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Efficacy of bioagents and botanicals against brown spot

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ABSTRACT

Efficacy of four bioagents and two botanicals namely Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens, Bacillus subtilis, neem leaf extract and neem oil were evaluated against brown spot of rice under field and lab condition. In field condition bioagents and botanicals were used as seed treatment and foliar spray. The seed treatment (ST) with Trichoderma viride @ 4g/kg seed + foliar spray (FS) with Pseudomonas fluorescens @ 10 g/liter of water was found effective with less disease severity of 34.39% and higher grain yield of 68.60 q/ha. Under in vitro condition in dual culture technique, Trichoderma viride was found most effective in inhabiting the growth of Helminthosporium oryzae (61.72%). In case of botanicals, neem oil @ 3% was observed best effective in inhibiting the growth of the pathogen (54.25%) under poisoned food technique.

Key words: *Brown spot, bioagents, botanicals and rice*

Brown spot of rice caused by *Helminthosporium oryzae* (Breda de Haan) Subram and Jain is one of the major fungal diseases of rice which occurs in almost all the rice growing areas (Singh, 2005). The disease is of great importance in several countries and has been reported to cause considerable loss. In India, this disease occurs sporadically. Recently its incidence has increased in cultivated varieties especially under rice – wheat cropping system (Geeta *et al.*, 2001). Continuous, inappropriate and indiscriminative use of chemicals is known to cause undesirable effects such as residual toxicity, development of pathogen resistance to fungicides, environmental pollution, health hazards to humans and animals and increased expenditure for plant protection (Ansari, 1995). Instead, plant pathologists have focused in developing environmentally safe, long-lasting and effective biocontrol and botanical methods for the management of plant diseases. Various fungal, bacterial biocontrol agents and botanicals are known for their mycoparasitic, antagonistic and antifungal mechanism for the control of fungal diseases particularly brown spot. The present study was undertaken to evaluate the efficacy of fungal, bacterial and botanicals as seed treatment and foliar spry as well as to test the mycoparasitic, antagonistic and antifungal activity of certain fungal, bacterial and plant extracts against *Helminthosporium oryzae*, the causal organism of brown spot of riceunder *in vitro* condition.

MATERIALS AND METHODS

The field experiment was conducted in randomized block design (RBD) with plot size 2×2m^2 and there were three replications for each treatment. The experiment was conducted with taking variety Pusa Basmati 1121 during summer season of 2013 and 2014. Treatments taken were *T. viride* (ST) + *Pseudomonas fluorescens* (FS) @ 4 g/kg seed + 10 g/liter, T. *viride* (ST) +*Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter; *T . harzianum* (ST) +*Pseudomonas fluorescens* (FS) @ 4 g/kg seed + 10 g/liter; *T. harzianum* (ST) + *Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter; *Pseudomonas fluorescens* (ST) + *T. viride* (FS) @ 4 g/kg seed + 10 g/liter; *Pseudomonas fluorescens* (ST) + *T. harzianum* (FS); @ 4 g/kg seed + 10 g/liter, *Bacillus subtilis* (ST) + *T. viride* (FS) @ 4 g/kg seed + 10 g/liter; *Bacillus subtilis* (ST) + *T. harzianum* @ 4 g/kg seed + 10 g/liter, Neem leaf extract (ST) + neem

oil (FS) @ 4 g/kg seed + 10 g/liter; neem oil (ST)+neem leaf extract (FS) @ 4 g/kg seed + 10 g/ liter and control. Seed treated before sowing of seedling and foliar spray (FS) of bioagents and botanicals were initiated, three times at an interval of 15 days starting from 30 days after sowing just after appearance of the disease.

The pure culture of pathogen was collected from IARI, New Delhi. Mass multiplication of pathogen was carried out in sorghum grains. Grains of sorghum was soaked in the boiling water for 30 minutes and then autoclaved at 15 ibs/inch^2 pressure for two consecutive days. On cooling of medium each flask was inoculated with mycelial bit (3 mm in diameter) of *Helminthosporium* oryzae under aseptic condition and culture was incubated at 25 ±2°C until the whole medium is covered with mycelium (Surulirjaan and Kandhari, 2005).

The experimental field was ploughed twice and soil was brought to a fine tilth. On completion of field preparation and levelling the total field was divided in to 33 plots. Inoculum prepared by mass culturing was incorporated in the soil @ 25g/plot and mixed well, 10 days after transplanting. (Surulirjaan and Kandhari, 2005).

Disease severity was determined by estimating the percentage of infected surface area of rice leaves in the laboratory. It was estimated by adopting the following formula.

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

Disease severity was estimated according to the disease index established by 0 to 9 SEC scale (Anonymous, 2011).

Aqueous extract of plant parts such as leaves, rhizomes were prepared by using the standard method as given by Gerard *et al*, (1994) and neem oil collected from Department of Plant Pathology. The plant extract thus prepared and neem oil were tested against *Helminthosporium oryzae* using poisoned food technique.

Potato Dextrose Agar (PDA) with 2% agar was used as culture medium. Varying amounts of the plant extracts and neem oil were added to the sterilized molten PDA to get a final concentration of 3%, 5%, 7% and 1%, 2%, 3% respectively. The poisoned PDA was poured into sterile petriplates and allowed to set. Four replications were maintained for each concentration, inoculated with 5 mm disc of fungal culture taken from actively growing mycelium of *Helminthosporium oryzae* and the plates were kept upside down. The plates were incubated at 26 ± 1°C. Plates without plant extracts were served as control. The growth was determined at regular intervals for 72 hrs after inoculation.

All the bioagent isolates were collected from Department of Plant Pathology. The isolates were maintained on PDA and NA slants. Then all the isolates of bioagents were tested against the brown spot pathogen. Sterilized and molten Potato Dextrose Agar medium and Nutrient Agar medium were poured in sterilized petriplates and allowed to solidify. Then 5 mm diameter disc of test pathogen i.e. *Helminthosporium oryzae* was cut from the edge of growing colonies and placed at the edge of the petriplates containing Potato Dextrose Agar medium and Nutrient Agar medium. Similarly 5 mm diameter mycelia disc of bioagents was cut from the edge of growing colonies and placed just at the opposite end of the disc of *Helminthosporium* oryzae. In control, only 5mm disc of *Helminthosporium oryzae* was inoculated at the centre of the plate containing Potato Dextrose Agar medium and Nutrient Agar medium. The paired culture plates were incubated at 26 ± 1°C and observed regularly for 72 hrs after inoculation of bioagents. *In vitro* screening of antagonists by per cent growth inhibition was recorded syadopting following formula (Vincent, 1947).

$$\text{\% growth inhibition} = \frac{\text{(Control length - treated pathogen length) x 100}}{\text{Control length}}$$

The grain yield in each treatment was recorded after harvesting of the crop, transformed into quintal per hectare. The disease severity was transformed into per cent and analyzed statistically. The per cent growth inhibiting of pathogen was also analyzed statistically.

RESULTS AND DISCUSSION

The result (Table 1) showed that the highest brown

Table 1. Efficacy of fungal, bacterial bioagents and botanicals (in field condition) on disease intensity and yield of rice

	Treatments	Disease intensity (%)	Yield q/ha
T_0	Control	44.64[a]	59.33[h]
T_1	*T. viride* (ST) + *Pseudomonas fluorescens* (FS) @ 4 g/kg seed + 10 g/liter	34.39[g]	68.60[a]
T_2	*T. viride* (ST) + *Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter	36.33[e]	64.88[c]
T_3	*T. harzianum* (ST) + *Pseudomonas fluorescens* (FS) @ 4 g/kg seed + 10 g/liter	36.68[e]	62.33[e]
T_4	*T. harzianum* (ST) + *Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter	37.41[cd]	61.90[e]
T_5	*Pseudomonas fluorescens* (ST) + *T. viride* (FS) @ 4 g/kg/seed + 10 g/liter	35.41[f]	67.68[b]
T_6	*Pseudomonas fluorescens* (ST) + *T. harzianum* (FS), @ 4 g/kg/seed + 10 g/liter	36.75[de]	61.94[e]
T_7	*Bacillus subtilis* (ST) + *T. viride* (FS) @ 4 g/kg seed + 10 g/liter	36.41[e]	63.66[d]
T_8	*Bacillus subtilis* (ST) + *T. harzianum* @ 4 g/kg seed + 10 g/liter	37.94[c]	60.86[e]
T_9	Neem leaf extract (ST) + neem oil (FS) @ 4 g/kg seed + 10 g/liter	38.64[b]	60.66[f]
T_{10}	Neem oil ((ST) + neem leaf extract (FS) @ 4 g/kg seed + 10 g/liter	39.30[b]	60.53[g]
CD (P<0.05)		0.691	0.467

*Average of two years with 3 replications.
T. = *Trichoderma*, ST = Seed Treatment and FS = Foliar Spray.

spot disease severity (13.61%) was recorded in control treatment (T_0) which was statistically superior to all other treatments and lowest brown spot severity (34.39%) was recorded in treatment T_1 where *T. viride* (ST) + *Pseudomonas fluorescens* (FS) @ 4 g/kg seed + 10 g/liter of water, spraying of *Pseudomonas fluorescens* @ 10 g/liter of water for three times at an interval of 15 days starting from 30 days after sowing were made and it was statistically at par followed by *Pseudomonas fluorescens* (ST) + *T. viride* (FS) @ 4 g/kg/seed + 10 g/liter (T5) where is disease severity 35.41% and *T. viride* (ST) + *Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter (T_2), *Bacillus subtilis* (ST) + *T. viride* (FS) @ 4 g/kg seed + 10 g/liter (T_7), *T. harzianum* (ST) + *Pseudomonas fluorescens* (FS) @ 4 g/kg seed + 10 g/liter (T_3) and *Pseudomonas fluorescens* (ST) + *T. harzianum* (FS), @ 4 g/kg seed + 10 g/liter (T_6) which was statistically similar where is disease severity 36.33%, 36.41%, 36.68% and 36.75% respectively, *T. harzianum* (ST) + *Bacillus subtilis* (FS) @ 4 g/kg seed + 10 g/liter (T_4) and *Bacillus subtilis* (ST) + *T. harzianum* @ 4 g/kg seed + 10 g/liter (T_8) which was statistically similar where is disease severity 37.41% and 37.94%. The highest disease severity was observed in treatment with neem leaf extract (ST) + neem oil (FS) @ 4 g/kg seed + 10 g/liter (T_9) and neem oil ((ST) + neem leaf extract (FS) @ 4 g/kg seed + 10 g/liter (T_{10}) which was statically similar where is disease severity 38.64% and 39.30% over control. The results obtained may be represented as follows:

$$\mathbf{T_1 \leq T_5 \leq T_2 \leq T_7 \leq T_3 \leq T_4 \leq T_8 \leq T_9 < T_{10} < T_0}$$

Biswas *et al.* (2008) reported better control of brown spot of paddy in seed treatment with bioagents. Similarly spray of *Pseudomonas fluorescens* at weekly interval @ 10g per liter was found effective in reducing the disease severity by 24.5%, (Josi *et al*., 2007)

The results (Table 1) showed that highest grain yield (68.60q/ha) was recorded in treatment T_1 where *T. viride* (ST) + *Pseudomonas fluorescens* (FS) @ 4 g/kg seed + 10 g/liter of water, *Pseudomonas fluorescens* @ 10 g/liter of water for three times at an interval of 15 days starting from 30 days after sowing were made and the lowest yield was recorded in control treatment T_{10} (60.53 q ha^{-1}). Sarkar *et al.* (2014) reported that the *Trichoderma* spp is best in controlling of brown spot of rice and increasing the grain yield Pannu *et al*. (2007) observed that spray with *Pseudomonas fluorescens* at weekly interval @ 10g per liter was found effective in increasing the grain yield (64.9 q ha^{-1}).

The two botanical namely neem leaf extract and neem oil were tested *in vitro* against the pathogen under the food poison technique at 3%, 5% , 7% (neem leaf extract) and 1%, 2% and 3% (neem oil) concentration. Table 2 indicates the results

Table 2. Efficacy of botanicals against radial growth and percent inhibition of Helminthosporium oryzae at different intervals

Treatments	Radial growth (mm)			Percent inhibition (%)		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
7% neem leaf extract	13.50^{ab}	25.25^{bc}	45.25^{bc}	8.73^{e}	39.69^{c}	53.88^{b}
5% neem leaf extract	11.25^{d}	23.50^{cd}	43.50^{cd}	20.90^{b}	44.04^{b}	51.66^{c}
3% neem leaf extract	10.75^{d}	21.52^{de}	41.50^{ef}	27.11^{a}	48.58^{a}	49.74^{d}
1% neem oil	13.25^{bc}	25.75^{b}	45.75^{b}	9.74^{d}	39.50^{c}	49.89^{d}
2 % neem oil	12.00^{cd}	23.25^{cde}	43.25^{de}	18.74^{c}	44.57^{b}	51.73^{c}
3 % neem oil	11.75^{d}	21.25^{e}	41.25^{f}	20.57^{b}	49.35a	54.75^{a}
control	14.75^{a}	42.00^{a}	90.00^{a}	-		
CD (P<0.05)	1.460	2.003	1.847	0.707	0.808	0.389

*Average of 4 replications.

of different botanicals on pathogen at different concentrations. It was recorded that at 3% concentration of neem oil the growth of pathogen was inhibited to the maximum of 54.75% followed extent by neem leaf extract 7% (53.88%), neem oil 2% (51.73%) and neem leaf extract 5% (51.66%) while neem oil 1% and neem leaf extract 3% expressed minimum growth inhibition that is 49.89 and 49.74 respectively. Previous studies shows that neem products are best for management of brown spot disease in laboratory as well as in field. Similarly, neem plant extract and neem oil were reported to have antifungal activity (Devi and Chhetry 2012).

Four bioagents such as fungal (*T. viride* and *T. harzianum*) and bacterial (*Pseudomonas fluorescens* and *Bacillus subtilis*) bioagents were tested against *Helminthosporium oryzae* by dual plate technique. The results (Table 3) showed that at 72hrs maximum inhibition (61.72%) of the pathogen was recorded where bioagent *T. viride* was used, where as the minimum inhibition (52.50%) was recorded where the bioagent *Bacillus subtilis* was used. The point of contact between the pathogen and antagonists were recorded at 3 day of inoculation. Gomathinayagam *et al.* (2010) reported that the *T. viride* showed maximum inhibition % against brown spot of rice (*Bipolaris oryzae*) found in lab condition. Srivastava and Singh (2012) antagonistic activity of *T. viride* found in maximum growth inhibition percent against agriculture crop pathogen like brown spot and sheath blight of rice.

From the results it can be concluded that fungal, bacterial abiogenes and botanicals were found efficiency in reducing the mycelial growth of the pathogen under *in vitro* condition. Application of

Table 3. Efficacy of fungal and bacterial bioagents against radial growth and percent inhibition of Helminthosporium oryzae at different intervals

Treatments	Radial growth (mm)			Percent inhibition (%)		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
H. o + B.s	13.00^{b}	26.00^{b}	43.50^{b}	7.37^{d}	36.52^{d}	52.50^{c}
H.o + T. h	12.00^{bc}	22.00^{bc}	42.00^{bc}	14.52^{b}	47.45^{b}	53.67c
H.o + T.v	11.50^{c}	18.25^{c}	35.75^{d}	21.52^{a}	57.47^{a}	61.72^{a}
H.o + *P.f*	12.25^{bc}	19.00^{bc}	39.00^{c}	13.45^{c}	46.37^{c}	56.50^{b}
control	14.50^{a}	42.00^{a}	90.00^{a}	-	-	-
CD (P<0.05)	1.382	7.361	3.204	0.598	0.749	1.343

*Average of 4 replication.

H.o = Helminthosporium oryzae, T.v = Trichoderma viride, T. h = Trichoderma harzianum, P.f = Pseudomonas fluorescens and *B.s = Bacillus subtilis.*

bioagents and botanicals although *in vivo* experiment some bioagents showed some promise in controlling the brown spot of rice. In this experiment, it has been established that for the management of brown spot of rice, seed treatment with *Trichoderma viride* @ 4g/kg of seeds + spraying of *Pseudomonas fluorescens* @ 10g/litre of water for three times at an interval of 15 days starting from 30 days after sowing may be recommended for trial on farmer's field in different location before giving recommendation to the farmer.

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