



# In vitro study of Fungicides in controlling *Helminthosporium oryzae* causal organism of Leaf brown Spot of Rice

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Received 12<sup>th</sup> September 2015, revised 25<sup>th</sup> September 2015, accepted 6<sup>th</sup> October 2015

## Abstract

Leaf brown spot is a serious disease of rice causing considerable yield losses. Among various control measures the uses of chemical fungicides have been found highly effective. In vitro studies were carried out to evaluate the efficacy of fungicides in inhibiting the mycelial growth of *H. oryzae* pathogen causal organism of leaf brown spot of rice. All the fungicidal formulations at various concentrations were found significant in reducing the radial growth of the fungus. Among the tested fungicide Bavistin @ 1500 ppm was found significantly effective in inhibiting the radial growth of mycelium and second best treatment was Hinosan that was found at par with each other at the same concentration after 144 hrs of incubation.

**Keywords:** Leaf brown spot, rice, fungicide, in vitro.

## Introduction

Brown spot disease of rice caused by *Helminthosporium oryzae* (*Cochliobolus miyabeanus*) also known as *Dreschlera oryzae* causes severe yield loss that reaches up to 90% in certain areas<sup>1</sup>. Brown spot pathogen can infect both seedlings and mature plants and thus a serious menace to the rice crop. Brown leaf spot disease considered seed borne, and air borne, leaf spot vary in size and circular to oval in shape depending upon the environmental conditions.

The smaller spots are dark brown to reddish brown and larger spots have dark brown margin and reddish brown to grey centers. Damage by brown spot is particularly noticeable when crop nutritionally deficient in potassium<sup>2</sup>. The various methods prevail for rice disease control but the most important control tactics used worldwide includes use of resistant varieties and chemical control<sup>3</sup>. Rice disease management strategies mainly aim at prevention of outbreak or epidemics through the use of host plant resistance and chemical pesticides<sup>1</sup>.

Fungicide treatment is one of the low cost control measures available for brown spot management but host plant resistance is most economical<sup>4</sup>. Synthesizing and characterizing a new molecule to be used as fungicide involves several steps. Initially the new lead molecule is tested in-vitro for its efficacy against the target pathogen and then it is characterized under field condition to ascertain its efficacy against the target disease and to finalize the most effective dose/rate that can be used for the control of the target disease<sup>3</sup>. Therefore the study has been carried out to evaluate the efficacy of fungicide in least possible dose concentration to inhibit the mycelial growth of *H. oryzae*

under laboratory conditions.

## Material and Methods

**Single spore isolation from leaf surface:** The leaves of highly susceptible traditional variety Dehula were collected showing the typical lesion of brown spot from Agriculture field for single spore isolation. The spores were picked up from the spots of the leaf surface with the help of sterilized needle and a suspension of the spores was prepared in sterilized water. About 15 ml of the suspension of brown spot pathogen was poured into petri dishes containing potato dextrose medium at 24<sup>o</sup>C<sup>5</sup>. During this period, the germinated spores were marked by glass marker pen on petri dishes by examining under low power microscope. Small blocks of the medium containing single spores were cut by means of an inoculating needle and transfer into petri dishes containing medium and pure fungal culture was maintained for *in vitro* study.

**In vitro experiments:** In vitro studies of five fungicides viz. Bavistin, Hinosan, Aureofungin sol, Danzole plus and Propineb 70 WP (table-1) were conducted over untreated check for evaluating the efficacy against *H.oryzae*. Poisoned food techniques were employed for evaluating the sensitivity of test fungus<sup>6</sup>. The fungicides were taken in concentrations of 1000 and 1500 ppm and were incorporated aseptically in potato dextrose agar medium. The amended medium was then poured in sterilized Petri dishes. 5 mm disc of the test fungus were cut from the margins of old test culture was then placed centrally in each of the petri dishes. The inoculated plates were incubated at room temperature and the colony diameter was recorded after incubation at an interval of 24 hr.

**Table-1**  
**Chemical fungicides tested for mycelial inhibition of *H. oryzae***

| Technical Name            | Trade Name      | a.i (g)      | Formulation Dosage/ha | Dilution in water (l) |
|---------------------------|-----------------|--------------|-----------------------|-----------------------|
| Carbendazim 5%GR          | Bavistin        | 0.62 kg      | 12.5 kg               | 00                    |
| Ediphenphos 50% EC        | Hinosan         | 250-300 (ml) | 500-600 (MI)          | 750-1000 l            |
| Aureofungin 46.15 W.V. SP | Aureofungin sol | 00           | 0.005%                | 500                   |
| Hexaconazole 5% EC        | Danzole plus    | 50g          | 1000g                 | 500                   |
| Propineb 70% WP           | Propineb 70 WP  | 1050-1400    | 1500-2000             | 500 lvol/ha           |

**Statistical analysis:** All Data were first subjected to analysis of variance (ANOVA). Comparisons among means were made using Duncan’s multiple range test ( $P = 0.05$ ).

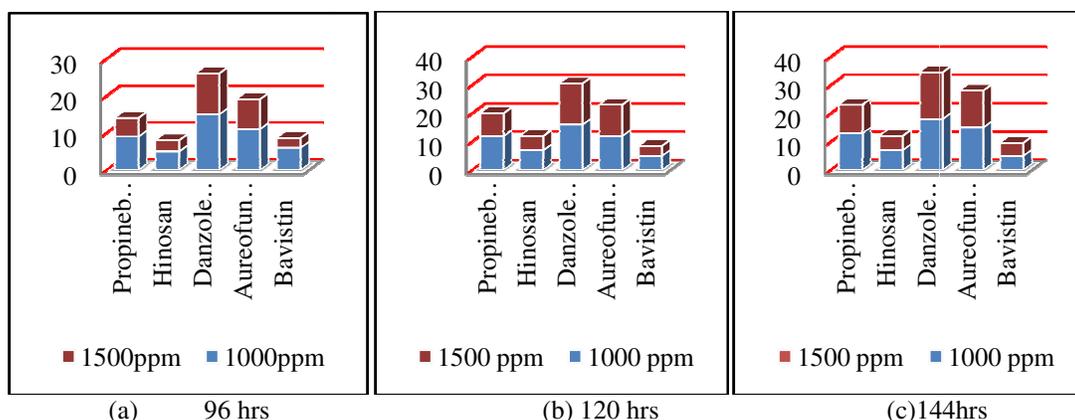
**Results and Discussion**

The bioefficacy of fungicide on radial growth of *H. oryzae* in different periodic interval (96, 120 and 144 hrs) were carried *in vitro*. All the tested fungicides and their different concentrations viz 1000 ppm and 1500 ppm, significantly inhibited the mycelial growth of *H. oryzae* *in vitro*. It was observed that the

rate of mycelial inhibition shows significant reduction with the passage of time. The data presented in table-2 and figure-1 reveals that fungicide Bavistin @ 1500 ppm was found highly effective in inhibiting the radial growth of mycelium in 96, 120 and 144 hrs. of incubation 2.6, 3.5 and 4.5mm, respectively. The Hinosan was the second best treatment that inhibited the radial growth in same concentration and periodic interval 3, 5 and 5mm over untreated check. The fungicide Propineb 70 WP and Aureofungin sol were found at par in checking the mycelial growth and Danzole plus shows least influence in inhibiting the mycelial growth of the fungus.

**Table-2**  
**Effect of fungicides on mycelial growth of *H. oryzae***

| Treatment       | Conc. (ppm) | Period of incubation (growth in mm) |         |         |
|-----------------|-------------|-------------------------------------|---------|---------|
|                 |             | 96 Hr                               | 120 Hr. | 144 Hr. |
| Propineb 70WP   | 1000        | 9                                   | 12      | 13      |
|                 | 1500        | 5                                   | 8       | 10      |
| Hinosan         | 1000        | 5                                   | 7       | 7       |
|                 | 1500        | 3                                   | 5       | 5       |
| Danzole plus    | 1000        | 15                                  | 16      | 18      |
|                 | 1500        | 11                                  | 14.5    | 16.5    |
| Aureofungin sol | 1000        | 11                                  | 12      | 15      |
|                 | 1500        | 8                                   | 11      | 13      |
| Bavistin        | 1000        | 6                                   | 5       | 5       |
|                 | 1500        | 2.6                                 | 3.5     | 4.5     |
| Control         |             | 20                                  | 27      | 38.5    |
| SEm±            |             | 5.31                                | 6.69    | 9.68    |
| CD (5%)         |             | 17.67                               | 22.25   | 32.18   |



**Figure-1**  
**Inhibition of mycelial growth of H.oryzae at various conc. of fungicides**

**Discussion:** All tested fungicides were effective against *H.oryzae* at higher concentration. The standard systemic fungicides were more pronounced compare to other products. The results of this research are in agreement with Ahmad et al<sup>7</sup> who evaluated four fungicides viz. Bavistin, Hinosan, Tilt 250 EC and Dithane M-45 against *Bipolarisoryzae* and observed that Dithane M-45 was the best with 100% reduction of the prevalence of the pathogen at 500 ppm as mycelia growth inhibition followed by Tilt 250 EC, Hinosan and Bavistin. Similarly Arshad et al<sup>4</sup> reported that Ridomil (Metalaxyl+Mancozeb) and Dithane M-45 (Mancozeb), at 50 ppm concentrations were more effective to control the mycelial growth of fungus in vitro. The result also goes in tune with Hunjan et al<sup>8</sup> who reported that new fungicides viz. trifloxystrobin + tebuconazole, tebuconazole, and propiconazole showed higher level of efficacy against *D. oryzae* pathogens of under laboratory conditions, however, thifluzamide (Spencer 24SC) was least effective against the diseases under the same condition.

The findings are in accordance with Rivero et al<sup>9</sup> who studied *in vitro* effect of different concentrations (300, 500, 700 and 1000 mg/L) of K1 (INCA) and SIGMA chitosans on fungal mycelial growth and observed that both products had an inhibitory effect on brown spot fungal growth at all evaluated concentrations and SIGMA chitosan showed a total inhibition at 1000 mg/L, besides its fungicide effect. The results also in close conformity with Ibiyam et al<sup>10</sup> who investigated the use of seed-dressing fungicides (Bavistin, Benlate, Fernasan-D, Apron Plus 50 DS and Dithane-M45), and soaking and slurry methods at various concentrations, for the control of seed-borne fungi of rice variety *in vitro* and observed that all the fungicides significantly inhibited the seed-borne fungi associated with the seeds of the variety at concentrations of 40g/ml, and 50mg/ml ( $P < 0.05$ ), in the soaking method, and at all the concentrations in the slurry method ( $P < 0.05$ ) along with percentage seed germination not below 71%, at all the concentrations in the two methods used. Shabana et al<sup>11</sup> tested the antifungal activity of three phenolic antioxidants (salicylic acid, benzoic acid and hydroquinone)

against *B. oryzae* at different concentrations *in vitro* and observed that benzoic acid or salicylic acid at 9 mM completely inhibited the growth of the pathogen.

### Conclusion

Therefore it can be concluded that all the fungicides involved for controlling brown spot pathogen of rice in vitro were found significant; however the least possible doses of these chemicals are advised for growers for a better results along with a sustainable agriculture.

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