antagonistic as fungal and bacterial bioagents are reported to induce the growth of various crop plants (Singh and Sinha, 2008; Singh *et al*., 2012). These responses may be due to: (i) Suppression of deleterious root micro – flora including those not causing obvious disease, (ii) Production of growth stimulating factors (hormones or growth factors) and / or (iii) Increased nutrient uptake takes through solubilization and sequestration of nutrients and / or enhanced root growth. Enhanced root development is also helpful in tolerating the biotic and abiotic stresses by the plants. Spore / cell suspension as well as dry powder has been used to coat the seeds with potential antagonists (Chao *et al.*, 1986)., For commercial purpose, dry powders of antagonists are used @ 3 to 8 g powder per kg seed based on seed size and formulations of antagonists (Mukhopadhyay, *et al.*, 1992). A large number of seed, seedling, root, stem, foliar and panicle diseases have been suppressed by seed treatment with antagonists. Mukhopadhyay (1996) reported increased growth of several crop plants following seed treatment with *T. harzianum* and *T. virens*. Antagonists also stimulate plant growth, even if there is no disease, which results in better yield (Mishra and Sinha, 2000). Different fungal and bacterial bioagents were found effective in promoting growth and reducing the disease

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severity of rice. In the present study, experiment was conducted to evaluate plant growth promoting activities of potential fungal and bacterial bioagents (effective against brown leaf spot of rice) on transplanted rice.

MATERIALS AND METHODS

The field trials were conducted in a randomized block design (RBD) with three replications and plot size of 4m2 (spacing 15cm X 20cm) on rice variety Pusa 1121 at Central Research Farm, Sam Higginbottom Institute of Agriculture Technology & Sciences Allahabad during Kharif 2013 and 2014. The doses of NPK fertilizers were applied and other cultural operations done as recommended for rice (Anon., 1996). Test plots was bounded all around to prevent the movement of water from one plot to another.

Seed treatment with bioagents formulation for raising nursery: Nursery was raised in separate plots in the field. Bioagents formulation (10^6 cfu / g) were applied as seed treatment (ST) at the rate 4 g / kg seed. Treated seeds were soaked in tap water for 12 h. For untreated check, seed were soaked in sterilized water. Plots were arranged in randomized and irrigated for puddling after puddling rice seed was sown in separate plots to raise nursery.

Preparation of bioagents formulation for foliar spray: The talc based fungal and bacterial bioagents was obtained from IPM Referral Lab, Department of Plant Pathology & Entomology SHIATS Allahabad. Spray of bioagents @ 10 g/l (10^6 cfu) was initiated, just after appearance the disease. The observation of disease severity was recorded near the maturity stage. Disease severity (DS) was estimated by following formula.

$$\text{Disease severity \%} = \frac{\text{Sum of all rating}}{\text{Maximum rating} \times \text{number of sample leaves}} \times 100$$

DS was estimated according to the disease index established by 0 to 9 scales (Anonymous 2011).

Decreasing the disease severity % was estimated by following formula

$$\text{Decreasing the disease severity \%} = \frac{\text{DS in control} - \text{DS in Treatments}}{\text{DS in control}} \times 100$$

Growth parameters, three plants were selected randomized of each treatments plant were uprooted for recording data on number of tillers per hills, root and shoot length, fresh root and shoot weight, dry root and shoot weight. The grain yield was recorded after harvesting of the crop transformed into quintal per hectare. Increasing grain yield (%) was estimated by following formula.

$$\text{Increasing yield \%} = \frac{\text{Yield in treatments} - \text{Yield in control}}{\text{Yield in cintrol}} \times 100$$

RESULTS AND DISCUSSION

Effect of bioagent formulations (applied as ST + FS) on shoot and root length and number of tillers per hill of rice plant 40 DAT

All bioagent formulations were significantly effective (72.86 to 83.80 cm) in increasing average shoot length over check (57.66 cm) (Table 1). Maximum shoot length (83.80 cm) was obtained by T_7 which is followed by T_6 (81.49 cm) and T_4 (79.35 cm). All the bioagent formulations were significantly effective (14.60 to 15.97 cm) in increasing root length over check (12.73 cm). Maximum root length (15.97 cm) was obtained with T_3 which is followed by T_4 (15.57 cm) and T_7 (14.76 cm). All bioagent formulations and chemical treatment was significantly effective (28.33 to 32.51) in increasing average numbers of tillers per hill over check (22.56). Maximum number of tillers per hill (32.51) in number of tillers per hill was obtained by T_7 which is followed by T_6 (31.42) and T_4 (28.62). Dubey (1995) reported a significant increase in root and shoot length, fresh and dry weight and yield of cultivated crops due to seed bacterization with *Pseudomonas*. Gopalakrishnan *et al* (2012) also reported the *Pseudomonas* is growth promoting bacteria which is responsible for cell elongation and root developments.

Effect of bioagent formulations (applied as ST + FS) on fresh and dry root weight, fresh and dry shoot weight of rice

Data presented in table 2, revealed that all bioagents were significantly effective in increasing fresh and dry root weight over check. Maximum fresh root weight (40.35 g) was exhibited by T_3 which is followed by T_4 (39.73 g) and T_7 (38.96 g). Maximum dry root weight (19.71 g) was shown by T_3. T_4 and T_7 were next in order to increasing dry root weight by 19.70 and 17.80 g, respectively. Significantly increased fresh and dry shoot weight over check. Maximum fresh shoot weight (199.17 g) was obtained by T_7 which is followed by T_6 (198.34 g) and T_4 (194.51 g). Similarly, maximum dry

shoot weight (49.70) was obtained by T_7. T_6 and T_4 were next in order to increasing dry shoot weight by 49.43 g and 48.32 g, respectively. The capacity of *Trichoderma spp*. to produce growth hormones such as auxins and gibberelins were reported as the main factor that contributes to the ability of *Trichoderma spp.* to support root growth and increase water absorption from soil (Contreras - Cornejo *et al*., 2009; Martínez - Medina *et al*., 2011). Increased fresh and dry weight of shoot, roots and nodules of broad bean was also reported by Yehia *et al.*, 1985 when seeds were coated with

Table 1. Effect of bioagents formulation (applied as seed treatments and foliar spray) on shoot length, root length and number of tillers of rice.

Treatments		Shoot length (cm)	Root length (cm)	Number of tillers
T_1	Control	57.66	12.73	22.56
T_2	*T. viride* (ST) + *P. fluorescens* (FS) @ 4 g/kg seed + 10 g/liter	78.21	14.73	28.57
T_3	*T. viride* (ST) + *Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter	77.56	15.97	28.40
T_4	*T. harzianum* (ST) + *P. fluorescens* (FS) @ 4 g/kg seed + 10 g/liter	79.35	15.57	28.62
T_5	*T. harzianum* (ST) + *B. subtilis* (FS) @ 4 g/kg seed + 10 g/liter	73.96	14.46	28.37
T_6	*P. fluorescens* (ST) + *T. viride* (FS) @ 4 g/kg/seed + 10 g/liter	81.49	14.66	31.42
T_7	*P. fluorescens* (ST) + *T. harzianum* (FS), @ 4 g/kg/seed + 10 g/liter	83.80	14.76	32.51
T_8	*B. subtilis* (ST) + *T. viride* (FS) @ 4 g/kg seed + 10 g/liter	72.86	14.60	28.33
T_9	*B. subtilis* (ST) + *T. harzianum* @ 4 g/kg seed + 10 g/liter	78.52	14.70	28.41
	C.D (0.05)	3.58	0.54	0.40

*Average of two year with 3replication.
T. = Trichoderma, P. = Pseudomonas, B. = Bacillus, ST = Seed Treatment and FS = Foliar Spray.

Table 2. Effect of bioagents formulation (applied as seed treatments and foliar spray) on fresh shoot, root, dry shoot and root weight of rice.

Treatments		Fresh root weight (g)	Dry root weight (g)	Fresh shoot weight (g)	Dry shoot weight (g)
T_1	Control	31.34	15.29	185.16	43.30
T_2	*T. viride* (ST) + *P. fluorescens* (FS) @ 4 g/kg seed + 10 g/liter	38.66	17.34	194.03	48.32
T_3	*T. viride* (ST) + *Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter	40.35	19.71	193.10	48.01
T_4	*T. harzianum* (ST) + *P. fluorescens* (FS) @ 4 g/kg seed + 10 g/liter	39.73	19.70	194.51	48.32
T_5	*T. harzianum* (ST) + *B. subtilis* (FS) @ 4 g/kg seed + 10 g/liter	38.59	17.24	193.00	48.31
T_6	*P. fluorescens* (ST) + *T. viride* (FS) @ 4 g/kg/seed + 10 g/liter	38.87	17.69	198.34	49.43
T_7	*P. fluorescens* (ST) + *T. harzianum* (FS), @ 4 g/kg/seed + 10 g/liter	38.96	17.80	199.17	49.70
T_8	*B. subtilis* (ST) + *T. viride* (FS) @ 4 g/kg seed + 10 g/liter	35.23	17.17	192.75	47.81.
T_9	*B. subtilis* (ST) + *T. harzianum* @ 4 g/kg seed + 10 g/liter	38.66	17.46	193.11	48.61
	C.D (0.05)	3.35	0.16	1.04	0.14

*Average of two year with 3replication.
T. = Trichoderma, P. = Pseudomonas, B. = Bacillus, ST = Seed Treatment and FS = Foliar Spray.

Table 3. Effect of bioagents formulation (applied as seed treatments and foliar spray) on disease severity and yield of rice.

Treatments		Disease intensity (%)	Reducing disease severity (%)	Yield q/ha	Increasing yield (%)
T_1	Control	48.68	-	30.47	-
T_2	*T. viride* (ST) + *P. fluorescens* (FS) @ 4 g/kg seed + 10 g/liter	38.12	21.69	40.96	34.42
T_3	*T. viride* (ST) + *Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter	38.07	21.79	41.38	35.80
T_4	*T. harzianum* (ST) + *P. fluorescens* (FS) @ 4 g/kg seed + 10 g/liter	36.67	24.67	42.41	39.18
T_5	*T. harzianum* (ST) + *B. subtilis* (FS) @ 4 g/kg seed + 10 g/liter	37.10	23.78	41.60	36.52
T_6	*P. fluorescens* (ST) + *T. viride* (FS) @ 4 g/kg/seed + 10 g/liter	34.67	28.77	42.96	40.99
T_7	*P. fluorescens* (ST) + *T. harzianum* (FS), @ 4 g/kg/seed + 10 g/liter	37.41	23.15	41.57	36.42
T_8	*B. subtilis* (ST) + *T. viride* (FS) @ 4 g/kg seed + 10 g/liter	36.81	24.38	41.69	36.82
T_9	*B. subtilis* (ST) + *T. harzianum* @ 4 g/kg seed + 10 g/liter	37.02	23.95	41.68	36.79
	C.D (0.05)	1.35		0.88	

*Average of two year with 3replication.
T. = Trichoderma, P. = Pseudomonas, B. = Bacillus, ST = Seed Treatment and FS = Foliar Spray.

T. viride. This may be because of production of growth stimulating factors by the bioagents (Doni *et al.* 214).

Effect of bioagent formulations (applied as ST + FS) on disease severity and yield of rice

Data indicate the table no 3, all bioagent formulations were significantly effective in reducing disease severity (38.12 to 34.67 %) over check (48.68 %). Maximum reducing disease severity (28.77 %) was exhibited by T_6 which is followed by T_4 (24.67 %), T_8 (24.38 %) and minimum reducing disease severity (38.12 %) was obtained by T_2. All bioagent formulations were significantly effective in increasing grain yield (42.96 to 40.96 q / ha) over check (30.47 q / ha). Maximum increase (40.99 %) in grain yield was exhibited by T_6 which was followed by T_4 (39.18 %) and T_8 (36.82 %). Biocontrol agent combination study Rao *et al.* (2013) *Pseudomonas fluorescens* and *T. harzianum* were found effective against brown leaf spot disease of paddy. Potential of *T. harzianum* and *T. viride* was also tested against brown spot of paddy where substantial decrease in disease severity was observed (Biswas *et al*., 2010). Kloepper *et al.* (1980) reported that *P. flourecens* and other plant growth promoting rhizobacteria enhance plant growth by showing antagonisms to potentially deleterious rhizoplane fungal and bacterial pathogens. The direct application of microorganism to seed or other plant part gives them a competitive advantage over pathogen that must compete for nutrients and sites for attachment prior to infection.

CONCLUSION

It was revealed from present study that bioagent formulations which were had potential for the management of brown spot disease were also exhibited plant growth promoting activity on rice plant when applied as combination of seed treatment and foliar application and resulted in significantly higher grain yield (40.96 to 42.96 g as compared to untreated check 30.47 g), even if there is no disease. Results of present investigation should be verified under field conditions.

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