

# Morphological, Pathological and Cultural Characteristics of *Magnaporthe Oryzae Triticum* Causing Blast of Wheat and its Fungicidal Control







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## ABSTRACT

*Magnaporthe oryzae Triticum* causing blast a devastating disease of wheat. An attempt was taken to study the morphological, pathological and cultural characteristics of *M. oryzae Triticum* and its *in-vitro* fungicidal control. Four isolates were isolated from infected wheat inflorescence. Conidia of the isolates were solitary, pyriform to obclavate, pale brown, smooth, granular to guttulate, 2-septate; hilum truncate, protruding and not darkened. Isolates were positive in pathogenicity test on wheat and caused characteristic eye shape lesions on leaves. The wheat blast isolates did not produce any blast symptom in rice. *M. oryzae Triticum* showed consistently better mycelial growth at pH 5.5, 29°C and in Oat Meal Agar medium. Efficacy of six fungicides such as Autostin 50WGD, Nativo 75WG, Diathane M 45, Khowin 50WP, Ridomil Gold MZ 68WG and Provax 200WP at concentrations of 50 ppm, 100 ppm and 150 ppm was evaluated against *M. oryzae Triticum* in *in-vitro*. Autostin 50WGD, Nativo 75WG and Knowin 50WP inhibited the growth of *M. oryzae Triticum* completely at all three concentrations. Therefore, these three fungicides may be suggested to control blast disease of wheat.

**Keywords:** Wheat blast, *Magnaporthe oryzae Triticum*, Characters and fungicides.

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### Highlights of this paper

- Wheat blast caused by the *Magnaporthe oryzae Triticum* is a devastating disease first reported in the State of Paraná in Brazil in 1985.
- While in Bangladesh, reported in 2016 for the first time in Asia. According to the International Maize and Wheat Research Center (CIMMYT), wheat blast is one of the most fearsome and intractable wheat diseases in recent decades.
- As the disease is new in Bangladesh, characterization of its pathogen is important to explore proper control measures. Morphological, pathological and cultural characteristics of the *M. oryzae Triticum* and its fungicidal control are described in present paper.

## 1. INTRODUCTION

Wheat blast caused by the *Magnaporthe oryzae Triticum* was first reported in the State of Paraná in Brazil in 1985 [1]. On the contrary, terrifying blast disease of wheat (*Triticum aestivum*) was spotted in Bangladesh in 2016 and this was the first occurrence in the Asia [2, 3]. About 15% of total wheat area in Bangladesh was affected, severely infection was observed in the districts of Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal and Bhola [3]. According to the International Maize and Wheat Research Center (CIMMYT), wheat blast is one of the most fearsome and intractable wheat diseases in recent decades [4]. Pathogen attacks on rachis. Typical symptoms include partially infected spikes that turn bleached (white) and are easily distinguishable from healthy plants. Examination of diseased plants showed the presence of elliptical, grayish to tan necrotic lesions with dark borders on the leaf often mixed with spot blotch disease lesions. Additionally, in some fields, blackening of lower nodes was observed. Grains from blast infected heads were small, shriveled, deformed, and had low test weight leading to serious yield losses [5]. Standing wheat crop was burnt in some areas. The phylogenetic analysis revealed that Bangladesh outbreak strains and the Brazil outbreak strains were the same phylogenetic lineage. The rapid open source genomic surveillance approach has revealed the precise identity of the infectious Bangladeshi fungus as the known wheat-infecting *M. oryzae* lineage and indicated that it is most likely originated from South America [3, 6, 7]. The pathogen is a new one in Bangladesh thus its proper characterization is important to find out proper control measures. Wheat blast is a sporadic disease, with the most extensive damage occurring during warm, wet years [8]. Wheat blast epidemics are reported to follow several days of continuous rains and temperatures from 18-25°C during flowering, followed by hot, sunny and humid days [9]. It was reported that wheat blast was seed-borne, airborne, likely on residues and infection spreads from plant to plant and it could survive on grasses to infect the next year's wheat crop [10, 11]. Different authors suggested that prophylactic fungicide application is effective to control this disease. Fungicides combining triazols with strobilurins can, under some situations, be effective in disease control at the heading stage Kohli, et al. [9]. Cruz, et al. [12] proposed that earlier fungicide applications might reduce *M. oryzae Triticum* inoculum from basal leaves and thus lower the risk of fungal infections on spikes. Another management strategy is seed treatment with fungicides. An effective seed treatment may eradicate *M. oryzae Triticum* primary inoculum and reduce sporulation [13]. Based on the above discussion the experiment was conducted to know the morphological, pathological and cultural characteristics of the *M. oryzae Triticum* and find out appropriate fungicides to control the pathogen.

## 2. MATERIALS AND METHODS

### 2.1. Isolation and Identification of Blast Pathogen

Fungal isolates were isolated from infected inflorescence of wheat collected from the farmers' field of Jessore and Rajbari district of Bangladesh. At the time of isolation, inflorescence parts were surface sterilized successively with 1% sodium hypochloride (NaOCl) for 2-3 min. The surface sterilized samples were placed in a petri dish laid

with sterilized blotter paper maintaining abundant moisture. After 7 days incubation at  $27\pm 1^\circ\text{C}$ , abundant conidia on aerial conidiophores were observed on the samples. Then conidia were aseptically transferred to PDA plate (potato 250 g, dextrose 20 g, agar 17 g, distilled water up to 1L). The petri dishes were incubated for 5-7 days at  $27\pm 1^\circ\text{C}$ . The fungus was purified on PDA medium following hyphal tip culture technique [14]. Identification of *M. oryzae Triticum* was performed under a compound microscope by observing detailed morphology and identified using appropriate keys [15-17]. The pure culture of the isolated *M. oryzae Triticum* was preserved in PDA slants at  $5\pm 1^\circ\text{C}$  as stock culture.

## 2.2. Pathogenicity Test

Four isolates of *M. oryzae Triticum* were tested for their pathogenicity in wheat and rice. The isolates were  $J_1$  and  $J_2$  isolated from inflorescence samples collected from Jassore and isolates  $R_1$  and  $R_2$  from samples collected from Rajbari, Bangladesh. Inoculum of isolated fungi was prepared by suspending pure colonies grown on PDA medium in sterilized distilled water separately in beakers, agitating and stirring upon mechanical stirrer for one hour and diluting it to make uniform fungal suspension of spores having  $10^5$  conidia  $\text{ml}^{-1}$ . Seedlings of BARI Gom25, BARI Gom26 and one rice variety BRRI Dhan28 were inoculated. When the seedlings were in three leaves stages, they were sprayed with a conidial suspension of four purified isolates  $J_1$ ,  $J_2$ ,  $R_1$  and  $R_2$ , until full wet. Non-inoculated controls were sprayed with only sterilized water. The inoculated plants were instantly covered with sterilized transparent polyethylene bags to maintain humidity. Plants were kept under natural light conditions at  $25\text{-}30^\circ\text{C}$  for the development of blast symptoms. Those bags were removed after 48 hrs and symptoms of infection were checked after 7 days of inoculation. The causal agent was confirmed after re-isolation of the pathogen from infected leaves.

## 2.3. Effect of pH on Growth of *M. Oryzae Triticum*

PDA medium amended with five levels of pH such as 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 was used. Inoculum (mycelial disc 4 mm diameter) taken from the margin of 7 days old culture of the isolates  $J_1$  and  $R_2$  grown on PDA were inoculated at the centre of petri plate containing PDA medium. Inoculated media were incubated at  $27\pm 1^\circ\text{C}$ , with each treatment replicated three times. After 10 days, colony diameter of each isolate was measured in two directions using a ruler.

## 2.4. Effect of Temperature on Growth of *M. Oryzae Triticum*

Growth of two isolates  $J_1$  and  $R_2$  was tested at five level temperatures 18, 22, 25, 29 and  $33^\circ\text{C}$  in PDA media. Mycelial disc of each isolate (4 mm in diameter) was taken from the edge of 7 days old colony grown on PDA and placed at the center of petri dishes containing freshly prepared PDA medium. Each treatment was replicated three times. Colony diameter (cm) of each isolate was measured after 10 days in two directions using a ruler.

## 2.5. Effect of Culture Media on Growth of *M. Oryzae Triticum*

The mycelial growth characters of isolate  $J_1$  and  $R_2$  were studied on five solid media. The media were mass media (Sucrose 10 g, Peptone 10 g, NaCl 5 g, Yeast Extract 5 g, Agar 17 g, Water 1 L), oat meal agar (Oat Meal 60 g, Glucose 20 g, Agar 17 g, Water 1 L), potato dextrose agar (Potato 200 g, Dextrose 20 g, Agar 17 g, Water 1 L), wheat meal agar (Wheat Meal 60 g, Sucrose 10 g, Agar 17 g, Water 1 L) and carrot agar (Carrot 200 g, Glucose 30 g, Agar 17 g, Water 1 L). The media were inoculated with 4 mm mycelial disc cut from the periphery of actively growing cultures of each *M. oryzae Triticum* isolate and incubated at  $27\pm 1^\circ\text{C}$ . Each treatment was replicated three

times. Colony diameter of each isolate on petri dishes was measured in two directions with a ruler at the 10<sup>th</sup> day of incubation.

### 2.6. Fungicidal Control of Radial Growth of *M. Oryzae Triticum*

Six fungicides namely Autostin 50WGD (Carbendazim), Nativo 75WG (Tebuconazole + Trifloxistobin), Diathane M-45 (Mencozeb), Khowin 50WP (Carbendazim), Ridomil Gold MZ 68WG (Mencozeb + Metalaxil), Provox-200WP (Carboxil + Thirum) were tested *in-vitro* to evaluate their effect on colony growth of *M. oryzae Triticum*. All fungicides were tested at 50, 100 and 150 ppm concentrations. Fungicidal suspensions of different concentrations were prepared by mixing required amount of each fungicide in warm PDA and autoclaved. The medium without fungicide served as control. Inoculation was performed using mycelial disc (4 mm diameter) taken from the margin of 7 days old cultures of J<sub>1</sub> and R<sub>2</sub> isolates. A mycelial disc of the fungus was placed reversely at the centre of the petri dish after solidification of PDA. Each treatment was replicated three times. The plates were incubated at 27±1°C and data on the radial colony diameter (cm) was recorded after 10 days of incubation. Radial growth of the fungus was measured by averaging the two measurements of diameters taken at right angles for each colony. Inhibition percentage of *M. oryzae Triticum* for different treatments was calculated.

### 2.7. Statistical Analysis

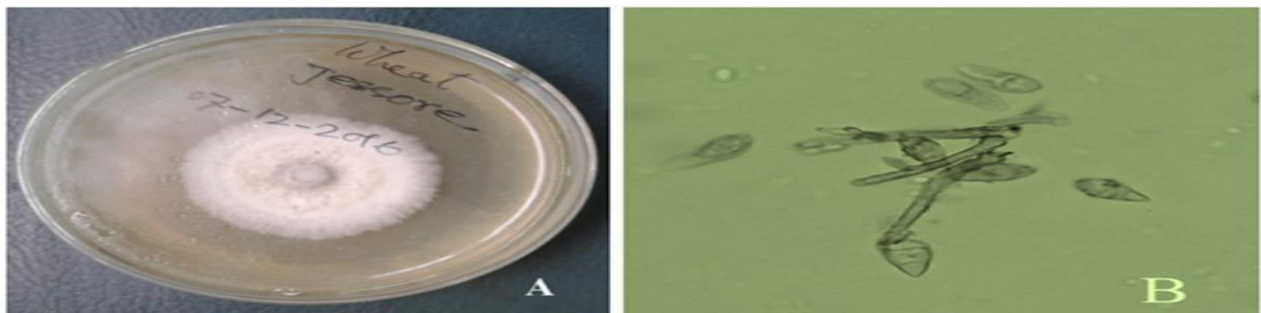
The data was analyzed by completely randomized design using Statistix 10 program. Treatment means were compared using Least Significant Difference (LSD) at 5% level of significance and sample data were calculated by using Microsoft Excel 2014.

## 3. RESULTS AND DISCUSSION

Four fungal isolates were isolated from infected wheat inflorescence. Isolates J<sub>1</sub> and J<sub>2</sub> were isolated from inflorescence collected from Jassore and isolates R<sub>1</sub> and R<sub>2</sub> were from inflorescence collected from Rajbari.

### 3.1. Morphology of Isolates

On PDA medium colonies exhibited concentric growth with abundant white aerial mycelia and pale grey sporulation at centre. Abundant white aerial mycelia were present and colony at the bottom were dark grey in color. Mycelium was consisting of smooth, hyaline, branched, septate hyphae. Conidiophores were solitary, erect, straight or curved, unbranched, medium brown, smooth, two to three septate; base arising from hyphae, not swollen, lacking rhizoids. Conidiogenous cells were integrated, terminal and intercalary, pale brown, smooth, forming a rachis with several protruding denticles. Conidia were solitary, pyriform to obclavate, pale brown, smooth, granular to guttulate, 2-septate, hilum truncate, protruding, unthickened, not darkened **Figure 1**.



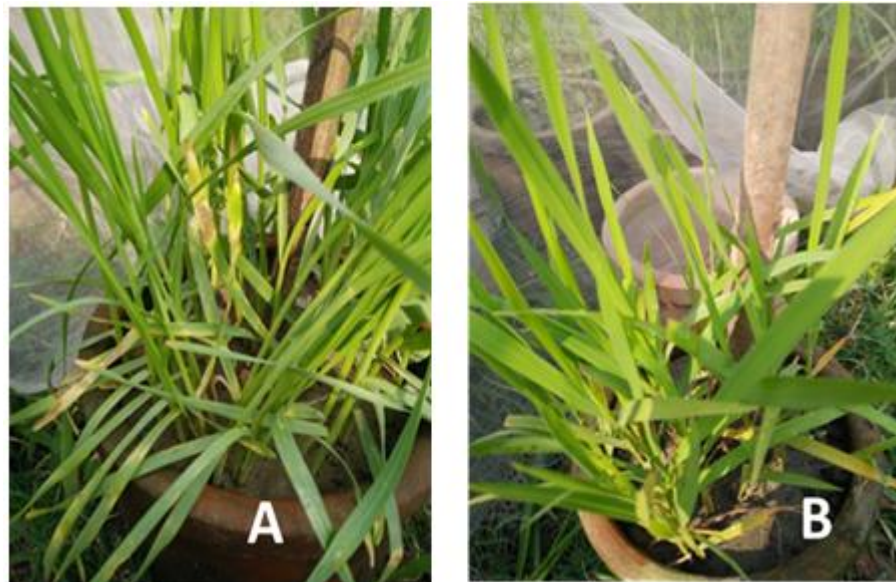
**Figure-1.** Wheat blast pathogen *Magnaporthe oryzae Triticum*, (A) Culture in PDA plate, (B) conidia and conidiophore observed under compound microscope.

### 3.2. Pathogenicity Test

Wheat blast isolates J<sub>1</sub>, J<sub>2</sub>, R<sub>1</sub> and R<sub>2</sub> developed typical blast lesions on leaves of the wheat varieties BARI Gom25 and BARI Gom26. Each of the isolates caused leaf infection on both the wheat varieties used in this study. Among the four isolates, R<sub>2</sub> was highly virulent followed by J<sub>1</sub> in terms of the average number of lesion per leaf. Re-isolation of pathogen from symptoms developed after inoculation confirmed the same pathogen. On the other hand, the wheat blast isolates J<sub>1</sub>, J<sub>2</sub>, R<sub>1</sub> and R<sub>2</sub> did not develop any blast symptom on the rice variety BRR1 Dhan28 Table 1. It was found that wheat blast pathogen developed characteristic eye shape lesions on wheat leaves, junction of leaf blade and leaf sheath. Mostly symptoms observed in lower leaves, several lesions coalesced and developed larger lesion Figure 2. The results suggested that chromosomal constituent of wheat and rice blast pathogen may bears some differences, so they do not cause compatible reaction on wheat and rice plants. It was previously reported that the rice pathogen population was not the source for wheat blast, based on lack of cross-infectivity of rice and wheat isolates [8, 18, 19]. The present results further supported by the findings of Farman [20]; Tosa, et al. [21]; Couch, et al. [22]; Tredway, et al. [23]; Klaubauf, et al. [24]; Paulo, et al. [25] they concluded that wheat blast isolates do not infect rice. Based on pathogenicity two isolates J<sub>1</sub> and R<sub>2</sub> were selected and preserved in test tubes containing PDA media for further study.

**Table-1.** Pathogenicity of *Magnaporthe oryzae Triticum* isolates isolated from wheat inflorescence sample.

Isolate codes	No. of lesion per leaf		
	BARI Gom25	BARI Gom26	BRR1 Dhan28
J <sub>1</sub>	4.18	4.96	0
J <sub>2</sub>	4.00	4.10	0
R <sub>1</sub>	3.82	3.90	0
R <sub>2</sub>	4.80	5.40	0



**Figure-2.** Blast diseases of wheat (A) Symptom develop after inoculation of fungus in wheat seedlings and (B) No symptom in case of rice seedlings.

### 3.3. Growth of *M. Oryzae Triticum* at Different pH Level

Significantly the highest mean mycelial growth of J<sub>1</sub> isolate (6.85 cm) was recorded at pH 5.5 followed by pH 5.0 (6.18 cm). The R<sub>2</sub> isolate produced the highest mycelial growth also at pH 5.5 (7.26 cm) followed by at pH 5.0 (6.52 cm). Mycelial growth of both the isolates was more than 5.00 cm at pH 4.5-6.0. Mycelial growth of R<sub>2</sub> isolate was also recorded 5.23 cm at pH 6.5. The least growth was observed at pH 7 for both the isolates J<sub>1</sub> (3.73 cm) and



R<sub>2</sub> (4.63 cm). This study indicated that isolates grows well at acidic pH ranged 5.0-5.5 Table 2. According to the Mijan [26] the growth of *P. grisea* increased with increase in pH from 3.5-6.5. The variation among the findings might be due to the difference in the sensitivity of the different isolates.

**Table-2.** Mycelial growth of *Magnaporthe oryzae Triticum* isolates at different pH range after 10 days of incubation at 27±1°C.

pH range	Colony diameter (cm)	
	J <sub>1</sub>	R <sub>2</sub>
4.5	5.78c	5.45cd
5.00	6.18b	6.52b
5.5	6.85a	7.26a
6.00	5.13d	5.65c
6.5	4.37e	5.23d
7.00	3.73f	4.63e
CV	1.81	2.23

Each value is an average of three replicates. Data followed by the same letter are not significantly different along the column ( $p < 0.05$ ).

### 3.4. Growth of *M. Oryzae Triticum* at Different Temperatures

Both the J<sub>1</sub> and R<sub>2</sub> isolates showed different responses to the different incubation temperatures. Increased mycelial growth was observed with increasing temperature from 18 to 29°C, but no growth was recorded at 33°C. The isolates J<sub>1</sub> and R<sub>2</sub> showed significantly highest colony diameter 6.78 cm and 7.00 cm, respectively at 29°C. Second highest growth was recorded at 25°C for both the isolates J<sub>1</sub> (5.65 cm) and R<sub>2</sub> (5.93 cm), which was followed by 22°C. The least growth was recorded at 18°C by the both isolates J<sub>1</sub> (3.88 cm) and R<sub>2</sub> (4.77 cm) Table 3. Kumar and Singh [27] studied the differential response of *Pyricularia grisea* isolates to temperature and reported maximum growth at 30°C. Netum, et al. [28]; Gashaw, et al. [29] recorded the better mycelium growth and sporulation of *P. grisea* on finger millet was at 25-30°C temperatures. The result of the present experiment agreed well with the findings of previous authors [27-29].

**Table-3.** Mycelial growth of *Magnaporthe oryzae Triticum* isolates at different temperatures after 10 days of incubation at 27±1°C.

Temperature (°C)	Colony diameter (cm)	
	J <sub>1</sub>	R <sub>2</sub>
18	3.88d	4.77d
22	4.65c	5.15c
25	5.65b	5.93b
29	6.78a	7.00a
33	0.00e	0.00e
CV	2.20	2.01

Each value is an average of three replicates. Data followed by the same letter are not significantly different along the column ( $p < 0.05$ ).

### 3.5. Growth of *M. Oryzae Triticum* at Different Solid Culture Media

J<sub>1</sub> and R<sub>2</sub> isolates showed significant variation in mycelial growth on different solid culture media. Among the media tested, oat meal agar showed the highest mycelial growth in both J<sub>1</sub> (5.50) and R<sub>2</sub> (5.92). The least mycelium growth was observed in wheat meal agar, 4.53 cm in case of J<sub>1</sub> and 4.92 cm in case of R<sub>2</sub>. The results of the experiments showed that oat meal agar medium favors the growth of *M. oryzae Triticum* much more than PDA medium Table 4. The higher preference for oat meal agar than PDA might be due to higher microelement contents in oat meat agar which are essential for good growth of *Pyricularia* species. Similar results were obtained by Meena [30] and Dar, et al. [31]. They suggested that oat meal agar as a suitable medium for growth and sporulation of

the pathogen. This possibly indicates that the pathogen has preference for certain nutritional components, which could be related to specificity for host.

**Table-4.** Mycelial growth of *Magnaporthe oryzae Triticum* isolates on different culture media after 10 days of incubation at  $27\pm 1^\circ\text{C}$ .

Media	Colony diameter (cm)	
	J <sub>1</sub>	R <sub>2</sub>
Potato dextrose agar	5.13bc	5.5b
Oat meal agar	5.50a	5.92a
Mass media	5.23ab	5.6ab
Wheat meal agar	4.53d	4.92c
Carrot agar	4.83cd	5.52ab
CV	3.85	4.09

Each value is an average of three replicates. Data followed by the same letter are not significantly different along the column ( $p < 0.05$ ).

### 3.6. Efficiency of Fungicides Against Growth of *M. Oryzae Triticum*

All six fungicides showed significant inhibitory effect on radial colony growth of *M. oryzae Triticum* on PDA plates at 50, 100 and 150 ppm concentration. The rate of inhibition varied with fungicides and their level of concentrations. Autostin 50WGD, Nativo 75WG and Khowin 50WP at 50, 100 and 150 ppm completely inhibited the mycelial growth of *M. oryzae Triticum* on PDA plate. Provax-200WP caused 66.58%, 71.29% and 80.44% inhibition of radial growth of at 50, 100 and 150 ppm, respectively. The reductions in radial colony diameter were 11.88, 17.32 and 27.72% under the treatment with Diathane M-45 at 50, 100 and 150 ppm concentration, respectively. Ridomil Gold MZ 68WG at three concentrations 50, 100 and 150 ppm gave 5.44, 8.91 and 33.66% reduction of radial growth, respectively Table 5, Figure 3. This result indicates that Autostin 50WGD (Carbandazim), Nativo 75WG (Tebuconazole) and Khowin 50WP (Carbandazim) were most effective against *M. oryzae Triticum* gave good control of the pathogen. The result of this experiments supported the findings of Saharan, et al. [32]. They found tricyclazole or carbendazim were effective for controlling seed inoculum in case of rice blast. Kohli, et al. [9] stated that Mixtures of triazoles (tebuconazole and metconazole) and strobilurins have been used effectively to control head blast in moderately resistant wheat varieties. The wheat Research Centre (WRC) of Bangladesh Agricultural Research Institute (BARI) recommended Nativo 75WG for controlling the blast disease of wheat [33]. The result of the present experiment supported the recommendation of WRC and at some extent agreed with the findings of Kohli, et al. [9] and Saharan, et al. [32]. Thus the results suggested that Autostin 50WGD, Nativo 75WG and Knowin 50WP are suitable for control of blast disease of wheat.

**Table-5.** Effect of Fungicides on the Radial growth of *Magnaporthe oryzae Triticum*.

Fungicides	Radial growth (cm)		
	50 ppm (mean $\pm$ SE)	100 ppm (mean $\pm$ SE)	150 ppm (mean $\pm$ SE)
Autostin 50WGD	0.00e $\pm$ 0.000	0.00e $\pm$ 0.000	0.00e $\pm$ 0.000
Nativo 75WG	0.00e $\pm$ 0.000	0.00e $\pm$ 0.000	0.00e $\pm$ 0.000
Diathane M-45	5.93c $\pm$ 0.067	5.57c $\pm$ 0.221	4.87b $\pm$ 0.089
Khowin 50WP	0.00e $\pm$ 0.000	0.00e $\pm$ 0.000	0.00e $\pm$ 0.000
Ridomil Gold MZ 68WG	6.37b $\pm$ 0.133	6.13b $\pm$ 0.120	4.47c $\pm$ 0.145
Provax-200WP	2.25d $\pm$ 0.029	1.93d $\pm$ 0.033	1.32d $\pm$ 0.060
Control	6.73a $\pm$ 0.089	6.73a $\pm$ 0.089	6.73a $\pm$ 0.089
CV	3.80	6.34	4.83

Each value is an average of three replicates. Data followed by the same letter are not significantly different along the column ( $p < 0.05$ ).

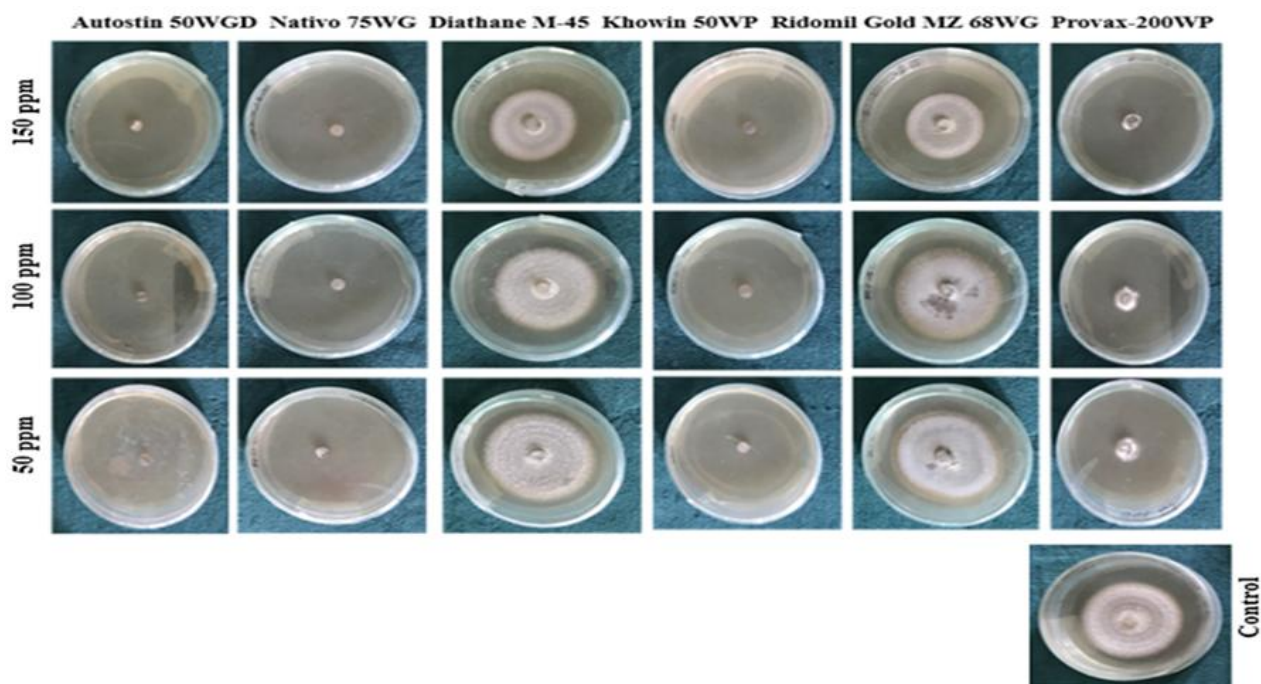


Figure-3. Effects of fungicides on radial colony growth of *Magnaporthe oryzae Triticum* at different concentrations.

#### 4. CONCLUSION

*Magnaporthe oryzae Triticum* isolates were pathogenic in wheat but not in rice. Higher growth of *M. oryzae Triticum* was recorded at pH 5.5, 29°C and in Oat Meal Agar media. Three fungicides namely Autostin 50WGD, Nativo 75WG and Knowin 50WP completely inhibited the growth of *M. oryzae Triticum* at 50, 100 and 150 ppm concentrations *in-vitro*. Therefore, it is suggested to use these three fungicides to control blast of wheat.

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