Effect of bioagent application time against bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Uyeda and Ishiyama) Dowson

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Abstract: Present investigation was carried out to test the effect of time of application on efficacy of *Trichoderma harzianum* and *Pseudomonas fluorescens* formulations against bacterial leaf blight of rice, under field condition. Foliar sprays with bioagent(s) in three time sequences i.e. 7 days before, simultaneously and 7 days after inoculation of *Xanthomonas oryzae* pv. *oryzae* inoculation significantly reduced disease severity over check during Kharif 2006 and 2007. Maximum reduction in bacterial leaf blight disease was obtained with the application of *T. harzianum* on 7 days before inoculation of pathogen during Kharif 2006 and 2007 and maximum increase in grain yield was obtained with the application of PBA-2 seven days before inoculation of pathogen. It was revealed that prophylactic spray of bioagents one week prior to pathogen inoculation was significantly (10^6 cfu/g) effective in reducing disease severity of bacterial leaf blight of rice as compared to chemical treatment.

Keywords: Bacterial leaf blight of rice, *Pseudomonas fluorescens*, Time of application, *Trichoderma harzianum*, *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

Bacterial leaf blight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Uyeda and Ishiyama) Dowson is the disease of great economic importance in all rice growing areas of the world and is particularly destructive in South East Asia (Mew *et al*., 1993 and FAOSTAT, 2002). The disease is known to occur in epidemic proportions in many parts of the world. The disease has been reported from almost all the rice growing states of India where it causes severe yield loss (Singh, 1972). Several bacterial plant pathogens are reported to exhibit antibiotic resistance (McManus *et al*., 2002). Non-availability of effective chemical control measures and inconsistent perform-ance of resistant rice varieties have forced the plant pathologists to new approaches of disease management. Interest in biological management has increased considerably in the recent past due to their added advantages over the other methods of disease management. Besides disease management, bioagents also stimulate plant growth, even if there is no disease, which results in better yield (Mishra and Sinha, 2000). Antagonistic potential of different bioagents against bacterial leaf blight of rice has been reported by several workers (Vidhyasekaran *et al*., 2001; Manmeet and Thind, 2002; Babu and Thind, 2005; Palaniyandi *et al*., 2006; Gangwar and Sinha, 2012a,b,c and Gangwar, 2013a,b). Time of application of bioagents may have effect on efficacy of bioagents as these are living entity and need a period of time for upsurge optimum population and establish on host. The level of management of disease depends on time of application of bioagents. Influence of time of application of bioagents in plant disease management was studied by several workers including Sindhan *et al*. (1997) and Vidhyasekaran *et al*. (2001). Present study was carried out to test the effect of time of application on efficacy of *T. harzianum* and *P. fluorescens* formulations against bacterial leaf blight of rice under field conditions.

MATERIALS AND METHODS

The experiment was conducted at Crop Research Center, G. B. Pant University of Agriculture and Technology, Pantnagar.
Field experiment: Layout of experimental plot was prepared according to randomized block design (RBD). Nursery of susceptible rice cultivar Jaya was raised separately and 21 days old seedling were transplanted in experimental plots. General agronomic practices were followed for cultivation of experimental plots. This experiment was carried out in Kharif sessions, 2006 and 2007.
Mass multiplication of fungal and bacterial bioagents and preparation of formulation: Two commercial formulations of *T. harzianum* (PBA-1) and *P. fluorescens* (PBA-2) obtained from Bio-control Laboratory, Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar and formulation of *T. harzianum* (rice leaf isolate), were evaluated. *Trichoderma* spp. was mass

multiplied on barnyard millet: *Echinocloa frumentacae* (local name: Jhangora) grains. Jhangora grains colonized by *Trichoderma* were air dried in open shade and ground with the help of Willy Mill to get fine powder. This powder was passed through 50 and 80 mesh sieves, simultaneously to obtain spore powder. The formulations of fungal bioagent was prepared by diluting spore powder with talcum powder (mesh = 350 with 95% whiteness) and 1% carboxyl methyl cellulose (CMC) to get desired concentration (10^6 cfu/g) of bioagents in the formulation.

Pathogen inoculation and application of bioagent formulations: Pathogen was inoculated at maximum tillering stage by clipping off the leaf tip @ 10^6 cell/ml inoculum (Kauffman *et al.*, 1973). *T. harzianum* formulation and three commercial formulations *viz.* PBA-2, PBA-3 and Sudocel were also used in the present study (Table 1). All bioagent formulations and chemical treatment [streptocycline (0.03 g/l water) + copper oxychloride (1 g/l water)] were applied against bacterial leaf blight disease on transplanted rice. Foliar spray of bioagents and chemical treatment were given on three time sequences *viz.* one week before, simultaneously and one week after pathogen inoculation. Bioagents were applied as foliar spray @ 10 g/l, while check plots were sprayed with water only. Each treatment was replicated thrice.

Data collection: Data was recorded as percent disease severity on artificially inoculated leaves (average 50 leaves/plot) at 21 and 28 days after treatment application. After harvesting, yield components (number of filled grains per plants, grain yield and 1000 grain weight) were recorded.

Statistical analysis: Statistical analysis of the data obtained from field experiment was done using appropriate programme as per the requirement of the experiment. The critical difference (CD) was calculated at 5% level of significance for comparison of difference between the means of different treatments.

RESULTS AND DISCUSSION

Effect of bioagent application time on disease severity: All three time of bioagent application significantly reduced (upto 53.80 and 54.72 %) mean disease severity over check during Kharif 2006 and 2007,
respectively (Table 2). Application of bioagents, 7 days before pathogen (*X. oryzae* pv. *oryzae*) inoculation was as effective as chemical treatment in reducing mean disease severity during Kharif 2006. All bioagent formulations were found to be significantly effective in reducing mean disease severity (53.88 to 44.11%) over check (71.33%). *Trichoderma harzianum* (44.11%) and PBA-2 (45.11%) were most effective and statistically equal to chemical treatment (43.00%) in order to reducing mean disease severity as compared to check. All

bioagent formulations were significantly effective in reducing mean disease severity over check at all the three times of bioagent application. All bioagent formulations inoculation (41.66 to 32.33%) were statistically as effective as chemical treatment (42.00%) when these were applied 7 days before pathogen inoculaion. *T. harzianum* (43.33%), PBA-2 (43.33%) and PBA-3 (48.33%) were also found to be statistically equal to chemical treatment (41.66) in reducing mean disease severity even when applied simultaneously with pathogen inoculation. *T. harzianum* exhibited maximum percent reduction (53.80, 39.47, and 21.62%) in disease severity at all three time of application (7 days before, simultaneously and 7 days after pathogen inoculation, respectively).

During Kharif 2007, all three time of bioagent application (61.72 to 44.63 %) were significantly effective in reducing mean disease severity over check (73.44 %). Mean disease severities in all treatments (56.50 to 44.11 %) were significantly lower than check (73.44 %). The effectivity of *T. harzianum* (44.11 %), PBA-2 (46.11 %) and PBA-3 (48.55) was statistically equal to chemical treatment (48.05 %). Application of bioagent formulations, 7 days before pathogen inoculation was significantly superior then chemical treatment in order to reducing disease severity. Statistically, *T. harzianum* (32.83 %) and PBA-2 (35.00 %) were found to be better than chemical treatment (43.33). Sudocel (44.33 %) and PBA-3 (40.33 %) was equally effective to chemical treatment (43.33 %) in decreasing disease severity when applied 7 days before pathogen inoculation. At simultaneous application of bioagent with pathogen inoculation, *T. harzianum* (42.16 %), PBA-2 (45.83 %) and PBA-3 (49.16 %) exhibited statistically equal effectivity to chemical treatment (42.50 %) in reduction of mean disease severity.

When bioagent formulations were applied 7 days after pathogen inoculation, PBA-3 (56.16 %), *T. harzianum* (57.33 %) and PBA-2 (57.50 %) was found equal to chemical treatment (58.33 %) in the reduction of disease severity but all of these were found significantly effective over check (75.00 %). Highest percent reduction (54.39 %) in disease severity was

Table 1. List of bioagent formulations and fungal/bacterial bioagants.

Bioagent formulations	Fungal/bacterial bioagants
Trichoderma harzianum	*T. harzianum* (rice leaf isolate)
PBA-2	*Pseudomonas fluorescens* PBAP-27
PBA-3	*T. harzianum* PBAT-43 + *P. fluorescens* PBAP-27
Sudocel*	*P. fluorescens*

* Obtained from market.

Table 2. Effect of time of application of bioagent formulations and chemical treatment on bacterial leaf blight disease severity (After 28 days of treatment application), applied as foliar spray under field conditions.

Treatments	Disease severity (%)							
	Kharif 2006				Kharif 2007			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
PBA-2	35.00 (36.27)	43.33 (41.13)	57.00 (49.02)	45.11 (42.14)	35.00 (36.26)	45.83 (42.57)	57.50 (49.31)	46.11 (42.71)
PBA-3	40.00 (39.21)	48.33 (44.04)	57.33 (49.22)	48.55 (44.15)	40.33 (39.42)	49.16 (44.52)	56.16 (48.54)	48.55 (44.16)
Sudocel	41.66 (40.19)	56.66 (48.83)	63.33 (52.79)	53.88 (47.27)	44.33 (41.73)	59.16 (50.28)	66.00 (54.38)	56.50 (48.80)
T. harzianum	32.33 (34.64)	43.33 (41.15)	56.66 (48.83)	44.11 (41.54)	32.83 (34.95)	42.16 (40.48)	57.33 (49.25)	44.11 (41.56)
Streptocycline + copper oxychloride	42.00 (40.39)	41.66 (40.19)	45.33 (42.31)	43.00 (40.96)	43.33 (41.16)	42.50 (40.68)	58.33 (49.80)	48.05 (43.88)
Check	70.00 (56.83)	71.66 (57.90)	72.33 (58.29)	71.33 (57.67)	72.00 (58.06)	73.33 (59.00)	75.00 (60.07)	73.44 (59.04)
Mean	43.50 (41.25)	50.83 (45.54)	58.66 (50.08)	51.00 (45.62)	44.63 (41.93)	52.02 (46.26)	61.72 (51.89)	52.79 (46.69)
CD at 5%	A (Time of bioagent application) = 2.89				A (Time of bioagent application) = 2.86			
	B (Treatments) = 4.09				B (Treatments) = 4.05			
	A × B = 7.09				A × B = 7.01			

*Mean of three replications; Figure in parentheses is angular transformed; T_1 = Application of bioagent, 7 days before pathogen inoculation; T_2 = Application of bioagent, simultaneously with pathogen inoculation; T_3 = Application of bioagent, 7 days after pathogen inoculation.

Table 3. Effect of time of application of bioagent formulations and a chemical treatment on number of filled grains per plant, applied as foliar spray under field conditions.

Treatments	No. of filled grains/ plant							
	Kharif 2006				Kharif 2007			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
PBA-2	2368	2247	1917	2177	2354	2225	2015	2198
PBA-3	2321	2267	1929	2172	2346	2168	1939	2151
Sudocel	2460	2252	2023	2245	2359	2212	1904	2158
T. harzianum	2361	2333	2092	2262	2378	2364	2133	2292
Streptocycline + copper oxychloride	2060	2045	1953	2019	2036	2155	1977	2056
Check	1872	1872	1872	1872	1882	1893	1806	1860
Mean	2240	2169	1964	2124	2254	2169	1962	2119
CD at 5%	A (Time of bioagent application) = 82				A (Time of bioagent application) = 84			
	B (Treatments) = 117				B (Treatments) = 120			
	A × B = ns				A × B = ns			

*Mean of three replications; T_1 = Application of bioagent, 7 days before pathogen inoculation; T_2 = Application of bioagent, simultaneously with pathogen inoculation; T_3 = Application of bioagent, 7 days after pathogen inoculation.

Table 4. Effect of time of application of bioagent formulations and a chemical treatment on grain yield (q/h), applied as foliar spray under field conditions.

Treatments	Grain yield (q/h)							
	Kharif 2006				Kharif 2007			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
PBA-2	30.77	28.40	27.93	29.03	28.48	27.25	26.79	27.50
PBA-3	29.82	26.50	26.03	27.45	27.32	28.23	26.49	27.35
Sudocel	28.87	26.50	25.08	26.82	27.17	26.41	25.26	26.28
T. harzianum	29.35	27.45	25.45	27.42	26.66	27.62	26.54	26.94
Streptocycline + copper oxychloride	26.03	26.50	25.56	26.03	25.52	26.22	25.77	25.84
Check	23.66	23.66	23.66	23.66	23.69	23.61	24.19	23.83
Mean	28.08	26.50	25.62	26.73	26.47	26.56	25.84	26.29
CD at 5%	A (Time of application) = 1.33 B(Treatments) = 1.88 A × B = ns				A (Time of application) = ns B(Treatments) = 1.60 A × B = ns			

*Mean of three replications; T_1= Application of bioagent, 7 days before pathogen inoculation; T_2= Application of bioagent, simultaneously with pathogen inoculation; T_3= Application of bioagent, 7 days after pathogen inoculation

Table 5. Effect of time of application of bioagents and a chemical treatment on 1000 grain weight, applied as foliar spray under field conditions.

Treatments	1000 grain weight (g)							
	Kharif 2006				Kharif 2007			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
PBA-2	26.00	25.27	25.02	25.43	26.16	25.27	25.22	25.55
PBA-3	25.88	25.70	24.61	25.39	25.75	25.31	24.95	25.34
Sudocel	25.79	25.13	24.56	25.16	25.68	25.13	24.51	25.11
T. harzianum	25.65	25.36	25.20	25.40	25.70	25.3	25.25	25.41
Streptocycline + copper oxychloride	23.18	24.40	21.83	23.14	23.47	24.36	21.80	23.21
Check	18.09	18.23	18.43	18.25	18.38	18.21	18.56	18.38
Mean	24.09	24.01	23.28	23.79	24.19	23.93	23.38	23.83
CD at 5%	A(Time of application) = 0.69 B(Treatments) = 0.98 A × B = ns				A (Time of application) = ns B(Treatments) = 1.06 A × B = ns			

*Mean of three replications; T_1= Application of bioagent, 7 days before pathogen inoculation; T_2= Application of bioagent, simultaneously with pathogen inoculation; T_3= Application of bioagent, 7 days after pathogen inoculation.

observed with *T. harzianum* when applied 7 days before pathogen inoculation. However, PBA-3 was exhibited highest reduction (25.11 %) in disease severity when applied 7 days after pathogen inoculation. Higher effectivity of some bioagents over others probably associated with its ability to grow and sporulate and therefore become established in large number on host surface.

Similar results were also observed by Gangwar and Sinha, 2012c under glasshouse conditions. Sindhan *et al.* (1997) also reported that 7 days pre-inoculation application of antagonists provided significantly greater protection of the bacterial leaf blight disease as compared to post-inoculation. Twenty four hours pre-inoculation application of rice phylloplane microflora was found significantly effective in reducing bacterial leaf blight disease as compared to 0 h and 24 h post-inoculation of in the studies of Saikia and Chowdhary (1992). Vidhyasekaran *et al.* (2001) observed that when the rice leaves were inoculated with *X. oryzae* pv. *oryzae* 4 days after *P. fluorescens* application, on the flower leaves. The disease intensity was decreased from 6.7 to 1.1 and when rice seeds were treated with the formulation of *P. fluorescence* PF 1 and sown, 30 day old seedling showed resistance to *X. oryzae* pv. *oryzae* and the disease intensity decreased from 6.8 to 1.2.

Effect of bioagent application time on filled grains per plant: Effect of all the three time of bioagent applications on increasing average number of filled grains per plant was significantly higher (2177 to 2262 and 2151 to 2292) than untreated check (1872 and 1860), during Kharif 2006 and 2007 respectively (Table 3). All bioagents (2177 to 2262) were found to be significantly effective over chemical treatment (2019) and check (1872) in increasing mean number of filled grains per plant, during Kharif 2006. When bioagents were applied 7 days before or simultaneously with pathogen inoculation, they showed significantly higher efficacy (2321 to 2460) as compared to chemical treatment (2060) in increasing mean number of filled grains per plant. When these bioagents were applied 7 days after pathogen inoculation, they (1917 to 2092) were significantly as effective as chemical treatment (1953) in increasing mean number of filled grains per plant. At 7 days before pathogen inoculation, maximum percent increase (31.38 %) in number of filled grains was obtained with Sudocel. *T. harzianum* resulted in maximum increase (11.73 %) in number of filled grains per plant even when applied 7 days after pathogen inoculation.

All bioagents were found to be significantly effective (2151 to 2292) in increasing mean number of filled grains per plant as compared to check (1860), during Kharif 2007. *Trichoderma harzianum* (2292) and PBA -2 (2198) showed higher effectivity over chemical treatment (2056) in increasing mean number of filled grains per plant. PBA-3 (2151) and Sudocel (2158)

were statistically at par with chemical treatment (2056). Application of bioagent formulations 7 days before (2254) and simultaneously with pathogen inoculation (2169) significantly increased mean number of filled grains per plant over chemical treatment (2036 and 2155, respectively). However, application of bioagent formulations 7 days after pathogen inoculation (1904 to 2133) was as effective as chemical treatment (1977). Maximum increase (26.35, 24.84 and 18.10%) in mean number of filled grains was recorded with *T. harzianum* at all three time of application (7 days before, simultaneously and 7 days after pathogen inoculation, respectively). Similarly, influence of bioagent application on number of filled grains was recorded by Gangwar and Sinha (2012c) under glasshouse conditions.

Effect of bioagent application time on grain yield and 1000 grain weight: All bioagent formulations were significantly effective in increasing mean grain yield (26.82 to 29.03 and 26.28 to 27.50 q/h) and 1000 grain weight (25.16 to 25.43 and 25.11 to 25.55 g) as compared to check, during Kharif 2006 and 2007 respectively (Table 4 and 5). Mean grain yield (25.62 to 28.08 q/h) and 1000 grain weight (23.28 to 24.19 g) at different time of bioagent application were also higher than check, during Kharif 2006 and 2007. Bioagent formulation, PBA-2 exhibits significantly higher mean grain yield (29.03 q/h) over chemical treatment (26.03 q/h) whereas, all other bioagent formulations resulted mean grain yield statistically equal to chemical treatment. All bioagent formulations gave significantly increased mean 1000 grain weight (25.16 to 25.43 g) over chemical treatment (23.14 g). When bioagent formulations were applied 7 days before pathogen inoculation, significantly higher mean grain yield (28.87 to 30.77 q/h) was recorded as compared to chemical treatment (26.03 q/h). Application of bioagent formulation simultaneously or 7 days after pathogen inoculation resulted in increasing mean grain yield were found to be statistically equal to chemical treatment. Increase in mean 1000 grain weight was significantly higher than chemical treatment (23.14 g), when bioagent formulations were applied 7 days before (24.09 g) and simultaneously with pathogen inoculation (24.01 g). Increase in mean 1000 grain weight was found statistically equal to chemical treatment when bioagent formulations were applied 7 days after pathogen inoculation. At all three time of application, PBA-2 exhibited maximum percent increase (30.05, 20.03 and 18.04%, respectively) in grain yield. Maximum percent increase in 1000 grain weight (43.72%) was recorded with PBA-2, when applied 7 days before pathogen inoculation. *T. harzianum* exhibited maximum increase (36.74%) in 1000 grain weight even when applied 7 days after pathogen inoculation.

All the bioagent formulations were significantly effective in enhancing mean grain yield (26.28 to 27.50 q/h) and 1000 grain weight (25.11 to 25.55 g),

during Kharif 2007. PBA-2 (27.50 q/h) showed significantly higher mean grain yield over chemical treatment (25.84 q/h) while, mean grain yield recorded with all other bioagent formulations (26.28 to 27.35 q/h) were statistically equal to chemical treatment. All the bioagent formulations were exhibited significantly higher mean 1000 grain weight (25.11 to 25.55 g) over chemical treatment (23.21 g). Maximum increase in grain yield (20.21%) and 1000 grain weight (42.96%) was observed with PBA-2, when applied 7 days before pathogen inoculation. PBA-2 resulted in maximum increase in grain yield (10.76%), while *T. harzianum* resulted in maximum increase in 1000 grain weight (36.48%) when applied 7 days after pathogen inoculation. Similarly, enhanced grain yield and 1000 grain weight was reported by Gangwar and Sinha (2012c) due to application of bioagent formulation 7 days before inoculation of pathogen under glasshouse conditions. Fungal and bacterial bioagent formulation were found to be effective in increasing grain yield and 1000 grain weight over check and chemical treatment in study carried out in glasshouse by Gangwar and Sinha (2012b).

Conclusion

On the basis of results of present investigation, it was concluded that different time of application had varied effect on efficacy of bioagent formulations. Prophylactic spray of bioagents one week before pathogen inoculation was significantly effective against bacterial leaf blight of rice as compared to chemical treatment. Application of bioagents one week prior to symptom appearance (7 days after pathogen inoculation) was effective against bacterial leaf blight as compared to check. However, the observed results need verification on large scale rice planting.

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