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MANAGEMENT OF BROWN SPOT DISEASE OF RICE BY USING SAFER FUNGICIDES AND SOME BIOAGENTS

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ABSTRACT
Rice is suffering from several fungal diseases among them brown spot caused by *Bipolaris oryzae* is important. In the present investigations the *in vitro* screening of fungicide were made against the *Bipolaris oryzae* by poisoned food technique and spore germination study and *in vitro* bio-control of established against pathogen by using dual plate technique, and finally management of Brown spot was done by using safer fungicides and some bioagents. For the management of the disease an experiment was conducted at Instructional Farm, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during 2010-11 and 2011-12 by using *Trichoderma viride* isolate 5 @ 100 kg, 200kg and 400 kg per ha, as basal application with FYM @1.2 ton at final land preparation, Seed treatment with Bavistin @ 1g/kg of seeds, Drenching of Bavistin @ 1g/litre of water as basal application, Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing, Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing and Seed treatment with Emissan-6@ 2g/kg of seeds.

INTRODUCTION

Rice (*Oryzae sativa* L) is the one of major cereal crops in India. The crop is suffering from number of fungal, bacterial, viral and mycoplasmal diseases. Among them the fungal diseases like blast (*Pyricularia oryzae*), brown spot (*Bipolaris oryzae*), stem rot (*Sclerotium oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*), bacterial diseases such as bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and viral diseases such as tungro (rice tungro virus) are most important. Losses due to rice diseases have been estimated to be 10-15% in general (kandhari, 2005). Individual diseases in certain cases have been reported to cause tremendous losses, even up to 100% (Chakraborty *et al*., 1998). Among the diseases Brown spot disease of rice caused by *Heminthosporium oryzae (Cochliobolus miyabeanus)* also known as *Dreschslera oryzae* which was first described by Bedi in 1960 later it was described by *Bipolaris oryzae* (Gangopadhyay and Padmanabhan, 1987) is most important. It causes severe yield loss in 1942 in West Bengal popularly known as Bengal famine and yield loss reaches upto 90% in certain areas. Rice disease management strategies mainly aim at prevention of outbreak or epidemics through the use of host plant resistance and chemical pesticides. The persistent, injudicious use of chemicals has toxic effects on non-target organisms and can cause undesirable changes in the environment. Most of these chemicals are to expensive for the resource poor farmers of West Bengal whose main cultivable crop is rice. Large scale and long term use of resistant cultivars is likely to result in significant shifts in the virulence characteristics of pathogen, culminating in resistant break down. However, research during the previous two decades indicates another potential option for rice disease management through the use of biocontrol agents (Mina *et al*., 2013; Gade , 2013; Ramteke *et al*., 2011 and Balai *et al*., 2013). A several biocontrol agents namely *Pseudomonas fluroscence*, *Bacillus subtilis*, *Trichoderma* spp. have been found effective against major rice diseases caused by fungal pathogens. (*Vasudevan et al*., 2002). The present investigation was undertaken for the management of Brown spot disease of rice by using safer fungicides and some bioagents

MATERIALS AND METHODS

Antagonistic study of *Trichoderma* sp. against *Bipolaris oryzae*

All the biocontrol isolates were collected from AICRP on Medicinal and Aromatic plants and Betelvine Laboratory, BCKV, Kalyani. The isolates were maintained on PDA slants at 5^0C. Then all the isolates of *Trichoderma* sp were tested against the isolate of the test pathogen i.e. *Bipolaris oryzae*. Sterilized and molten Potato Dextrose agar medium was poured in sterile petriplates and allowed to solidify. Then 6 mm diameter disc of test pathogen i.e. *Bipolaris oryzae* was cut from the edge of growing colonies and placed at the edge of the petriplates containing Potato Dextrose agar medium. Similarly 6 mm diameter mycelia disc of *Trichoderma* sp was cut from the edge of growing colonies and placed just at the opposite end of the disc of *Bipolaris oryzae* . In control

treatment only 6 mm disc of *Bipolaris oryzae* was inoculated at the centre of the plate containing Potato Dextrose agar medium. The paired cultures plates were incubated at 28 1°C and observed regularly for 6 days after inoculation of *Trichoderma sp* .The degree of antagonism was studied on a scale of classes 1-5 (Bell *et al.,* 1982).

Class-1 (R_1) = The antagonist completely over grew the pathogen and covers the entire medium surface.

Class-2 (R_2) = The antagonist over grew at least $2/3_3$ of the pathogen's surface.

Class-3 (R_3) = The antagonist colonized on 1/2 of the growth of pathogen.

Class-4 (R_4) = The pathogen and antagonist locked at the point of contact.

Class-5 (R_5) = The pathogen completely over grew the mycoparasite.

An isolate of the antagonist was considered to be antagonistic to the pathogen if the mean score for a given comparison (when rounded to the nearest class number) was < 2, but not highly antagonistic if the number was > 3. *In vitro* Screening of Antagonist by Percent Inhibition was recorded by measuring the growth of *Bipolaris oryzae*. Percent growth inhibition was recorded from the following formula.

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

Management of the disease under field condition

Field experiments were conducted at Instructional Farm, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during 2010-11 and 2011-12 to study the management of brown spot disease using safer fungicides and bio control agents. The site of the experiment located at south of tropic of cancer with 22p 93 ´ N latitude, 88p 53 ´ E longitude with an elevation of 9.75 m from mean sea level. Soil is of sandy loam type and having good irrigation facility. The Rice variety MTU -7029 was used for this experiment. Seeds of rice were sown on 25thJuly 2011. Size of the individual plot is 12 sq m (4m x 2m) planting density was 25 seedlings/ m^2. The spacing between the plant was 25 cm x 25 cm. Basal application of fertilizer of N, P_2O_5 and K_2O was performed at 40, 30 and 30 kg/ha, respectively, with an additional application at 30, 20 and15 kg/ha respectively at the flowering stage. The treatment which are used given in Table 1.

Four replications were used for each treatment. Disease severity (DS) and disease incidence (DI) were assessed. Brown spot disease incidence was calculated based on total number of plants present and number of plants showing typical symptoms in each by using the following formulae given by Wheeler (1969).

$$\% \text{ disease incidence} = \frac{\text{Total no. of plant} \times 100}{\text{Total no. of plants}}$$

Rice brown spot disease occurred naturally in the field. 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 (Anonymous 2011).

0 No. of plants showing any symptom

1 Lesion limited to 1/4th of leaf sheath i.e. (1-10%)

2 plants showing lower half of leaf sheath i.e. (11-20%)

3 Lesion limited to 1/2th of leaf sheath i.e. (21-30%)

4 Lesion increase slight to 1/2th of leaf sheath i.e. (31-40%)

5 Lesion limited to more than 1/2th of leaf sheath i.e. (41-50%)

6 Slight infection on lower 3rd leaf i.e. (51-60%)

7 Lesion limited to 3/4th of leaf sheath i.e. (61-70%)

8 slight infection on flag leaf i.e. (71-80%)

9 Lesion on tiller and severe infection on all the leaf i.e. (81-100%)

The grain yield in each treatment was recorded after harvesting of the crop and transformed into quintal per hectare. The cost: benefit ratio was also recorded.

The percent disease incidence and percent disease index were transformed by angular transformation and analyzed statistically. The yield data was also analyzed statistically.

RESULTS AND DISCUSSION

Screening of *Trichoderma* sp. isolates against isolate of *B. oryzae*

Seven isolates of *Trichoderma* spp. were tested against isolate of *Bipolaris oryzae* by dual plate technique. Rating of antagonism (Table.2) was recorded according to the modified Bell's ranking stated earlier. The results (Table 2) showed that at 6th day maximum inhibition (99.20%) of the pathogen was recorded where bioagent TV_5 was used where as the minimum inhibition (72.22%) was recorded where the bioagent TH_2 was used. When we consider the inhibition in Bell's scale it was revealed that except isolate TH_2 all the isolates were in R1 rating after 6 the day and TH_2 was in scale of R2 after 6th day of observation. The point of contact between the pathogen and antagontists were recorded at 3 day of inoculation.

Management of brown spot of rice

Percent disease incidence (2011)

The result (Table 3) showed that the highest Brown spot disease incidence (13.61%) was recorded in Control treatment (T9) which was statistically superior to all other treatments and lowest brown spot incidence (10.34%) was recorded in treatment T7 where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre 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 with all the treatments except in treatments T2 (*Trichoderma viride* isolate 5 @ 200 kg/ ha as basal application with FYM @ 1.2 ton at final land preparation),T3 (*Trichoderma viride* isolate 5 @ 400 kg/ha as basal application with FYM @1.2 ton at final

land preparation) and T9 (Control).

Percent disease incidence (year 2012)

Highest Brown spot disease incidence (25.42%) was recorded in Control treatment (T9) which was statistically superior to all other treatments and most effective treatment against brown spot incidence (17.24%) was recorded in treatment T1 where *Trichoderma viride* isolate 5 @ 100 kg/ ha were applied as basal application with FYM @1.2 ton at final land preparation and it was statistically at par with all the treatments except treatments T8 (Seed treatment with Emissan-6@ 2g/kg of seeds) and T9 (control) (Table 3).

Pooled of 2011 and 2012

Most effective treatment against brown spot incidence (14.30%) was recorded where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing (T7) were made which was statistically at par with all other treatments except T9 (control) treatment (Table 3) and recorded 26.69 % disease control. The control treatment (T9)

Table 1: Treatment used in field trial

Treatment	Details
T1	*Trichoderma viride* isolate 5@ 100 kg per ha as basal application with FYM @1.2 ton at final land preparation.
T2	*Trichoderma viride* isolate 5 @ 200kg per ha as basal application with FYM @ 1.2 ton at final land preparation.
T3	*Trichoderma viride* isolate 5 @ 400 kg per ha as basal application FYM @ 1.2 ton at final land preparation.
T4	Seed treatment with Bavistin @ 1g/kg of seeds.
T5	Drenching of Bavistin @ 1g/litre of water as basal application.
T6	Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing.
T7	Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing.
T8	Seed treatment with Emissan-6@ 2g/kg of seeds.
T9	Control

Table 2: Screening of *Trichoderma* spp. isolates against isolate of *B. oryzae*

Bioagent used	Percentage of inhibition at			Scale			
	4thday	5thday	6thday	Point of contact	Bell's scale modified		
					4th	5th	6th
TV_1	24.00	54.72	87.25	3	R4	R3	R1
TV_2	25.33	57.62	92.24	3	R4	R3	R1
TV_3	17.33	36.19	74.26	3	R4	R4	R2
TV_4	38.67	65.23	98.78	3	R4	R3	R1
TV_5	**34.67**	**66.19**	**99.20**	3	R4	R3	R1
TH_1	20.37	52.37	86.23	3	R4	R3	R1
TH_2	16.67	48.26	72.22	3	R4	R4	R2

Bell's ranking:
R_1 = The antagonist completely over grew the pathogen and cover the entire medium surface;
R_2 = The antagonist over grew at least $^2/_3$ of the pathogen's surface;
R_3 = The antagonist colonized on ½ of the growth of pathogen;
R_4 = The pathogen and antagonist locked at the point of contact and
R_5 = The pathogen over grew the mycoparasite.

Table 3: Management of brown spot of rice

Treatment	Percent disease incidence				Percent disease severity				Grain yield in q/ha			Cost:benefit ratio
	2011	2012	Pooled	%disease control	2011	2012	Pooled	%disease control	2011	2012	Pooled	
T1	11.5	17.24	14.37	26.33	15.11	14.10	14.55	29.00	34.37	33.21	33.79	3.92
T2	11.56	20.15	15.86	18.74	15.54	14.21	14.80	27.78	35.31	38.35	36.83	2.24
T3	11.79	20.98	16.38	16.05	15.91	15.52	15.69	23.42	35.31	36.61	35.96	1.21
T4	11.44	20.15	15.79	19.06	14.14	14.26	14.21	30.66	26.87	30.23	28.55	27.36
T5	10.64	20.04	15.34	21.40	15.73	11.79	13.55	33.89	34.37	34.00	34.18	1.21
T6	11.10	20.48	15.79	19.10	14.51	14.12	14.30	30.23	36.10	37.28	36.69	5.04
T7	10.34	18.27	14.30	26.69	11.88	14.04	13.08	36.17	36.56	38.46	37.51	1.17
T8	10.41	21.33	15.87	18.66	15.36	14.50	14.88	27.38	36.87	37.94	37.40	52.28
T9	13.61	25.42	19.51		19.78	21.06	20.49		20.00	24.56	22.28	
SEm±	0.41	1.29	0.85		1.53	0.76	1.10		3.57	4.23	3.90	
CD at 5%	1.20	3.78	2.49		4.47	2.22	3.22		10.43	11.43	10.93	
CV%	7.62	13.37	10.49		20.18	10.48	14.79		21.71	24.4	23.05	

T1= *Trichoderma viride* isolate 5 @ 100kg/ ha as basal application with FYM @1.2 ton at final land preparation ,T2= *Trichoderma viride* isolate 5 @ 200 kg/ ha as basal application with FYM @ 1.2 ton at final land preparation,T3= *Trichoderma viride* isolate 5 @ 400 kg/ha as basal application with FYM @1.2 ton at final land preparation, T4= Seed treatment with Bavistin @ 1g/kg of seeds.T5= Drenching of Bavistin @ 1g/ litre of water as basal application T6= Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing, T7= Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing, T8= Seed treatment with Emissan-6@ 2g/ kg of seeds,T9=control.*Avg no of 4 replication

recorded maximum disease incidence. The pooled results may be represented as follows:

$$T7 \leq T5 \leq T4 \leq T6 \leq T1 \leq T2 \leq T8 \leq T3 < T9$$

Percent disease severity (2011)

Highest Brown spot disease severity (19.78%) was recorded in Control treatment (T9) which was statistically at par with the treatments T2, T3, T5 and T8 where *Trichoderma viride* isolate 5 @ 750 kg/ ha as basal application with FYM @ 1.2 ton at final land preparation, *Trichoderma viride* isolate 5 @ 500 kg/ ha as basal application with FYM @1.2 ton at final land preparation, Drenching of Bavistin @ 1g/litre of water as basal application and Seed treatment with Emissan-6@ 2g/kg of seeds were made respectively. Treatment T7 where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/ litre of water as basal application + Spraying of Bavistin @ 1g/ litre of water for three times at an interval of 15 days starting from 30 days after sowing were made recorded the best treatment and it was statistically at par with the treatments. Most best treatment (11.88%) against brown spot incidence was recorded where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing (T7) which was statistically at par to all other treatments except the control treatment (T9).

Percent disease severity (2012)

The results (Table 3) revealed that highest Brown spot disease severity (21.06%) was recorded in Control treatment (T9) which was statistically superior to all other treatments and most effective treatment (14.04%) against brown spot incidence was recorded where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing (T7) which was statistically at par with all other treatments except the control treatment (T9).

Pooled of 2011 and 2012

The pooled analysis showed that the highest Brown spot disease severity (20.49%) was recorded in Control treatment (T9) which was statistically superior to all other treatments and most effective treatment (13.08%) against brown spot incidence was recorded where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing (T7) were made which was statistically at par to all other treatments except control treatments (Table 3) and recorded 36.17 % disease control over control treatment. The pooled results may be represented as follows:

$$T7 \leq T5 \leq T4 \leq T4 \leq T6 \leq T1 \leq T2 \leq T3 < T9$$

Yield (2011)

The results (Table 3) showed that highest yield (36.56q/ha) was recorded in treatment T7 where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre 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 (T9) (20.0q/ha).

Yield (2012)

Lowest yield (24.56q/ha) was recorded in T9 (control treatment) and the highest yield (38.46q/ha) was recorded (Table 3) in treatment T7 where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing were made.

Yield (pooled)

The pooled analysis of two years data revealed that the lowest yield (22.28q/ha) was recorded in T9 (control treatment) and highest yield was obtained where the traditional recommendation of Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing were made (T7) (Table 3). The results obtained may be represented as follows:

$$T7 \leq T8 \leq T6 \leq T2 \leq T3 \leq T5 \leq T1 \leq T4 < T9$$

Cost benefit ratio

The highest cost: benefit ratio was recorded where seed treatment with Emissan-6 was made and lowest cost: benefit ratio was recorded in treatment T7 where Seed treatment with Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/litre of water for three times at an interval of 15 days starting from 30 days after sowing were made (Table 3).

From the results it can be concluded that for the management of brown spot of rice application of bioagents is not so effective than the traditional recommendation of application of fungicides although *in vitro* experiment some bio agents 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 Bavistin @ 1g/kg of seeds + Drenching of Bavistin @ 1g/litre of water as basal application + Spraying of Bavistin @ 1g/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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