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

Canadian Journal of Agriculture and Crops
Vol. 4, No. 2, 218-227, 2019
e-ISSN: 2518-6655

(✪ *Corresponding Author*)

Debnath, B.[1]
Khan, A.A.[2]
Hossain, M.M.[3]
Rubayet, M.T.[4]
Miah, M.R.U.[5]

[1,2,3,4]*Department of Plant Pathology, Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.*
[1]*Email: bithedebnath1@gmail.com Tel: +8801677984448*
[2]*Email: ashraf@bsmrau.edu.bd Tel: +8801711193457*
[3]*Email: hossainmm@bsmrau.edu.bd Tel: +8801917043506*
[4]*Email: tanbir86plp@gmail.com Tel: +8801717313156*
[5]*Department of Entomology, Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.*
[5]*Email: ramizmiah@yahoo.com Tel: +8801552495584*

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.*

DOI: 10.20448/803.4.2.218.227
Citation | Debnath, B.; Khan, A.A.; Hossain, M.M.; Rubayet, M.T.; Miah, M.R.U. (2019). Morphological, Pathological and Cultural Characteristics of Magnaporthe Oryzae Triticum Causing Blast of Wheat and its Fungicidal Control. Canadian Journal of Agriculture and Crops, 4(2): 218-227.

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
History: Received: 22 August 2019/ Revised: 26 September 2019/ Accepted: 31 October 2019/ Published: 3 December 2019
Publisher: Online Science Publishing

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 Jassore 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±1°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±1°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±1°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 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 25-30°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±1°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°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±1°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 10th day of incubation.

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

Six fungicides namely Autostin 50WGD (Carbendazim), Nativo 75WG (Teboconazole + Trifloxistobin), Diathane M-45 (Mencozeb), Khowin 50WP (Carbendazim), Ridomil Gold MZ 68WG (Mencozeb + Metalaxil), Provax-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_1 and R_2 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 influorescence. Isolates J_1 and J_2 were isolated from influorescence collected from Jassore and isolates R_1 and R_2 were from influorescence 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.

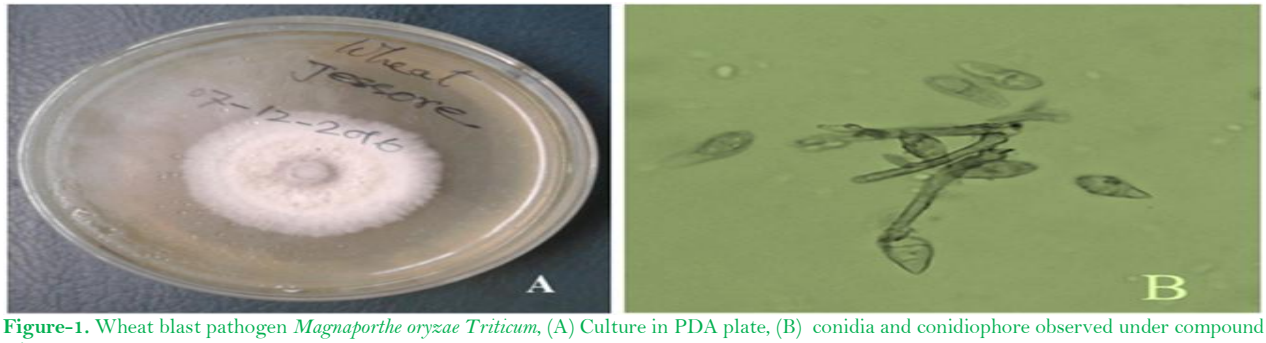

Figure-1. Wheat blast pathogen *Magnaporthe oryzae Triticum*, (A) Culture in PDA plate, (B) conidia and conidiophore observed under compound microscope.

3.2. Pathogenicity Test

Wheat blast isolates J_1, J_2, R_1 and R_2 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_2 was highly virulent followed by J_1 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_1, J_2, R_1 and R_2 did not develop any blast symptom on the rice variety BRRI 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_1 and R_2 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	BRRI Dhan28
J1	4.18	4.96	0
J2	4.00	4.10	0
R1	3.82	3.90	0
R2	4.80	5.40	0

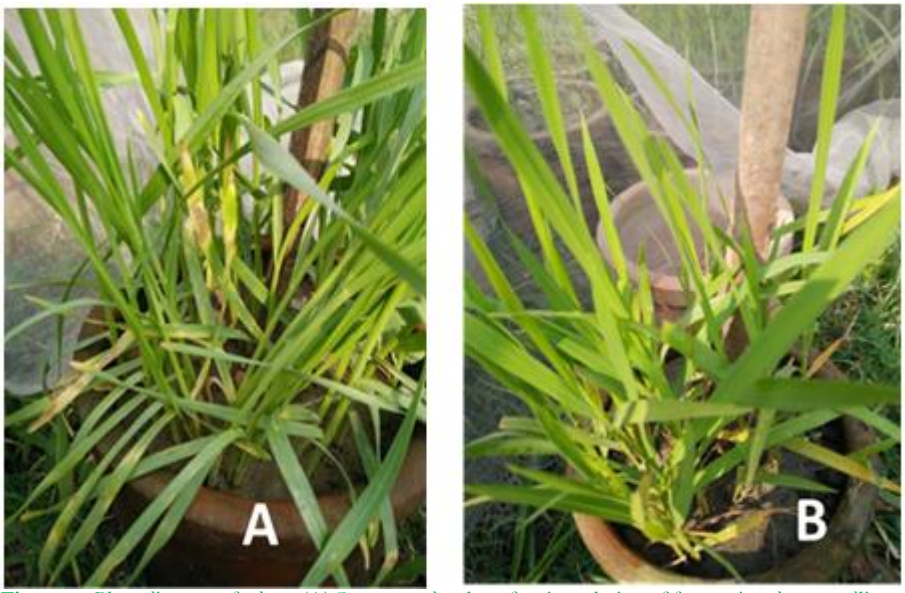

Figure-2. Blast diseases of wheat (A) Symptom develop after inoculation of fungus in wheat seedlings and (B) No symptom in case of rice seedlings.

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

Significantly the highest mean mycelial growth of J_1 isolate (6.85 cm) was recorded at pH 5.5 followed by pH 5.0 (6.18 cm). The R_2 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_2 isolate was also recorded 5.23 cm at pH 6.5. The least growth was observed at pH 7 for both the isolates J_1 (3.73 cm) and

R_2 (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_1	R_2
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_1 and R_2 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_1 and R_2 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_1 (5.65 cm) and R_2 (5.93 cm), which was followed by 22°C. The least growth was recorded at 18°C by the both isolates J_1 (3.88 cm) and R_2 (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_1	R_2
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_1 and R_2 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_1 (5.50) and R_2 (5.92). The least mycelium growth was observed in wheat meal agar, 4.53 cm in case of J_1 and 4.92 cm in case of R_2. 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±1°C.

Media	Colony diameter (cm)	
	J_1	R_2
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 (Teboconazole) 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 ± SE)	100 ppm (mean ± SE)	150 ppm (mean ± SE)
Autostin 50WGD	0.00e ± 0.000	0.00e ± 0.000	0.00e ± 0.000
Nativo 75WG	0.00e ± 0.000	0.00e ± 0.000	0.00e ± 0.000
Diathane M-45	5.93c ± 0.067	5.57c ± 0.221	4.87b ± 0.089
Khowin 50WP	0.00e ± 0.000	0.00e ± 0.000	0.00e ± 0.000
Ridomil Gold MZ 68WG	6.37b ± 0.133	6.13b ± 0.120	4.47c ± 0.145
Provax-200WP	2.25d ± 0.029	1.93d ± 0.033	1.32d ± 0.060
Control	6.73a ± 0.089	6.73a ± 0.089	6.73a ± 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 ($p<0.05$).

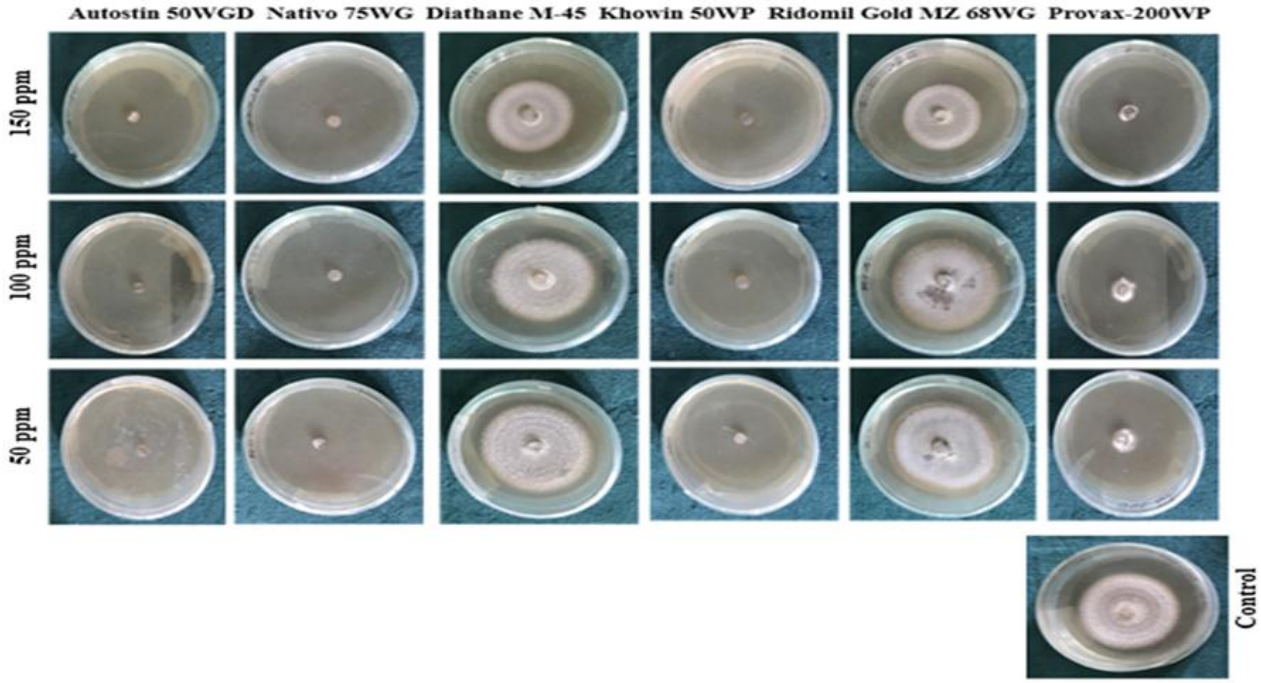

Figure-3. Effects of fungicides on radial colony growth of *Magnaporthe oryzae Triticum* at different concentrations.

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.

REFERENCES

[1] S. Igarashi, "Pyricularia in wheat. 1. Occurrence of Pyricularia sp in the state of Parana," *Fitopatol Bras*, vol. 11, pp. 351-352, 1986.

[2] E. Callaway, "Devastating wheat fungus appears in Asia for first time," *Nature News*, vol. 532, pp. 421-422, 2016.Available at: https://doi.org/10.1038/532421a.

[3] P. K. Malaker, N. Barma, T. Tiwary, W. J. Collis, E. Duveiller, K. Singh, A. K. Joshi, R. P. Singh, H.-J. Braun, and G. L. Peterson, "First report of wheat blast caused by M*agnaporthe oryzae* pathotype *triticum* in Bangladesh," *Plant Disease*, vol. 100, p. 2330, 2016.Available at: https://doi.org/10.1094/PDIS-05-16-0666-PDN.

[4] CIMMYT, *Wheat blast disease: A deadly and baffling fungal foe.* Texcoco, Mexico: International Maize and Wheat Improvement Center, 2016.

[5] R. A. Wilson and N. J. Talbot, "Under pressure: Investigating the biology of plant infection by *Magnaporthe oryzae*," *Nature Reviews Microbiology*, vol. 7, pp. 185-195, 2009.Available at: https://doi.org/10.1038/nrmicro2032.

[6] M. T. Islam, D. Croll, P. Gladieux, D. M. Soanes, A. Persoons, P. Bhattacharjee, M. S. Hossain, D. R. Gupta, M. M. Rahman, and M. G. Mahboob, "Emergence of wheat blast in Bangladesh was caused by a South American lineage of *Magnaporthe oryzae*," *BMC biology*, vol. 14, p. 84, 2016.Available at: https://doi.org/10.1186/s12915-016-0309-7.

[7] M. A. Sadat and J. Choi, "Wheat blast: A new fungal inhabitant to Bangladesh threatening world wheat production," *The Plant Pathology Journal*, vol. 33, pp. 103-108, 2017.Available at: https://doi.org/10.5423/ppj.rw.09.2016.0179.

[8] A. Urashima, S. Igarashi, and H. Kato, "Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil," *Plant Disease*, vol. 77, pp. 1211-1216, 1993.Available at: https://doi.org/10.1094/pd-77-1211.

[9] M. Kohli, Y. Mehta, E. Guzman, L. De Viedma, and L. Cubilla, "*Pyricularia* blast–a threat to wheat cultivation," *Czech J. Genet. Plant Breed*, vol. 47, pp. S130-S134, 2011.Available at: https://doi.org/10.17221/3267-cjgpb.

[10] C. Greer and R. Webster, "Occurrence, distribution, epidemiology, cultivar reaction, and management of rice blast disease in California," *Plant Disease*, vol. 85, pp. 1096-1102, 2001.Available at: https://doi.org/10.1094/pdis.2001.85.10.1096.

[11] A. K. Chowdhury, M. S. Saharan, R. Aggrawal, P. K. Malaker, N. Barma, T. Tiwari, E. Duveiller, P. K. Singh, A. K. Srivastava, and K. Sonder, "Occurrence of wheat blast in Bangladesh and its implications for South Asian wheat production," *Indian Journal of Genetics and Plant Breeding (The)*, vol. 77, pp. 1-9, 2017.Available at: https://doi.org/10.5958/0975-6906.2017.00001.3.

[12] C. D. Cruz, J. Kiyuna, W. W. Bockus, T. C. Todd, J. P. Stack, and B. Valent, "Magnaporthe oryzae conidia on basal wheat leaves as a potential source of wheat blast inoculum," *Plant Pathology*, vol. 64, pp. 1491-1498, 2015.Available at: https://doi.org/10.1111/ppa.12414.

[13] W. W. Bockus, C. D. Cruz, J. P. Stack, and B. Valent, "Effect of seed treatment fungicides on sporulation of *Magnaporthe oryzae* from wheat seed, 2014," *Plant Dissese Managagement Report*, vol. 9, p. ST0004, 2015.Available at: doi:10.1094/PDMR09.

[14] J. Tuite, *Plant pathological methods - fungi and bacteria*. Minneapolis, Minnesota: Burgess Publication Co, 1969.

[15] H. L. Barnett and B. B. Hunter, *Illustrated genera of imperfect fungi*, 4th ed. Minneapolis: Burgessbub. Co, 1972.

[16] B. Rubert and P. Streets, *The diagnosis of plant disease*. Arizona, USA: The University of Arizona Press, 1982.

[17] S. B. Mathur and O. Kongsdal, *Common laboratory seed health testing methods for detection fungi*. Switzerland: Published by the ISTA, 2003.

[18] A. S. Prabhu, M. C. Filippi, and N. Castro, "Pathogenic variation among isolates of *Pyricularia oryzae* infecting rice, wheat, and grasses in Brazil," *Tropical Pest Managagement*,, vol. 38, pp. 367-371, 1992.Available at: https://doi.org/10.1080/09670879209371729.

[19] M. Monsur, M. Ahmed, A. Haque, Q. Jahan, T. Ansari, M. Latif, N. Borma, M. Ali, M. Kabir, and B. Banik, "Cross infection between rice and wheat blast pathogen *Pyricularia oryzae*," *Bangladesh Rice Journal*, vol. 20, pp. 21-29, 2016.

[20] M. L. Farman, "*Pyricularia grisea* isolates causing gray leaf spot on perennial ryegrass (*Lolium perenne*) in the United States: Relationship to *P. grisea* isolates from other host plants," *Phytopathology*, vol. 92, pp. 245-254, 2002.

[21] Y. Tosa, K. Hirata, H. Tamba, S. Nakagawa, I. Chuma, C. Isobe, J. Osue, A. Urashima, L. Don, and M. Kusaba, "Genetic constitution and pathogenicity of *Lolium* isolates of *Magnaporthe oryzae* in comparison with host species-specific pathotypes of the blast fungus," *Phytopathology*, vol. 94, pp. 454-462, 2004.

[22] B. C. Couch, I. Fudal, M.-H. Lebrun, D. Tharreau, B. Valent, P. Van Kim, J.-L. Nottéghem, and L. M. Kohn, "Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice," *Genetics*, vol. 170, pp. 613-630, 2005.

[23] L. Tredway, K. Stevenson, and L. Burpee, "Genetic structure of *Magnaporthe grisea* populations associated with St. Augustinegrass and tall fescue in Georgia," *Phytopathology*, vol. 95, pp. 463-471, 2005.

[24] S. Klaubauf, D. Tharreau, E. Fournier, J. Z. Groenewald, P. W. Crous, R. P. De Vries, and M.-H. Lebrun, "Resolving the polyphyletic nature of *Pyricularia* (Pyriculariaceae)," *Studies in Mycology*, vol. 79, pp. 85-120, 2014.

[25] C. C. Paulo, L. C. Vanina, L. N. M. Joao, and A. M. Bruce, "The recent emergence of wheat blast in Brazil," *McDonald Wheat Blast Asilomar Fungal Genetics*, vol. 10, 2015.

[26] H. M. D. Mijan, "Studies on blast disease of rice caused by *Pyricularia grisea* (Cooke) Sacc. in upland areas," M.Sc. Thesis, University of Agricultural Sciences, Dharwad, 2000.

[27] A. Kumar and R. A. Singh, "Differential response of *Pyricularia grisea* isolates from rice, finger millet and pearl millet to media, temperature, pH and light," *Indian Journal of Mycology and Plant Pathology* vol. 25, pp. 238-242, 1995.

[28] R. S. Netum, A. N. Bahadur, R. K. S. Tiwari, and U. Tiwati, "Effect of different culture media, carbon source, nitrogen source, temperature and pH, level on the growth and sporulation of *Pyricularia grisea* isolate from finger millet," *Research Journal of Agricultural Sciences*, vol. 4, pp. 83-86, 2013.

[29] G. Gashaw, T. Alemu, and K. Tesfaye, "Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelial growth inhibition of *Pyricula riagrisea* isolates using biological antagonists and fungicides *in vitro* condition," *Journal of Applied Bioscience*, vol. 73, pp. 5883- 5901, 2013.

[30] B. S. Meena, "Morphological and molecular variability of rice blast pathogen Pyricularia grisea (Cooke) Sacc," M.Sc. Thesis, Dharwad University of Agricultural Sciences, Dharwad, 2005.

[31] M. S. Dar, S. Hussain, A. B. Darzi, and S. H. Bhat, "Morphological variability among various isolates of *Magnaporthe grisea* collected from paddy growing areas of Kashmir," *International Journal of Pharmaceutical Sciences Review and Research*, vol. 7, pp. 90-92, 2011.

[32] M. S. Saharan, S. C. Bhardwaj, R. Chatrath, P. Sharma, A. K. Choudhary, and R. K. Gupta, "Wheat blast disease-An overview," *Journal of Wheat Research*, vol. 8, pp. 1-5, 2016.

[33] WRC, *Wheat blast disease and its management. Leaflet Published by Wheat Research Centre.* Gazipur, Bangladesh: Bangladesh Agricultural Research Institute, 2017.