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## Impact of different fungicides combination against brown leaf spot (*Drechslera oryzae*) of rice under the *in vitro* and *in vivo*

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**Abstract**

*Drechslera oryzae* is the causal agent of rice brown spot disease and is responsible for significant economic losses. In order to control this disease, four fungicides were tested (carbendazim (Bavistin) 50 WP, carboxin (Vitavax) 50 WP, propiconazole (Tilt) 25 EC and hexaconazole (Contaf) 25 EC). The antifungal activity of the tested substances were investigated against *D. Oryzae* at different concentrations *in vitro*, as well as the efficacy of their exogenous application in controlling rice brown spot disease under field conditions. *In vitro*, propiconazole (Tilt) at 500 ppm maximum inhibited (96.58 %) the growth of *D. oryzae*. Under field conditions, seed treatment (ST) with Bavistin @ 0.2 g a.i/kg and foliar spray (FS) with Tilt @ 1ml/liter led to a significant reduction in disease severity (37.26%), in addition to a significant increase in the grain yield (55.49 %) and its components.

**Keywords:** Bavistin, Contaf, *Drechslera oryzae*, Tilt and Vitavax.

**1. Introduction**

Rice (*Oryza sativa* L.) has been regarded as one of the most important cereal crops and a major food grain contributor to the total world food grain basket. There is continuous increase in global demand of rice which is expected to reach 852 million tonnes by the year 2035 from its present production status of 676 million tonnes, and in order to produce 176 million tonnes of more rice to fill this deficit, there is a need to enhance the productivity of rice from 10 to 12.5 tonnes/hectare [1]. But with the introduction of high yielding rice cultivars, the crop is prone to several diseases among which brown spot (*Drechslera oryzae*) sheath blight (*Rhizoctonia solani*), sheath rot (*Fusarium moniliforme*) and blast (*Parycularia oryzae*) have assumed serious problem in the last few year. However brown spot (*Drechslera oryzae*) disease is major problem of rice, which causes severe yield loss that reaches up to 90 % in certain areas [2]. Apart from reducing plant growth and yield, they are also responsible for causing grain discoloration at maturity, thus reducing market value. At present, there are very limited strategies for the control of brown spot and cultivars with an adequate level of resistance are not available [3]. Fungicide treatment is one of the low cost control measures available for brown spot management but host plant resistance is most economical. Application of fungicides for the control of brown spot is the most effective management option, but under high disease pressure effective control is not achieved [4]. Earlier recommended fungicide such as zineb etc. do not provide satisfactory disease control under heavy inoculum pressure and along fungicide are also not effective against brown spot. The present study undertaken to impact of different fungicides combination as a seed treatment and foliar spray against brown leaves spot of rice under field and lab condition.

**Method and Material**

The experiment was carried out during *kharif* season of 2013 and 2014 at Central Research Farm, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Allahabad. The research farm is situated on the right side of Allahabad Rewa road at 20 degree and 15<sup>0</sup> North, 60<sup>0</sup> 03 east longitude and is about 129.2 cm above sea level. The site selected was uniform, cultivable with typical sandy loam soil having good drainage. Disease severity (percent infection) was recorded on the basis of 0 to 9 scale [5]. The percent disease intensity (PDI) was computed using the following formula:

$$PDI \% = \frac{\text{Sum of all unmerical rating}}{\text{Total number of observations} \times \text{Maximum rating}} \times 100$$

### Mass multiplication of pathogen

The pure culture of pathogen was isolated from the infected leaf and collected from central field of plant pathology SHUATS Allahabad. 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/inch<sup>2</sup> pressure for two consecutive days. On cooling of medium each flask was inoculated with mycelial bit (3 mm in diameter) of *Drechslera oryzae* under aseptic conditions and culture was incubated at 25+2 °C until the whole medium is covered with mycelium [6].

### In- vitro evaluation of fungicides against *D. Oryzae*

The efficacy of fungicides viz., carbendazim (Bavistin) 50 WP, carboxin (Plantvex) 50 WP, propiconazole (Tilt) 25 EC and hexaconazole (Contaf) 25 EC were tested at different concentrations of 100, 200, 250 and 500 ppm against *D. Oryzae* by poisoned food technique [7]. The efficacy of each fungicide was expressed as percent inhibition of mycelia growth over control calculated by using the formula suggested by [8].

$$PI\% = \frac{MC - MT}{MC} \times 100$$

Where, MC = Mycelial growth in control, MT = Mycelial growth in treatment.

### Field evaluation of fungicides against brown spot of rice

The field trials were conducted in a randomized block design (RBD) with three replications and plot size of 4m<sup>2</sup> (spacing 15cm X 20cm) on rice variety Pusa 1121. The doses of NPK fertilizers were applied and other cultural operations done as recommended for rice [9]. Test plots was bounded all around to prevent the movement of water from one plot to another. Inoculum prepared by mass culturing was incorporated in the soil @ 25g/plot and mixed well, before 10 days of transplanting [6]. Nursery was raised in separate plots in the field. Fungicide carboxin and carbendazim were applied as seed treatment (ST) at the rate 0.2 g/kg seed. Treated seeds were soaked in tap water for 12h. 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. propiconazole (Tilt) and hexaconazole (Contaf) applied as foliar spray and carboxin and carbendazim applied as seed treatments and foliar spray. Treatments combinations namely carboxin (ST) @ 0.2g a.i/kg seed + Tilt (FS) @ 1ml/liter (T2), carboxin (ST) @ 0.2g a.i/kg seed + hexaconazole (FS) @ 1ml/liter (T3), carboxin (ST) @ 0.2g a.i/kg seed + carbendazim (FS) @ 1g/liter (T4), carbendazim (ST) @ 0.2g a.i/kg seed + Tilt (FS) @ 1ml/liter (T5), carbendazim (ST) @ 0.2g a.i/kg seed + hexaconazole (FS) @ 1 ml/liter (T6) and carbendazim (ST) @ 0.2g a.i/kg seed + carboxin (FS) @ 1g/liter (T7) was compared against brown spot under artificial inoculation conditions (T1). Spray of fungicides was done in first week of August after the appearance of disease symptoms. Observations regarding disease severity were recorded periodically and yield/plot was calculated at harvest of crop. Percent increasing yield was calculated using the formula: [10].

$$\frac{b-c}{c} \times 100$$

Where, b = estimate of yield obtained in protected plot and c = estimate of yield obtained in unprotected plot.

### Result and Discussion

#### In vitro evaluation of fungicides against *B. Oryzae*

The data recorded revealed that among the different fungicides tested against *D. oryzae*, propiconazole was observed to be most effective with 96.58, 83.00, 74.00 and 63.85 % inhibition at 500, 250, 200 and 100 ppm concentrations, respectively, as compared to control (Table 1). The minimum percent inhibition was exhibited by carboxin (26.27 %) at 100 ppm concentration. However, there were significant differences among the different fungicides tested at various concentrations. [11] Had reported that propiconazole under *in - vitro* conditions was most effective in inhibiting the mycelia growth of *D. Oryzae* with percent inhibition of 95.98 at 500 ppm concentration. [12] Also reported that propiconazole at 500 ppm concentration significantly reduced the mycelia growth of *D. Oryzae*.

#### Performance of fungicide combination (applied as ST + FS) on disease severity and yield.

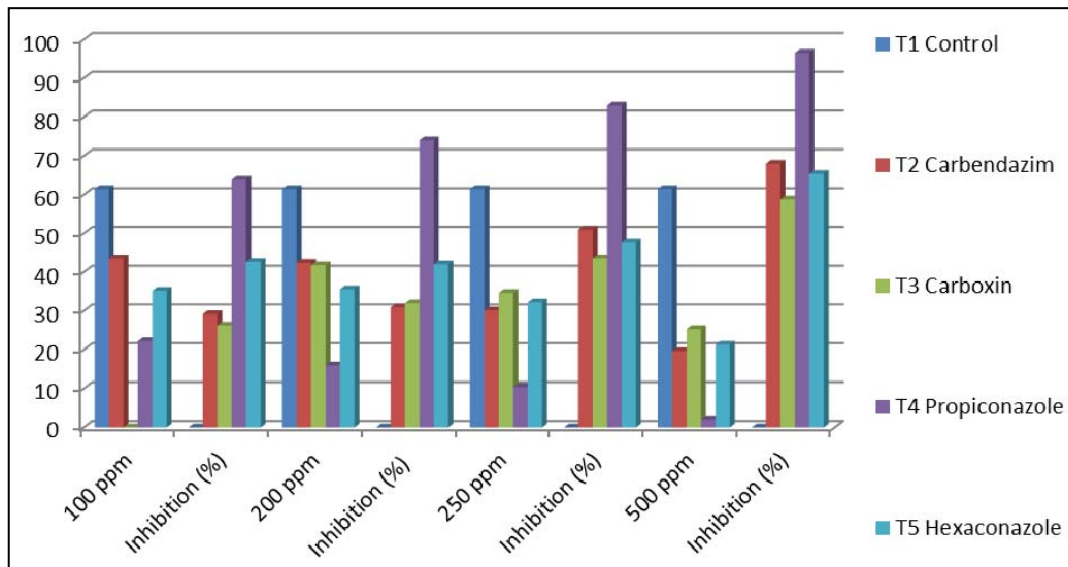
Data indicate the table no 2, all fungicide combination were significantly effective in disease severity (range 37.08 to 30.54 %) over check (48.68%). Maximum reducing disease severity percent (37.26 %) was exhibited by T<sub>5</sub> where Bavistin (ST) @ 0.2 g a.i/kg seed + Tilt (FS) @ 1ml/liter which is followed by T<sub>2</sub> (35.21%), T<sub>6</sub> (31.08%) and minimum reducing disease severity (23.82%) was obtained by T<sub>7</sub> where Bavistin (ST) @ 0.2g a.i/kg seed + Vitavax (FS) @ 1g/liter. Treatment T<sub>7</sub>, clear indicate that the seed treatment with Bavistin is good but foliar spray with Vitavax is not effective. All fungicide combination were significantly effective in increasing grain yield (range 41.00 to 47.38 q / ha) over check (30.47q/ha). Maximum increasing grain yield percent (55.49 %) was exhibited by T<sub>5</sub> Bavistin (ST) @ 0.2 g a.i/kg seed + Tilt (FS) @ 1 ml/liter which was followed by T<sub>2</sub> (47.94%) and T<sub>6</sub> (45.12%) and minimum increasing grain yield percent (34.55%) was obtained by T<sub>7</sub> where Bavistin (ST) @ 0.2 g a.i/kg seed + Vitavax (FS) @ 1 g/liter. [13] also reported that the seed treatment through Bavistin and foliar spray with Tilt 1 ml/liter, best performance against brown spot of rice and also increasing the yield. [14] Reported propiconazol (Tilt) as the most promising fungicide that provided 47.5% and 26.5% reduction in disease incidence and severity, respectively along with 7.7% increase in grain yield. [10, 15] evaluate the Propiconazole 1 ml/liter foliar spray effectively against brown spot disease severity.

From the findings of the investigation, it was revealed that the all fungicide combination showed better performance against the brown spot diseases. Fungicide combination, Bavistin (ST) @ 0.2 g a.i/kg seed + Tilt (FS) @ 1 ml/liter was the best performance among all the treatments against brown spot disease and also increased the yield of rice.

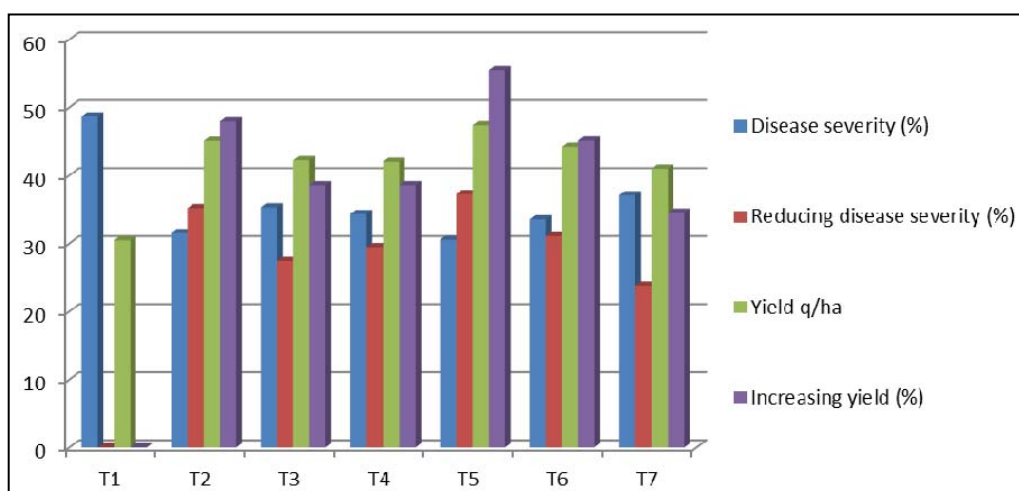
**Table 1:** Chemical fungicides tested for mycelial inhibition of *B. Oryzae*.

Mean radial growth (mm) of <i>Bipolaris oryzae</i> at different concentrations								
Treatments	100 ppm	Inhibition (%)	200 ppm	Inhibition (%)	250 ppm	Inhibition (%)	500 ppm	Inhibition (%)
T <sub>1</sub> Control	61.50 <sup>a</sup>	0.00	61.50 <sup>a</sup>	0.00	61.50 <sup>a</sup>	0.00	61.50 <sup>a</sup>	0.00
T <sub>2</sub> Carbendazim	43.56 <sup>b</sup>	29.17	42.49 <sup>b</sup>	30.91	30.15 <sup>c</sup>	50.97	19.67 <sup>c</sup>	68.01
T <sub>3</sub> Carboxin	45.34 <sup>b</sup>	26.27	41.83 <sup>b</sup>	31.98	34.67 <sup>b</sup>	43.62	25.34 <sup>b</sup>	58.79
T <sub>4</sub> Propiconazole	22.23 <sup>d</sup>	63.85	15.99 <sup>d</sup>	74.00	10.45 <sup>d</sup>	83.00	2.10 <sup>d</sup>	96.58
T <sub>5</sub> Hexaconazole	35.23 <sup>c</sup>	42.71	35.63 <sup>c</sup>	42.06	32.20 <sup>bc</sup>	47.64	21.30 <sup>bc</sup>	65.36
CD (0.05%)	4.54		2.82		3.66		4.34	

\*Average of four replication

**Fig 1:** Radial growths (mm) of *Bipolaris oryzae* at different concentrations and inhibition %**Table 2:** Impact of fungicide combination (applied as ST + FS) on disease severity and yield of rice.

Treatments	Disease severity (%)	Reducing disease severity (%)	Yield q/ha	Increasing yield (%)
T <sub>1</sub> Control	48.68 <sup>a</sup>	0.00	30.47 <sup>c</sup>	0.00
T <sub>2</sub> Vitavax (ST) @ 0.2g a.i/kg seed + Tilt (FS) @ 1ml/liter	31.54 <sup>bc</sup>	35.21	45.08 <sup>ab</sup>	47.94
T <sub>3</sub> Vitavax (ST) @ 0.2 g a.i/kg seed + Contaf (FS) @ 1 ml/liter	35.32 <sup>bc</sup>	27.44	42.22 <sup>b</sup>	38.56
T <sub>4</sub> Vitavax (ST) @ 0.2 g a.i/kg seed + Bavistin (FS) @ 1g/liter	34.34 <sup>bc</sup>	29.45	42.00 <sup>b</sup>	38.56
T <sub>5</sub> Bavistin (ST) @ 0.2g a.i/kg seed + Tilt (FS) @ 1ml/liter	30.54 <sup>c</sup>	37.26	47.38 <sup>a</sup>	55.49
T <sub>6</sub> Bavistin (ST) @ 0.2 g a.i/kg seed + Contaf (FS) @ 1 ml/liter	33.55 <sup>bc</sup>	31.08	44.22 <sup>ab</sup>	45.12
T <sub>7</sub> Bavistin (ST) @ 0.2g a.i/kg seed + Vitavax (FS) @ 1g/liter	37.08 <sup>b</sup>	23.82	41.00 <sup>b</sup>	34.55
CD (0.05%)	6.01		4.98	

**Fig 2:** Impact of fungicide combination on disease severity and yield of rice.**Reference**

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