

ORIGINAL ARTICLE

Efficacy Of Fungal, Bacterial Bioagents and Botanicals Against Brown Spot (*Helminthosporium Oryzae*) of Rice (*Oryzae Sativa*)

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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 was 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) *Trichoderma viride* @ 4g/kg seed + foliar spray (FS) *Pseudomonas fluorescens* @ 10 g/liter water was found effective with less disease severity of 34.39% and higher grain yield 68.60 q/ha. In vitro condition it was observed dual culture technique, *Trichoderma viride* was most effective inhibiting the growth of *Helminthosporium oryzae* (61.72%). Botanicals was observed, neem oil @ 3% was best effective inhibiting growth of pathogen (54.25%) was found in food poison technique.

Key words: Brown spot, bioagents, botanical, *Helminthosporium oryzae* and rice.

INTRODUCTION

Brown leaf spot of rice caused by *Helminthosporium oryzae* (Breda de Haan) Subram and Jain is one of the major fungal disease of rice which occurs in almost all the rice growing areas [1]. The disease is of great importance in several countries and has been reported to cause considerable losses. In India, disease occurs sporadically. Recently its incidence has increased on cultivated varieties especially under rice – wheat cropping system [2]. Continuous, inappropriate and non-discriminative 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 [3]. Instead, plant pathologists have focused their attention to develop environmentally safe, long-lasting and effective biocontrol and botanical methods for the management of plant diseases. Among various fungal, bacterial biocontrol agents and botanicals are known for their mycoparasitic, antagonistic and antifungal mechanism for the control of fungal disease (brown spot). The present study was undertaken to evaluate the efficacy of fungal, bacterial and botanical as a seed treatment and foliar spray as well as *in vitro* to test the mycoparasitic, antagonistic and antifungal activity of certain fungal, bacterial and plant extracts against *Helminthosporium oryzae*, the causal organism of brown leaf spot of rice.

MATERIALS AND METHODS

Field condition and treatments

The field experiment was conducted in randomized block design (RBD) with plot size 2×2m² and there were three replication for each treatment. The experiment was conducted on variety Pusa Basmati 1121 during summer season 2013 and 2014. Treatment was *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 botanical were initiated, three times at an interval of 15 days starting from 30 days after sowing just appearance of disease.

Mass multiplication of pathogen

The pure culture of pathogen was collected from IARI New Delhi. Mass multiplication of pathogen was carried out on sorghum grains. Grain of sorghum was soaked in the boiling water for 30 minutes and then autoclaved at 15 lbs/inch² 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 conditions and culture was incubated at 25 ± 2°C until the whole medium is covered with mycelium Surulirjaan and Kandhari [4].

Inoculation of pathogen

The experimental field was ploughed twice and soil was brought to a fine tilth. On completion of field preparation and leveling the total field was divided in to 33 plots. Inculum prepared by mass culturing was incorporated in the soil @ 25g/plot and mixed well, 10 days transplanting. Surulirjaan and Kandhari [4].

Assessments of disease severity

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

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

DS was estimated according to the disease index established by 0 to 9 scales [5].

IN VITRO TEST

Efficacy of botanical study against *Helminthosporium oryzae*

Aqueous extract of plant parts such as leaves, rhizomes were prepared by using the standard method as given by Gerard Ezhilan *et. al.* [6] 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 plants extract 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 was maintained for each concentration were 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 was served as control. The growth was determined at regular intervals for 72 hrs after inoculation.

Efficacy of fungal and bacterial antagonists against. *Helminthosporium oryzae*

All the bioagents 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 pathogen i.e. *Helminthosporium oryzae*. Sterilized and molten Potato Dextrose agar medium and Nutrient Agar was poured in sterile 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. 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 treatment only 5mm disc of *Helminthosporium oryzae* was inoculated at the centre of the plate containing Potato Dextrose agar medium and Nutrient Agar. The paired cultures plates were incubated at 26 ± 1°C and observed regularly for 72 hrs after inoculation of bioagents. In vitro Screening of antagonist by percent inhibition was recorded from the following formula [7].

$$\% \text{ growth inhibiting} = \frac{(\text{Control length} - \text{Reated pathogen length}) \times 100}{\text{Control length}}$$

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

RESULTS AND DISCUSSION**Field evaluation (The mean of two years viz. 2013 and 2014)****Disease severity**

The result (Table 1) showed that the highest Brown spot disease severity (13.61%) was recorded in control treatment (T₀) which was statistically superior to all other treatments and lowest brown spot severity (34.39%) was recorded in treatment T₁ 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 (T₂), *Bacillus subtilis* (ST) + *T. viride* (FS) @ 4 g/kg seed + 10 g/liter (T₇), *T. harzianum* (ST) + *Pseudomonas fluorescens* (FS) @ 4 g/kg seed + 10 g/liter (T₃) and *Pseudomonas fluorescens* (ST) + *T. harzianum* (FS), @ 4 g/kg seed + 10 g/liter (T₆) which was statically 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 (T₄) and *Bacillus subtilis* (ST) + *T. harzianum* @ 4 g/kg seed + 10 g/liter (T₈) which was statically similar where is disease severity 37.41% and 37.94%. The highest disease severity was observed in treatment neem leaf extract (ST) + neem oil (FS) @ 4 g/kg seed + 10 g/liter (T₉) and neem oil (ST) + neem leaf extract (FS) @ 4 g/kg seed + 10 g/liter (T₁₀) which was statically similar where is disease severity 38.64% and 39.30% over control. The results obtained may be represented as follows:

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

Biswas *et al.* [8] reported better control of brown leaf spot of paddy in seed treatment with bioagents. Similarly spray of *Pseudomonas fluorescens* at weekly interval @ 10g per liter found effectively in reducing the disease severity 24.5%, Josi *et al.* [9].

Yield (The mean of two years viz.2013 and 2014)

The results (Table 1) showed that highest yield (68.60q/ha) was recorded in treatment T₁ 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 T₁₀ (60.53q/ha). Sarkar *et al.* [10] reported that the *Trichoderma* spp is best control of brown spot of rice.

Efficacy of botanicals against brown spot pathogen of rice in vitro

The two botanical namely neem leaf extract and neem oil were tested in vitro against the pathogen by the food poison technique at the 3%, 5% ppm, 7% (neem leaf extract) and 1%, 2% and 3% (neem oil) concentration. Table 2 indicates the results of different botanical on pathogen at different concentrations. It was recorded that at 3% concentration, neem oil highest inhibited the growth of pathogen (54.75%) followed 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 also found that neem product are best for management of brown spot disease in laboratory as well as in field. Similarly report that the emulated the antifungal activity of neem plant extract and oil maximum inhibition percent, O. Jitendiya Devi and G.K.N. Chhetry [11].

Efficacy of fungal and bacterial antagonists against *Helminthosporium oryzae* (in vitro)

Four bioagent 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.* [12] reported that the *T. viride* maximum inhibition % against brown spot of rice (*Bipolaris oryzae*) found in lab condition. Ritu Srivastava and Diwakar Singh [13] antagonistic activity of *T. viride* found in maximum growth inhibition percent against agriculture crop pathogen like brown spot of rice and sheath blight of rice.

From the results it can be concluded that for the efficacy of fungal, bacterial and botanical against brown spot of rice showed that the reducing the mycelial growth of pathogen *in vitro* experiment. Application of bioagents and botanical although *in vivo* experiment some bioagents showed some promise in controlling the pathogen of 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.

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

Treatments		Disease intensity (%)	Yield q/ha
T ₀	Control	44.64 ^a	59.33 ^b
T ₁	<i>T. viride</i> (ST) + <i>Pseudomonas fluorescens</i> (FS) @ 4 g/kg seed + 10 g/liter	34.39 ^e	68.60 ^a
T ₂	<i>T. viride</i> (ST) + <i>Bacillus subtilis</i> (FS) @ 4 g/kg seed + 10 g/liter	36.33 ^e	64.88 ^c
T ₃	<i>T. harzianum</i> (ST) + <i>Pseudomonas fluorescens</i> (FS) @ 4 g/kg seed + 10 g/liter	36.68 ^e	62.33 ^e
T ₄	<i>T. harzianum</i> (ST) + <i>Bacillus subtilis</i> (FS) @ 4 g/kg seed + 10 g/liter	37.41 ^{cd}	61.90 ^e
T ₅	<i>Pseudomonas fluorescens</i> (ST) + <i>T. viride</i> (FS) @ 4 g/kg/seed + 10 g/liter	35.41 ^f	67.68 ^b
T ₆	<i>Pseudomonas fluorescens</i> (ST) + <i>T. harzianum</i> (FS), @ 4 g/kg/seed + 10 g/liter	36.75 ^{de}	61.94 ^e
T ₇	<i>Bacillus subtilis</i> (ST) + <i>T. viride</i> (FS) @ 4 g/kg seed + 10 g/liter	36.41 ^e	63.66 ^d
T ₈	<i>Bacillus subtilis</i> (ST) + <i>T. harzianum</i> @ 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 ^e
C.D (0.05)		0.691	0.467

*Average of two year with 3replication.

T. = *Trichoderma*, ST = Seed Treatment and FS = Foliar Spray.

Table 2: Efficacy of botanical against radial growth and percent inhibition of *Helminthosporium oryzae* at different interval.

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	49.35 ^a	54.75 ^a
control	14.75 ^a	42.00 ^a	90.00 ^a	-	-	-
C.D (0.05)	1.460	2.003	1.847	0.707	0.808	0.389

*Average of 4 replication.

Table 3: Efficacy of fungal and bacterial against radial growth and percent inhibition of *Helminthosporium oryzae* at different interval.

Treatments	Radial growth (mm)			Percent inhibition (%)		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
<i>H. o</i> + <i>B. s</i>	13.00 ^b	26.00 ^b	43.50 ^b	7.37 ^d	36.52 ^d	52.50 ^c
<i>H. o</i> + <i>T. h</i>	12.00 ^{bc}	22.00 ^{bc}	42.00 ^{bc}	14.52 ^b	47.45 ^b	53.67 ^c
<i>H. o</i> + <i>T. v</i>	11.50 ^c	18.25 ^c	35.75 ^d	21.52 ^a	57.47 ^a	61.72 ^a
<i>H. o</i> + <i>P. f</i>	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	-	-	-
C.D (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*.

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