ASSESSMENT OF WHEAT BLAST INTENSITY IN SOUTH WEST OF BANGLADESH AND CHARACTERIZATION OF *MAGNAPORTHE ORYZAE TRITICUM* ON DIFFERENT CULTURE MEDIA

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BY

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Dedicated To

My Beloved Parents and The Farmers who feed the nation.

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ABSTRACT

This study was carried out to determine the incidence and severity of wheat blast, isolation and pathogenicity of *Magnaporthe oryzae triticum* (MoT) isolates and evaluation on different culture media for their growth and cultural characteristics. Wheat blast were assessed in 30 farmers' fields in two districts during the Rabi cropping season in February to March 2019, in major wheat growing areas viz. Meherpur, Mujibnagar and Chuadanga. The results of the assessment revealed that the wheat blast incidence and severity varied from low to high depending on the survey sites and crop varieties. The highest incidence and severity, 100% for all areas were recorded in BARI Gom-24 (Prodip) followed by BARI Gom 26 and BARI Gom 28 in entire three districts. A total of 15 MoT isolates were identified and characterized based on their growth parameters. Among them, ten isolates such as ME MoT13, ME MoT14, ME MoT15, ME MoT16, MU MoT13, MU MoT14, MU MoT15, MU MoT16, CH MoT11 and CH MoT12 were evaluated on the eleven culture media such as Oat meal agar, Corn meal agar, Prune agar, Yeast extract agar, V5 agar, Wheat straw agar, Potato dextrose agar, Potato carrot agar, Wheat seed extract agar, Wheat flour agar and Potato sucrose agar. MoT isolates showed comparatively higher mean radial mycelial growth on OMA (51mm) followed by Yeast extract agar (48.33mm) and Wheat flour agar (44.57mm) at 4 DAI. Minimum mean radial mycelial growth was recorded on CMA (16.2mm). The radial mean mycelial growth was also sufficient on PDA, PSA and Wheat straw agar.

Key words: Wheat blast, incidence, severity, culture media, *Magnaporthe oryzae triticum*

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CHAPTER 1

INTRODUCTION

Introduction

Wheat (*Triticum aestivum*) is one of the world's most important grains and a leading source of calories and plant protein in human foods (Curtis, 2002). Wheat is synonymous with food for a large part of the global food security and population of developing countries (ICAR). It is the primary staple food in North Africa and the Middle East and is gaining popularity in Asia. Wheat originated from the nearby Levant region of the east but is now cultivated worldwide. It grows in more than 70 countries on 5 continents (Dixon, 2007) and is the most widely grown crop in the world. In 2017, world production of wheat was 771.7 million tonnes, making it the third most produced cereal (FAOSTAT, 2017). Wheat is grown on more than 1.5 million hectares, which is larger than any other crop. The four largest producers of wheat in 2017 are China (134.3 million tonnes), India (98.5 million tonnes), Russia (85.9 million tonnes) and the United States (47.3 million tonnes) (FAOSTAT, 2017).

Wheat blast is basically a head disorder. Common Symptoms caused by *Magnaporthe oryzae triticum* (MoT) have also been described, from small elliptical lesions to complete bleaching and empty spikes (Igarashi *et al*., 1986, Igarashi 1990). Infected plants show signs of common wheat blast with partial or less obvious bleaching of the spike Showing of diseased trees as they are blackened, with a dark border on the leaves, on the rachis of diseased plants, with gray to tan necrotic lesions often present. 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 (Malakar, 2016).

In February 2016, Bangladesh was identified as the first Asian country to have the origin of the alarming wheat blast disease caused by a South American lineage of a hemibiotrophic filamentous fungus *Magnaporthe oryzae Triticum* (MoT) pathotype

(Callaway, 2016; Islam *et al*., 2016; Malaker *et al*., 2016). With the first emergence of Bangladesh, wheat blast has entered Asia, which accounts for about 42% of the world's wheat production. The first occurrence of wheat blast during 2015-16 cropping season was restricted to eight districts of Bangladesh (Islam *et al*., 2016). However, the outbreak spread to eight more neighboring districts: Magura, Faridpur and Rajshahi in 2016-17, 2017-18 cropping season. According to the data of the Bangladesh Department of Agriculture Extension total area of wheat cultivation in the blast affected districts during the year 2015-16 and 2016-17 was 99,259 and 47,278 hectares, respectively.

Infected wheat fields were burnt, which contributed to a 15% reduction in wheat production in nine infected districts (Islam *et al*., 2016; Malakar *et al*., 2016). Despite this reduction, the total wheat production in Bangladesh has increased slightly (35000 MT, 2.7%) in the total of 2016 compared to 2015. Total Harvesting Fields Growth and Yield (1.0-1.5 m / ha) contributed to the total wheat production in 2016 wheat Production (Production, Supply and Distribution [PSD] Online). Importantly, 100% of the seed proportions of the state-owned Bangladesh Agricultural Development Corporation (BADC) in the affected districts (private 355 Ha) were completely cleared by the Ministry of Agriculture to destroy the pathogen inoculum. Farmer wheat fields that were severely damaged (up to 100%) were also burned (Islam *et al*., 2016).

It is now clear that wheat blast disease is well established in at least one country in Asia (Bangladesh). The incidence of the disease has certainly decreased in the amount, location, and size of the infected area. However, the new incidence of infection in new regions indicates gaps in the control of wheat blast and the spread. Since this wheat killer is in progress, the need for rapid development of effective management methods, including the development of blast-resistant varieties using modern biotechnological approaches, including genome editing is essential before it becomes catastrophizing (Islam, *et al*., 2019).

Castruagudin *et al*. (2016) used four culture media such as CMA, MEA, OA and PDA for showing the growth of PoT. They showed that maximum growth was occurred on PDA media. But that work was not sufficient to evaluate the isolates. So I used eleven culture media for showing radial mycelial growth and other characteristics of the colony. Keeping the review in mind the present research work was conducted with the following objectives.

I. To determine the incidence and severity of wheat blast in selected areas of southwestern wheat growing regions of Bangladesh.
II. To isolate, identify and pathogenicity study of wheat blast pathogen *Magnaporthe oryzae triticum* (MoT).
III. To find out the morphological and cultural diversity of different isolates of *M. oryzae triticum in* eleven different culture media *in-vitro*

CHAPTER II

REVIEW OF LITERATURE

Blast caused by the heterothallic ascomycete *Magnaporthe oryzae* (Hebert) Barr. (Anamorph: *Pyricularia grisea* is the most serious disease of wheat and can cause severe losses in most wheat growing environments. Rapid changes in the virulence properties of these pathogens pose an ongoing threat to existing blast resistant varieties. Therefore, a deep surveillance about the virulence spectrum of the pathogen is needed. In this chapter, the variability of the existing *Magnaporthe oryza triticum* in different agricultural areas and them *in vitro* morphological and cultural characteristics on different culture media were reviewed.

2.1 Historical Events

The disease has spread widely in South American countries such as Brazil, Argentina, Bolivia, Panama and Paraguay since the 1980s. It was first noticed in the Brazilian state of Parana in 1985, where six commercial municipalities in six municipalities were severely damaged. In 1996, the first explosion outside Brazil was reported in Bolivia, the most important region for global production, in the Santa Cruz Division (Perello *et al.*, 2015). The wheat explosion reached the Itapia and Alto Parana sections of Paraguay in 2002 and the province of Formosa in northeastern Argentina in 2007 (Malakar, 2016). The pest has previously been present in the United States (2011) in the state of Kentucky but is no longer. This strain was found in a research plot from the University of Kentucky and is believed to have developed as a low strain through a host jump from the annual ryegrass pathogen.

After its origin in Parana, the pathogen followed the agricultural expansion to the warm Cerrado areas of central-western Brazil, arriving in Minas Gerais in 1990 (Lima, 2004), (Prabhu *et al.*, 1992) and Brasilia in 1993 (Anjos *et al.*, 1996), spreading about 1200 km north from its origin. MoT also invaded new wheat agroecosystems located 1700 km to the north-west of Paraná, arriving in Bolivia in 1996 (Barea and Toledo, 1996)

and eastern Paraguay in 2002. It also spread to cooler regions 1200 km south-west of Parana, reaching Chaco and Corrientes provinces in Argentina in 2007 (Perello *et al*., 2015). The alarming appearance of wheat blast in Bangladesh in 2016 greatly increased the urgency to understand this disease, including its causes and consequences. The invasion of wheat blast into Bangladesh in 2016 (Callaway, 2016; Islam *et al*., 2016) and its possible spread into India in 2017 (Government of India, 2016) brought wheat blast to the attention of Asian governments and the international community of plant pathologists, exposing an urgent need to develop strategies to contain the spread of this destructive pathogen (Islam *et al*., 2016; Malaker *et al*., 2016; McDonald and Stukenbrock, 2016; Sadat and Choi, 2017; Sharma, 2017; Singh *et al*., 2016).

Due to the importation of wheat into the country, the wheat blast strain can be transported from South America to Bangladesh via man-made transport. A local Bangladeshi newspaper reported that seeds imported for consumption in Brazil in 2015 were seen as unhealthy and could be infected with blast diseases.

2.2 Survey on the incidence and severity of the disease

Malakar *et al*, (2016) reported that wheat blast was observed for the first time outside of South America during the 2015-16 cropping season in Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola and several districts in the south of Bangladesh.

Perello *et al*., (2015) carried out an experiment in vitro and they estimated severity 10, 13 and 17 days after inoculation. Leaf infections ranged from 2.7 to 64.3%. The leaf is symptomless under control condition. They observed that leaf infections were up to 43.1%, 50% and 63.1%, respectively.

At adult plant stage, diagrammatic scale recommended by the International Rice Research Institute (IRRI, 1996), with modifications, for wheat diseases is used where zero is – absence of symptoms; 1 (one) - 0.1 to 4%; 2 (two) - 5 to 10%; 3 (three) - 11 to 25%; 4 (four) - 26 to 50%; and 5 (five) - 51 to 100% of infected leaf area. A standard

area diagram set (SADS) was develop and tested by Rios *et al*. (2013) to quantify the severity of wheat blast. It has ten levels of wheat blast severity from 0.1, 1, 5, 10, 22,32, 42, 52, 62 and 72%.

Trindade *et al.,* (2006) proposed a scale that is based on according to the point at which the pathogen has penetrated the rachis and affected the length of the spike. The score 0 referred to nil visual symptoms, 1 for 25 % of the spike showing symptoms; 2 for 50%, for 75% and for 100% length of spike affected.

According to the data of the Department of Agricultural Extension of Bangladesh, total area under wheat cultivation in the blast affected districts during2015-16 and 2016-17 was 99,259 and 47,278 hectares, respectively. In the 2017-18, wheat cultivation area in Bangladesh was 0.349 million hectares which is only 79% of the previous year and lowest in three decades.

Islam *et al*. (2016) reported that the severity of the wheat blast and its associated yield losses are in different districts.The highest percentage of infected wheat fields was observed in Meherpur (70 %) followed by Chuadanga (44 %), Jessore (37 %), Jhenaidah (8 %), Bhola (5 %), Kushtia (2 %), Barisal (1 %), and Pabna (0.2 %). Yield losses in different affected districts varied.

The highest average yield loss was recorded in Jhenaidah (51%), Chuadanga (36%), Meherpur (30%), Jessore (25%), Barisal (21%), Pabna (18%), Kushtia (10%) and Bhola (3%). Although the average yield reduction across the district was less than 51%, in the individual cases the yield loss was as high as 100% (BADC).

Cruz *et al*. (2017) stated that the first occurrence of wheat blast affects about 15% of Bangladesh's total wheat area. However, it is increasing day by day. In some areas it reaches 100%.

Gomes *et al*. (2017) observed that in all genotypes there was a higher incidence of blast in the field. Higher incidence was observed when the highest doses of inoculum were applied (20 to 30%).At the lowest inoculum dose (5%) for genotype VI 98053 and CD 104, incidence above 20% were obtained.

A disease surveillance program related to wheat explosion was organized in mid-February 2017 in collaboration with CIMMYT and CU in the United States. Of the 103 survey sites, 33 sites were infected with wheat blast. Overall disease prevalence in 2017 was relatively low compared to previous season with low disease severity (5-10%) (CYMMYT, 2017).

2.3 Isolation of *Magnaporthe oryzae triticum*

Perello *et al*. (2015) isolated *pyricularia* in a humid chamber for 12 hours, the accumulation of these infected plants resulted in the production of pyriform conidia from the typical symptom of fungi such as *Pyricularia*. Single-spore isolation of the fungus was obtained by transferring the conidia to acidified 1.5% water agar and subsequently to the single, germinated conidia by transferring to V8 juice agar and OMA medium.

The infected tissues used by Urishama *et al.* (2004) were cut into small pieces and placed in Petri dishes and kept at 25° C for 24 h in moist conditions. Single-conidial isolation was made in potato dextrose agar (PDA). For long-term storage, the pure cultures were incubated with sterilized barley Seeds, dried completely at 25℃, and stored in a plastic case with silica gel at 5° C.

Warda (2004) conducted an experiment in which oat meal agar (40 g rolled oats, 5 g sucrose, and 16 g agar and 1000 ml distilled water) and potato dextrose agar media (200 g peeled potatoes, 20 g dextrose, 20 g of agar and 1000 ml of distilled water) were used for the detachment pathogen isolation. The leaves of infected wheat cultivars were cut into small pieces (less than 1.0 cm in size) and surface was sterilized with 1% sodium hypochlorite for 1min. The distillation was followed by 3 washings with distilled water. Then the plant pieces were lined with moist filter paper in petri dishes, and they were incubated at 250 ° C for 24 hours to encourage sporulation.

Monsur *et al.* (2016) isolated the fungus by following steps. Blast infected spikelets were sterilized by soaking with Clorox (5%) for 3-5 seconds and then in ethanol (70%) for 1 minute. Sterilized spikelets were gently rinsed with sterile water and dried samples

with sterile tissue paper. Dried sterile spikelets were put on water-soaked filter paper (Whatman International Ltd. Maidstone, England.) and incubated at 26°C for sporulation. Single spore was collected and cultured on prune agar medium.

2.4 Pathogenicity test of *M. oryzae* strains

Monsur *et al.* (2016) used wheat blast isolates WBL002 and WBL0011that developed typical blast symptoms on leaves of the wheat varieties BARI Gom25 and BARI Gom26. Both of the isolates caused the highest level (100%) of plant and leaf infection on both wheat varieties used in this study.

Islam *et al.* (2016) studied about ten strains BTJP 3-1, BTJP 3-2, BTJP 3-3, BTJP 4- 1, BTJP 4-2, BTJP 4-3, BTJP 4-4, BTJP 4-5, BTJP 4- 6, and BTJP 4-7. Artificial inoculation of wheat seedling leaves using conidia of two isolates (BTJP 3-1 and BTJP 4-1) produced characteristic symptoms five days after inoculation. Initially, a diamond-shaped, water-soaked lesion in green leaves was observed, which gradually turned into an eye-shaped lesion, with a tan or gray colored center. At a later stage, the spots enlarged, spread to entire leaves, and killed the leaves. No difference in symptoms was observed on wheat seedlings of the cultivars Shatabdi and Prodip and between the two isolates (BTJP 3-1 and BTJP 4-1). Similar disease symptoms and sporulation were observed on leaves of artificially inoculated goosegrass (Eleusine indica) and barley (Hordeum vulgare). Terminal infection stages were characterized by a massive production of hyaline to pale gray, pyriform, and asexual conidia on aerial conidiophores.

2.5 Variation of blast isolates on Different Media

Islam *et al.* (2016) studied about ten strains BTJP 3-1, BTJP 3-2, BTJP 3-3, BTJP 4- 1, BTJP 4-2, BTJP 4-3, BTJP 4-4, BTJP 4-5, BTJP 4- 6, and BTJP 4-7 using a single-conidia isolation method On potato dextrose agar (PDA) plates, the predominant morphology of the isolates was gray to white aerial mycelia with an olive or brown center. After 14–21 days of inoculation, the center of the culture became black.

Castruagudin *et al.* (2016) examined a representative subgroup of 30 isolates were grown on Corn Meal Agar (CMA), Malt Extract Agar (MEA), Oatmeal Agar (OA), Potato Dextrose Agar (PDA), and Synthetic Nutrient-poor Agar (SNA). Mycelium in SNA on disinfected barley seeds is smooth, hyaline, branched, septate hyphae, featuring 1.5-2 mm diam. In CMA colonies with moderately dark gray aerial mycelium with irregular margins, there are sometimes dark aerial mycelium with sporulation in circles. In MEA, the colonies present several variants: sometimes white white aerial mycelia within the contract growth ring with gray sporulation in the center. Colonies in OA with gray aerial mycelium and sporulation in dense circles; Sometimes surface mycelia were white or cream, showing frequent growth, up to 7.9 cm diameter. The opposite is dark gray. PDA colonies exhibited many variations in culture, often with frequent growths: abundant white aerial mycelia and pale gray sporulation in the center; Bottom white aerial mycelia dark gray mycelia, 7 cm diameter with white aerial mycelia. On SNA the colonies with dark green centres with sparse pale brown margins; or pale grey at the Centre and sparse pale brown margins; reverse dark green to black at the Centre and with pale brown margins.

Rabindramalaviya (2014) used four culture media to study the mycelial growth of the *M. oryzae in vitro*. Of these, PDA Media supports the highest mycelial growth after 16 hours followed by Richard's agar medium. The sporulation of M. *oryzae* was found after 16 hours on potato dextrose agar medium and Richard's agar medium.

CHAPTER 3

MATERIALS AND METHODS

The present research work was carried out both in field and laboratory condition to determine Disease incidence, severity including variation of different isolates on different growth media during the time between February 2018 to April 2019. The details of the materials and methodology adopted during this study are described here under the following points.

3.1 Survey and experimental sites

The survey was made in the farmer's wheat field which are affected by wheat blast disease at Meherpur and Mujibnagar upazilla in Meherpur districts and Chuadanga sadar upazilla in Chuadanga districts in Bangladesh during January to April, 2018 and 2019 (Fig. 1). The laboratory experiment was conducted from February 2018 to April 2019 in the Plant Pathology Laboratory, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207.

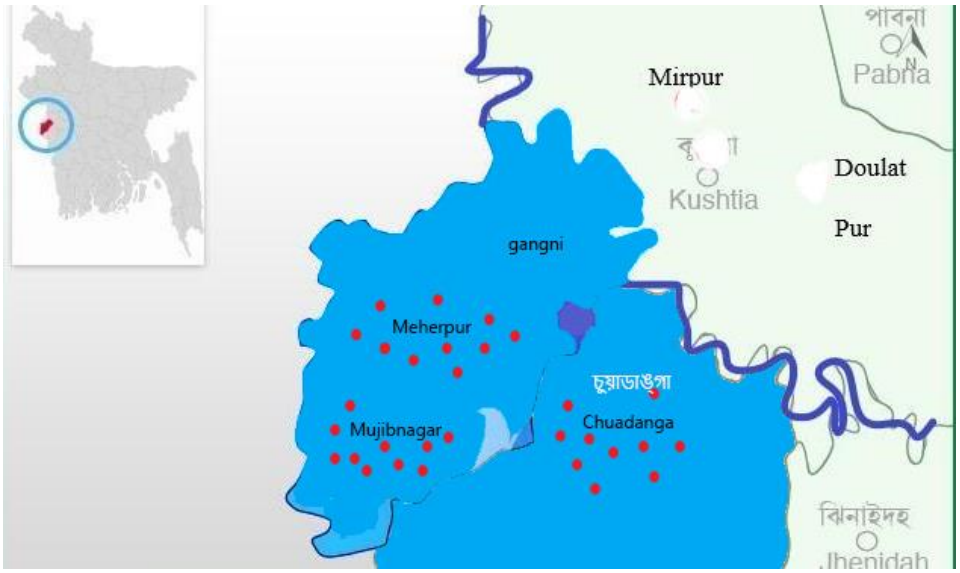

Fig. 1. Survey area and sampling sites (●) of wheat blast infected south western region of Bangladesh

(http://en.ntvbd.com/bangladesh/181607/Mud-collapse-kills-two-children-in-Kushtia)

3.2 Determination of disease incidence and severity

At first Visual symptoms of disease were assessed. The survey was conducted using simple random sampling method, within at 3-4 km intervals on wheat fields along the main and accessible road sides. The Wheat blast incidence and severity were recorded along the two diagonal 'X' fashion of the fields at five random spots using (1×1 m^2) quadrants and used to calculate the average values. Totally, 30 farmer's wheat fields were surveyed at critical growth stage of the crop during which the blast symptoms reached its maximum severity level. From each locality, 10 farmer's wheat fields were selected.

$$\text{Prevalence (\%)} = \frac{\text{Number of fields affected by the disease}}{\text{Total number of fields assessed}} \times 100$$

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number (healthy and infected)of plants.}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Area of panicle tissue affected by disease}}{\text{Total area of panicle}} \times 100$$

The scale proposed by Trindade *et al.* (2006) was used for deciding the severity. It is based according to the point at which the pathogen has penetrated the rachis and affected the length of the spike. The score 0 referred to nil visual symptoms, 1 for 25 % of the spike showing symptoms; 2 for 50%, 3 for 75% and 4 for 100% length of spike affected. (Table 1).

Maciel *et al*. (2013) photographed the infected spikes. The blast severity was ascertained by using resources of the software Image. A diagrammatic scale was prepared and the disease severity values were 3.5, 7.5, 21.4, 30.5, 43.8, 57.5, 68.1, 86.0, and 100.0%.

Table 1. Scale used to calculate of wheat blast severity.

Scale	% Severity values
0	Nil Visual symptoms
1	25% length of the spike infected
2	50% length of the spike infected
3	75% length of the spike infected
4	100% length of the spike infected

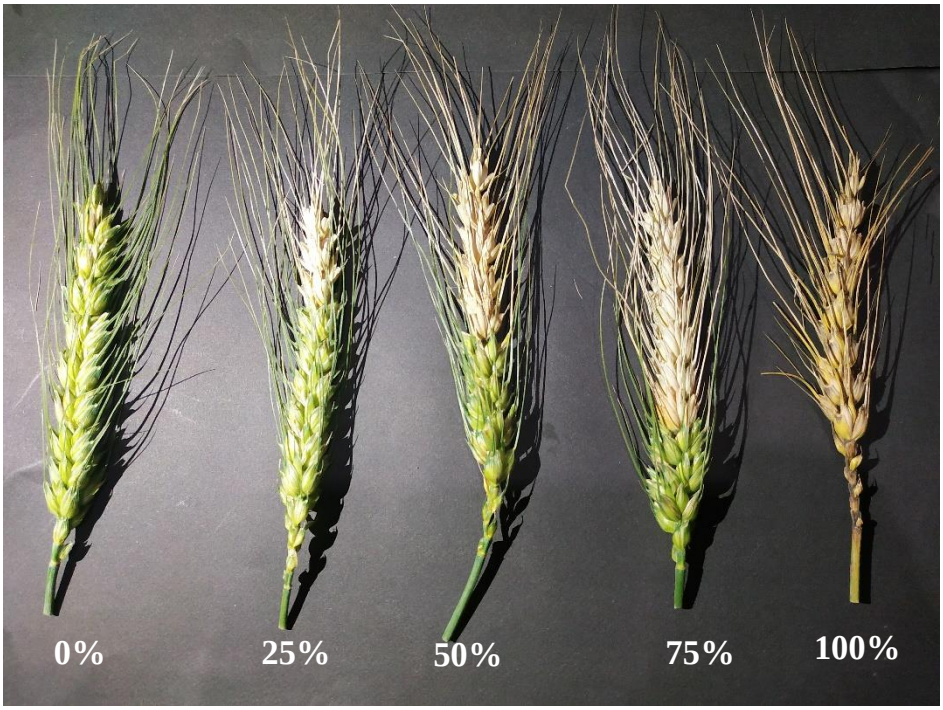

Figure 2. Disease Severity scale

3.3 Sampling

Sampling was carried out in different areas of Meherpur, Mujibnagar and Chuadanga Sadar upazilas by taking wheat blast infected panicle (Fig. 2). About five random spots (1 ×1 m) were selected for each field. At least 3 samples were collected from each spot. Samples were kept into brown paper envelopes. After sample collection, all required information was labeled on brown paper envelopes, such as a field site, farmer's name, variety, date of collection etc. Samples were kept in a refrigerator for 3 hours and at 4℃. When all the work was done, the samples were taken out of the refrigerator and transferred to the Plant Pathology Laboratory of Sher-e-Bangla Agricultural University for isolation, identification, and characterization.

3.4 Isolation and identification of pathogen

Infected rachis was used for isolation of *Magnaporthe oryzae triticum*. At first glumes were removed from rachis and whole rachis was cut into small pieces (12-15cm). Then they were surface sterilized by using 1% sodium hypochlorite for 1 min dipping into it following by rinsing with distilled water at least 3 times. After sterilization they were placed into previously sterilized petri dishes having whatmann filter paper. Small

amount of distilled water was sprayed over the sample and lid was closed. Then the petri dishes were incubated at 25℃ for 24 hours. Water agar (20g agar and 1000ml distilled water) and PDA (200g peeled potato, 20g Agar, 20g Dextrose and 1000ml distilled water) were used for isolation of pathogen. After incubation, infected rachis was examined under sterio-dissecting microscope for observation of pathogenic growth and sporulation around the infected lesion having blackish, bushy appearance. A sterilized pin pointed needle was used to pick up conidia from the sporulated portion and put into water agar (WA) and incubated petri dishes for 2 days at 25℃ for growth and development of mycelia. After 2 days' mycelia were recultured on Potato Dextrose Agar (PDA) containing oramycin-K (antibacterial) by hyphal tip culture technique. Petri dishes containing PDA incubated 25-27℃ for 3-4days with alternating 12 hours light and 12hours darkness. Cultural and Morphological study was done following Castroagudin *et al.* (2016). Slides were made from the culture and observed under light microscope on the basis of conidia or spore for identifying *Magnaporthe oryzae triticum.* After identification the blast isolates designated as MoT denotes and number denotes representative of sites based on different isolates of the blast pathogen *P. oryzae* and they were purified using single spore (mono conidial) technique (Hoang *et al.*, 1999).

3.5 Maintenance of isolates of *Magnaporthe oryzae triticum* (MoT)

The fungus was sub cultured on Potato dextrose agar media and kept at 26±2℃ for 15 days. Subsequent, subculturing of isolates was done at an interval of 20 days. Such isolates were stored in a refrigerator at 4℃. The cultures were revived monthly.

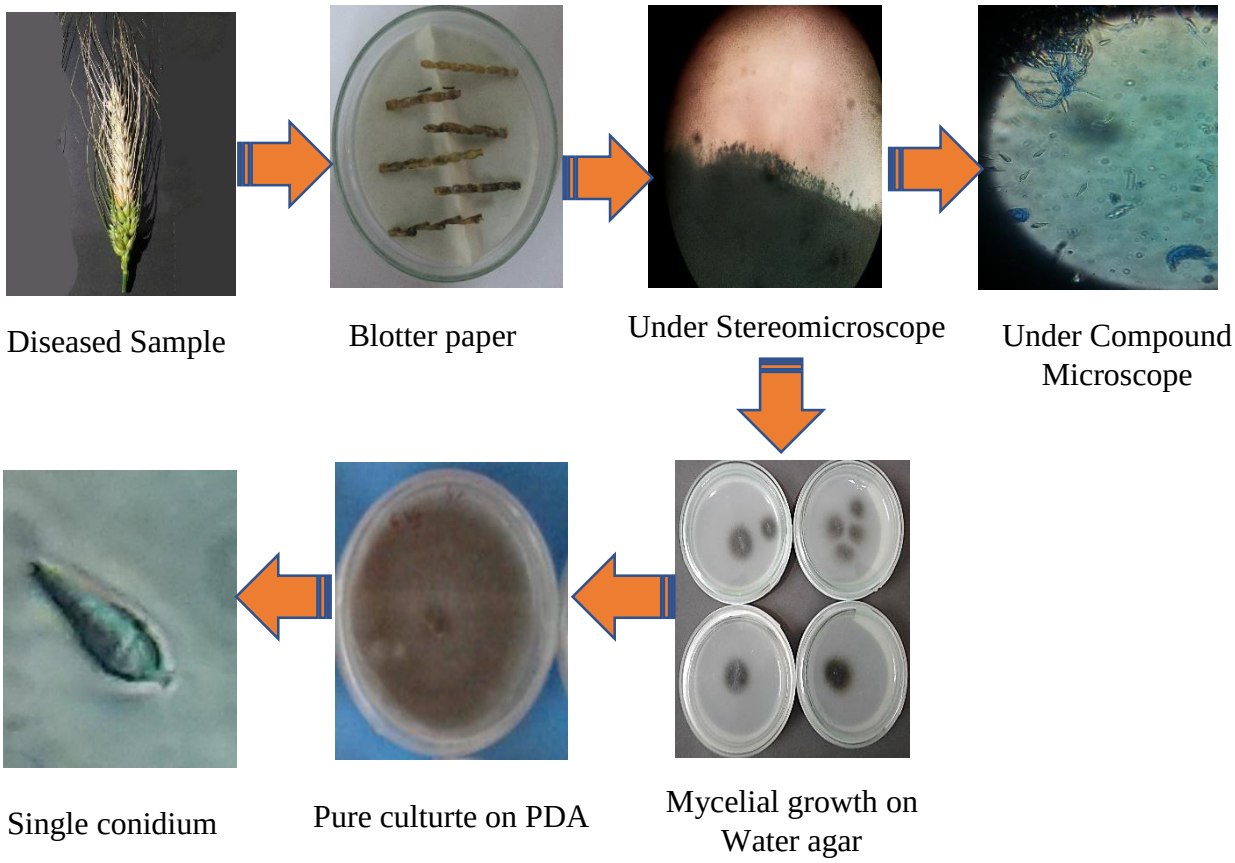

Figure 3. Flow chart of isolation and identification of wheat blast pathogen MoT

3.6 Pathogenicity test

After isolation and identification of different isolates of *Magnaporthe oryzae triticum* it was essential to go pathogenicity test for verifying the pathogen. For that purpose, 8 pots were sterilized properly. Soils were collected from the field of Sher-e-Bangla agricultural university and sterilized. The pots were filled up with the soil. Disinfected viable seeds of the variety BARI GOM 26 were sown in sterilized soil in pots with 5-6 plants per pot. When the seedlings were three weeks old, they were inoculated with spore suspension about 40–50 ml of spore suspension adjusted to 1×10^6 spores/ml with the help of hemacytometer. The inoculated plants were covered with polyethylene bags to keep 100% relative humidity and then kept in a humid chamber for 48 hours at room temperature (25 ± 10℃). Periodic monitoring was performed for the development of symptoms on the leaf seven days after inoculation. A re-isolation was made and the

identity of the fungus was confirmed according to the original description. The experiment was conducted on CRD with three replications.

Fig. 4. Artificially inoculated plant showing typical wheat blast symptom on leaves

3.7 Variation among the isolates of *Magnaporthe oryzae triticum* (MoT)

3.7.1 Morphological variation

Spores of different isolates of *M. oryzae triticum* were measured directly from infected host tissue mounted in lactophenol on a clean slide. The spores were thoroughly mixed with lactophenol to obtain a uniform spread, and then a cover slip was placed. The slides were measured under high power objective (40x) using an ocular microscope. The average size of the slides was then determined and the size of the slides was recorded. A microphotograph was taken to show the typical spore morphology of the blast pathogen.

3.7.2 Cultural variation of *M. oryzae triticum*

Growth characters were recorded on eleven different solid media viz. Oat meal agar (OMA), Corn meal agar (CMA), Prune agar (PA), Yeast extract agar (YEA), V5 agar, Wheat straw agar (WSA), Potato dextrose agar (PDA), Potato carrot agar (PCA), Wheat seed polished agar (WSPA), Wheat flour agar (WFA) and Potato sucrose agar (PSA). Fifteen to twenty ml of each of the medium was poured into each of sterilized Petri plates. Inoculation was made by transferring the five mm disc of mycelial mat, taken from the periphery of 15 days old culture of each of the ten isolates. Each treatment was

replicated thrice. The plates were incubated at 25 ± 10℃. Observation on colony radial growth was taken at 4 DAI (days after inoculation) and 8 DAI. Other cultural characters *viz*., rate of growth, type of margin, colony color and sporulation were also recorded.

The composition and procedures for preparation of the media used in this experiment were followed as explained by Ainsworth (1971) and Tuite (1969). The chemical composition of each medium is as follows.

Composition of OMA

Ingredients	
Oat meal	40 g
Agar	20 g
Distilled water	1 L

Preparation of OMA

Oat meal and agar were added into 800 ml water in a conical flask. The volume was increased up to 1000 ml. Oat meal and agar were melted by heat and sterilized at 121℃ at 15 PSI for 1 hour.

Composition of CMA

Ingredients	
Corn meal agar	17 g
Distilled water	1 L

Procedure of CMA

17 g Corn meal agar was added to 1000ml water and heated for some times for melting, then sterilized in autoclave at 121℃ at 15 PSI for 1 hour.

Composition of Prune agar

Ingredients	
Prune	4 piece
Lactose	5 g
Yeast	2 g
Agar	20 g
Distilled water	1 L

Procedure of Prune agar

5 piece prune was boiled into 900 ml water and extract was filtered into a conical flask through a muslin cloth. Lactose and yeast were dissolved in the solution. Agar was also added and volume up to 1 L and sterilized at 121℃ at 15 PSI for 1 hour.

Composition of Yeast extract agar

Ingredients	
Yeast extract	2 g
Sucrose	20 g
Agar	20 g
Distilled water	1 L

Procedure of Yeast extract agar

Yeast extract, sucrose and agar were added to 1000ml water and dissolved thoroughly, then sterilized the media at 121℃ at 15 PSI for 1 hour.

Composition of V5 agar

Ingredients	
Potato	1g
Tomato	1g
Carrot	1g
Spinach	1g
Water cress	1g
Distilled water	1oo ml

Procedure of V5 agar

All vegetables were cut into small pieces and blended properly with water. The extract was filtered through a muslin cloth. This is stock solution.

I used 50 ml from the stock solution and added 20 g agar. Then volume the solution up to 1000 ml and sterilized it at 121℃ at 15 PSI for 1 hour.

Composition of Wheat straw agar

Ingredients	
Wheat straw	50 g
Dextrose	20 g
Agar	20 g
Distilled water	1 L

Procedure of wheat straw agar

Wheat straw was cut into small pieces and blended smoothly with water. The extract was filtered through cloth. Dextrose and agar were dissolved with it and volume up to 1 L, then sterilized in an autoclave at 121℃ at 15 PSI for 1 hour.

Composition of Potato dextrose agar

Ingredients	
Potato	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1 L

Procedure of Potato dextrose agar

First, potatoes were peeled off and cut into small pieces. Then they were boiled and extract was filtered through the muslin cloth. The dextrose was dissolved in the solution. Later sterilized the solution at 121℃ at 15 PSI for 1 hour.

Composition of Potato carrot agar

Ingredients	
Carrot	200 g
Potato	250 g
Agar	20 g
Distilled water	1 L

Procedure of Potato carrot agar

at first, potato and carrot peeled off and cut into small pieces. Then they were boiled and extract was filtered through the muslin cloth. The agar was dissolved in the solution and sterilized at 121℃ at 15 PSI for 1 hour.

Composition of Wheat seed extract agar

Ingredients	
Wheat seed	20 g
Agar	20 g
Distilled water	1 L

Procedure of Wheat seed extract agar

Wheat seed was separated from the glumes, washed properly and blended with water. The extract was filtered and Agar was dissolved into the solution, then sterilized.

Composition of Wheat flour agar

Ingredients	
Complete flour	15 g
Yeast	4 g
Agar	20 g
Distilled water	1 L

Procedure of Wheat flour agar

Wheat flour, yeast and agar were added to water and dissolved properly. The solution was sterilized at 121℃ at 15 PSI for 1 hour.

Composition of Potato sucrose agar

Ingredients	
Potato	200 g
Sucrose	20 g
Agar	20 g
Distilled water	1 L

Procedure of PSA media

At first potatoes were peeled and cut into small pieces. They were boiled into water and the extract was filtered through muslin cloth. Sucrose and Agar were dissolved thoroughly and sterilized the solution at 121℃ at 15 PSI for 1 hour.

Figure 5. Media preparation (A) OMA (B) CMA (C) Prune agar (D) Yeast extract agar (E) V5 agar (F) Wheat straw agar (G) PDA (H) PCA (I) Wheat seed polished agar (J) Wheat flour agar (K) PSA.

3.8 Experimental design

At first, a total 15 isolates were grown and characterized on PDA, PSA and OMA petri plates were randomized in three replications. Total 135 petri plates with three replications. Later on 10 isolates were characterized on eleven media in three replications. The experiment was laid out in Completely Randomized Design (CRD).

3.9 Statistical analysis

The prevalence, incidence and severity data were analyzed by using the descriptive statistical analysis (means) over district / localities and altitudinal ranges. Analysis of data on other parameters were subjected to perform by statistical analysis using Statistical Tool for Agricultural Research (STAR) software used in Complete Randomized Design with three replications. Grouping of isolates were made for each of the parameters based on critical difference (CV) value at five per cent (5%) level of significance. All the isolates which fall under one range were considered to be on pair with each other, while the isolates coming under different range were significant with respect to each other.

CHAPTER 4

RESULTS AND DISCUSSION
RESULTS

The results of the investigation on variability of wheat blast pathogen *Magnaporthe oryzae triticum* are presented here under. The experiments were conducted in the south western region of Bangladesh and the laboratory in Department of Plant Pathology, sher-e-Bangla Agricultural University, Sher-e-Bangla nagar, Dhaka 1207 during February 2018 to April 2019.

4.1 Survey, surveillance and collection of different isolates of *Magnaporthe oryzae triticum*

Survey for the diseased specimens with typical blast symptoms of wheat was carried out during Robi season from different agro climatic zones of Meherpur and Chuadanga districts. The infected samples were collected and used for the isolation of *M. oryzae triticum*.

4.2 Symptomatology

Leaf blast symptoms

Magnaporthe oryzae triticum produced dark grey lesions on mature leaf. Common eye shaped lesions with light grey center and dark brown margin can be observed in severely affected wheat leaves.

Symptoms on head

Wheat spikes infected with blast usually have symptoms of bleached head. Most of the spikes become completely bleached and grey from the blast infection on Spike's neck.

4.3 The prevalence, incidence and severity of wheat blast disease

A survey was conducted during January to April, 2018 and 2019, for the assessment of prevalence, incidence and severity of blast disease in different south western wheat growing regions in Bangladesh including Meherpur, Mujibnagar and Chuadanga (Table 2, 3 and 4**)**. Survey was conducted in 30 sites of 3 upazila. During survey, the wheat blast prevalence was observed on 25% in Meherpur sadar upazilla, 15% in Mujibnagar upazilla and 5% in Chuadanga upazilla (Table 2). The result of the assessment revealed

that the intensity of the disease varies from slight to high intensity depending on the agro-ecological and cultivar differences. In the survey areas three wheat varieties *viz.* BARI Gom 24 (Prodip), BARI Gom 26 and BARI Gom 28 were found to be infected by wheat blast. The most dominant cultivar grown by the farmers in the South west of Bangladesh was BARI Gom 24 (Prodip) but the other cultivars were also introduced which were grown in some farmer's fields. The highest incidence and severity was scored in variety Prodip and it was up to 100% in few fields of Meherpur, Mujibnagar and Chuadanga (Table 2, 3 and 4). Some fields were also burned. The lowest incidence and severity was found in BARI Gom 28 (26%) at Pirojpur village and BARI Gom 26 (28%) at Buripota village in Meherpur upazilla, respectively (Table 3). In Mujibnagar upazilla, lowest incidence and severity was observed in BARI Gom 26 (29%) at Maniknagar and BARI Gom 28 (40%) at Bollobpur, respectively (Table 4). In Chuadanga sadar upazilla, minimum incidence was recorded in case of BARI Gom 24 (29%) at Korimpur and Ibrahimpur village. Lowest severity 40% was observed in BARI Gom 28 at Kaliabokri village (Table 5).

A **B**

C **D**

E **F**

Figure 6. Blast infected wheat field in Meherpur (A & B), Mujibnagar (C & D) and Chuadanga (E & F)

Table 2. Prevalence of wheat blast in three survey areas

Survey area		Number of fields inspected	Number of fields infected	Prevalence (%)
District	Upazilla			
Meherpur	Sadar	120	30	25
	Mujibnagar	100	15	15
Chuadanga	Sadar	100	5	5

Table 3. Incidence and severity of spike blast (*Magnaporthe oryzae triticum*) disease of wheat at different locations in Meherpur district of Bangladesh in 2019

Villages	Name of variety	Blast disease		
		Incidence (%)	Severity (%)	Score
Kathalpota	BARI Gom-28	45 cd	52 c	2
Sonapur	BARI Gom-24(Prodip)	100a	100 a	4
Pirojpur	BARI Gom-28	26 d	45 cd	2
Nurpur	BARI Gom-24(Prodip)	75 b	71 b	3
Kola	BARI Gom-24(Prodip)	75 b	85 b	3
Raypur	BARI Gom-24(Prodip)	40 cd	31 de	1
Tungi	BARI Gom-24(Prodip)	100 a	100 a	4
Rajapur	BARI Gom-24(Prodip)	60 bc	55 c	2
Buripota	BARI Gom-26	30 d	28 e	1
Gohorpur	BARI Gom-28	40 cd	34 d	1
CV (%)		28.19	18.92	
LSD (0.05)		21.4367	14.5321	

Means with the same letter are not significantly different.

Table 4. Incidence and severity of spike blast (*Magnaporthe oryzae triticum*) disease of wheat at different locations in Mujibnagar district of Bangladesh in 2019

Villages	Name of variety	Blast disease		
		Incidence (%)	Severity (%)	Score
Gopalpur	BARI Gom-24(Prodip)	99 a	80 ab	3
Jotarpur	BARI Gom-26	29 d	45 d	2
Mohajonpur	BARI Gom-24(Prodip)	81 b	75 abc	3
Bollobpur	BARI Gom-28	34 d	40 e	2
Maniknagar	BARI Gom-26	29 d	50 cde	2
Komorpur	BARI Gom-28	52 c	70 bcd	3
Shibpur	BARI Gom-24(Prodip)	100 a	100 a	4
Bagoan	BARI Gom-24(Prodip)	53 c	70 bcd	3
Poranpur	BARI Gom-24(Prodip)	83 b	80 ab	3
Voborpara	BARI Gom-24(Prodip)	55 c	50 cde	2
CV (%)		13.33	30.07	
LSD (0.05)		10.4823	25.36473	

Means with the same letter are not significantly different.

Table 5. Incidence and severity of spike blast (Magnaporthe oryzae triticum) disease of wheat at different locations in Chuadanga district of Bangladesh in 2019

Villages	Name of variety	Blast disease		
		Incidence (%)	Severity (%)	Score
Sutipur	BARI Gom-28	99 a	80 ab	3
Korimpur	BARI Gom-24(Prodip)	29 d	45 de	2
Hematpur	BARI Gom-24(Prodip)	81 b	75 abc	3
Kaliabokri	BARI Gom-28	34 d	40 e	2
Ibrahimpur	BARI Gom-24(Prodip)	29 d	50 cde	2
Alokdi	BARI Gom-24(Prodip)	52 c	70 bcd	3
Charulia	BARI Gom-24(Prodip)	100 a	100 a	4
Hogoldanga	BARI Gom-24(Prodip)	53 c	70 bcd	3
Vogratpur	BARI Gom-26	83 b	80 ab	3
Valaypur	BARI Gom-26	55 c	50 cde	2
CV (%)		12.49	31.24	
LSD (0.05)		9.8548	26.4431	

Means with the same letter are not significantly different.

4.4 Wheat grain yield parameter of Healthy panicle vs Blast panicle

In Meherpur upazilla for healthy plant, highest amount of seed per panicle was recorded in Sonapur (BARI Gom 24), it was 48.33 and lowest seed per panicle was observed in Kola, it was 29.00 seed per panicle. Seed weight per panicle was maximum in Tungi (BARI Gom 24), it was 2.37 g. Lowest weight of seed per panicle was 1.69g in Kola. Highest weight of thousands seed was 59.61g and lowest weight of thousand seed was 46.33g was seen in Pirojpur and Sonapur village, respectively. For blast disease, maximum seed per panicle was 27.67 in Raypur (BARI Gom 24) and minimum seed per panicle was 19.00 in Kathalpota (BARI Gom 28). Thousand seeds weight was also lower than healthy plant. 1000 seeds weight was maximum in Tungi (31.70g) and lowest seed weight was 27.73g in Nurpur (Table 6). In Chuadanga sadar upazilla, highest seed per panicle was 41.00 in Alokdi (BARI Gom 24) village for healthy plant that was more than highest seed per panicle in diseased plant in Alokdi and Charulia (BARI Gom 24) village (22 seed per panicle). Maximum weight of seed per panicle was 3.43g in Charulia and minimum weight was 1.18g in Sutipur (BARI Gom 28) and Karimpur (BARI Gom 24) which was also more than blast plant. In case of blast affected plant weight of seed per panicle was high in Ibrahimpur (0.85g) and lowest was observed in Kaliabokri (BARI Gom 28) (0.29g). Thousand seeds weight in healthy plant (maximum 65.43g in Alokdi and minimum 33.43g in Hogoldanga) was also higher than blast affected plant (maximum 32.65g in Alokdi and minimum 21.76g in Charulia) (Table 7). In Mujibnagar upazilla, same things were happened in every parameter. The healthy plant produced more grain than the blast affected plant. In healthy plants, highest seed per panicle was recorded in variety BARI Gom 24 in Shibpur (40) and lowest seed per panicle was observed in variety BARI Gom 28 in Komorpur (32) . Seed weight per panicle was maximum in 2.29g in Shibpur and minimum in 1.83g in Komorpur. Highest weight of thousand seeds was seen in Shibpur (60.93g) and lowest weight was observed 28.48g in Shibpur. In case of blast affected plant, highest number of seed per panicle was 22 in Jotarpur and lowest number of seed per panicle was 18 in Bollobpur and Shibpur. Weight of seed per panicle and 1000 seed weight was also maximum in Maniknagar (0.63g and 30.86g, respectively). The lowest number of seed per panicle was 18 in Bollobpur and Shibpur (Table 8).

Table 6. Number of seed/panicles, weight of seed/panicle, 1000-seed weight (wt.) of wheat seeds collected from healthy and bleached panicle as influenced by sites and variety in Meherpur sadar upazilla

Villages	Varieties	Healthy			Blast		
		No. of seed/ panicle	Seed wt.(g)/ panicle	1000 seed wt.(g)	No. of seed/ panicle	Seed wt.(g)/ panicle	1000 seed wt.(g)
Kathalpota	BARI Gom-28	41.00 d	2.20 c	52.42 c	19.00 e	0.53 f	31.39 b
Sonapur	BARI Gom-24(Prodip)	48.33 a	2.27 b	46.33 f	19.33 e	0.60 e	30.06 d
Pirojpur	BARI Gom-28	30.33 fg	1.81 e	59.61 a	24.33 bc	0.74 c	28.84 f
Nurpur	BARI Gom-24(Prodip)	44.00 c	2.24 b	50.82 d	21.67 d	0.60 e	27.73 g
Kola	BARI Gom-24(Prodip)	29.00 g	1.69 f	57.88 b	23.00 cd	0.74 c	30.82 c
Raypur	BARI Gom-24(Prodip)	32.67 e	1.89 d	57.80 b	27.67 a	0.84 a	29.66 e
Tungi	BARI Gom-24(Prodip)	46.00 b	2.37 a	49.33 e	25.00 b	0.81 b	31.70 a
Rajapur	BARI Gom-24(Prodip)	31.00 ef	1.83 e	57.69 b	24.67 bc	0.69 d	30.99 c
CV %		2.81	0.9661	1.37	5.00	2.16	0.4487
LSD (0.05)		1.8359	0.0341	1.2763	1.9987	0.0260	0.2342

Means with the same letter are not significantly different.

Table 7. Number of seed/panicles, weight of seed/panicle, 1000-seed weight (wt.) of wheat seeds collected from healthy and bleached panicle as influenced by collection sites and variety in Chuadanga sadar upazilla

Villages		Healthy			Blast		
	Variety	No. of seed/ panicle	Seed wt.(g)/ panicle	1000 seed wt.(g)	No. of seed/ panicle	Seed wt.(g)/ panicle	1000 seed wt.(g)
Sutipur	BARI Gom-28	34.00 c	1.18 c	34.84 f	19.67 c	0.45 d	21.87 c
Korimpur	BARI Gom-24(Prodip)	34 c	1.18 c	34.45 g	20 bc	0.44 d	22.25 c
Hematpur	BARI Gom-24(Prodip)	40.67 a	2.46 b	62.25 c	21 bc	0.63 c	30.28 b
Kaliabokri	BARI Gom-28	27 e	1.24 c	35.31 e	11.67 e	0.29 e	22.15 c
Ibrahimpur	BARI Gom-24(Prodip)	38 b	2.33 b	61.08 d	23.67 a	0.85 a	32.50 a
Alokdi	BARI Gom-24(Prodip)	41 a	3.06 a	65.43 a	22.33 ab	0.74 b	32.65 a
Charulia	BARI Gom-24(Prodip)	38.67 b	3.43 a	63.55 b	21.67 abc	0.73 b	21.76 c
Hogoldanga	BARI Gom-24(Prodip)	30.33 d	1.25 c	33.43 h	15 d	0.31 e	32.12 a
CV %		2.99	11.34	0.3098	6.99	2.68	1.22
LSD (0.05)		1.8359	0.3955	0.2616	2.3436	0.0257	0.568

Means with the same letter are not significantly different.

Table 8. Number of seed/panicle, weight of seed/panicle, 1000-seed weight (wt.) of wheat seeds collected from healthy and bleached panicle as influenced by collection sites and variety in Mujibnagar sadar upazilla

Villages	**Varieties**	**Healthy**			Blast		
		No. of seed/ panicle	Seed wt.(g)/ panicle	1000 seed wt.(g)	No. of seed/ panicle	Seed wt.(g)/ panicle	1000 seed wt.(g)
Gopalpur	BARI Gom-24(Prodip)	32.33 d	2.03 cd	57.42 f	20.67 a	0.62 a	29.52 c
Jotarpur	BARI Gom-26	38.00 ab	2.25 a	59.25 c	22.00 a	0.60 ab	30.63 b
Mohajonpur	BARI Gom-24(Prodip)	36.67 bc	2.12 b	57.42 f	20.67 a	0.61 a	29.68 c
Bollobpur	BARI Gom-28	35.33 c	2.08 bc	60.33 b	18.00 b	0.56 bc	28.49 d
Maniknagar	BARI Gom-26	37.00 bc	2.22 a	59.15 d	21.67 a	0.63 a	30.86 a
Komorpur	BARI Gom-28	32.00 d	1.83 e	58.09 e	20.67 a	0.63 a	29.54 c
Shibpur	BARI Gom-24(Prodip)	39.67 a	2.29 a	60.93 a	18.00 b	0.53 c	28.48 d
Bagoan	BARI Gom-24(Prodip)	33.00 d	1.98 d	59.11 d	21.00 a	0.62 a	29.58 c
CV %		3.35	1.77	0.0567	6.81	4.18	0.4029
LSD (0.05)		2.0602	0.0645	0.0578	2.3963	0.0434	0.2064

Means with the sane letter are not significantly different.

Blast Healthy

Fig. 7. Blast and healthy panicle in blast infected wheat field

Fig. 8. Rachis, infected grains and glumes from bleached and tagged wheat spike after threshing

4.5 Cultural and morphological variability among *Magnaporthe oryzae triticum* (MoT) Isolates

Study on cultural characteristics of isolates of *M. oryzae triticum* was carried out on different culture media in two sessions. At first three solid media with fifteen isolates were used *viz*., Potato dextrose agar, Oatmeal agar and Potato sucrose agar. In second session I used eleven media with 10 isolates respectively as described in 'Material and Methods'. The different parameters considered for assessing the variability among the isolates were *viz.*, colony diameter, colony color, colony margin, surface elevation of the pathogen.

4.6. Mean mycelial diameter, growth rate and Growth characteristics of 15 isolates on three solid media

4.6.1 Potato dextrose agar media

Colonies were ashy white to ash color sometime white color, smooth or rough margin, cottony surface. Some isolates viz. ME MoT15, CH MoT11, CH MoT12, CH MoT13 having velvety surface. Most of the isolates had good growth, few had very poor growth. CH MoT13 ME MoT17, ME MoT18, MU MoT15 isolate showed medium growth. Almost all isolates produced pyriform, hyaline to pale olive conidia. Highest mycelial growth was found 38.33 mm and maximum growth rate per day was observed 12.78 mm in ME MoT17 isolate at 3 DAI, and highest mycelial growth was 47 mm and growth rate was 11.75 mm per day was observed in MU MoT16 at 4 DAI. Minimum mycelial growth was seen 16 mm in CH MoT14 isolate at 3 DAI and 26mm in the same isolate at 4 DAI. (Table 9 and 10).

4.6.2 Potato sucrose agar media

Most of the isolates viz. ME MoT14, ME MoT15, ME MoT18, MU MoT15, MU MoT16, CH MoT 11, CH MoT 12 showed ashy white colony. ME MoT13, ME MoT17, ME MoT13, ME MoT14 showed whitish, cottony surface colony. Rough margin was observed in isolates ME MoT15, ME MoT 17, ME MoT18, MU MoT16, MU MoT17, CH MoT12, CH MoT13 and CH MoT14. Others isolate showed smooth margin. Highest mycelial growth was found 38 mm and 46 mm in isolate ME MoT18 at 3 DAI and 4DAI, respectively. Lowest mycelial growth and growth rate was seen in ME

MoT15, it was 24mm and 8mm at 3 DAI, and 30 mm and 7.50 mm at 4 DAI. Rest of the isolates showed medium mycelial growth (Table 11 and 12).

4.6.3 Oat meal agar

Whitish to grey color colony that was not raised, smooth margin and medium growth was found in almost all isolates except ME MoT14 having rough margin. Conidia were hyaline to pale olive color with pyriform shape. Highest mycelial growth and growth rate was recorded 39.50 mm and 13.17 mm at 3 DAI and 47.67 mm and 11.91 mm at 4 DAI in isolate MU MoT13. Low radial mycelial growth was found 31.67 mm and growth rate was 10.75 mm was observed in MU MoT17 at 4 DAI. ME MoT13, ME MoT14, MU MoT 14, MU MoT15, CH MoT11 showed sufficient mycelial growth at both 3 DAI and 4 DAI (Table 13 and 14).

Table 9. Radial mycelial growth and growth rate/day of 15 isolates of *Magnaporthe oryzae triticum* (MoT) on Potato Dextrose Agar (PDA) media

MoT Isolate	Radial mycelial growth (mm)		Mycelial growth rate/day (mm)	
	3 DAI	4 DAI	3 DAI	4 DAI
ME MoT13	33.33 cd	41.33 cd	11.11 cd	10.33 cd
ME MoT14	35.33 bc	43 bc	11.78 bc	10.75 bc
ME MoT15	32 d	41 cd	10.67 d	10.25 cd
ME MoT16	32 d	41.33 cd	10.67 d	10.33 cd
ME MoT17	38.33 a	45 ab	12.78 a	11.25 ab
ME MoT18	35 bc	40 d	11.67 bc	10 d
MU MoT13	35 bc	40.33 d	11.67 bc	10.08 d
MU MoT14	34.50 bc	40 bc	11.50 bc	10 d
MU MoT 15	33.33 cd	42 cd	11.11 cd	10.50 cd
MU MoT16	37 ab	47 a	12.33 ab	11.75 a
MU MoT17	23.33 e	33 e	7.78 e	8.25 e
CH MoT11	35 bc	42 cd	11.67 bc	10.50 cd
CH MoT 12	26 e	30 fg	8.67 e	7.50 fg
CH MoT 13	20 f	29.67 g	6.67 f	7.42 g
CH MoT 14	16 g	26 h	5.33 g	6.50 h
CV (%)	5.70	3.59	5.69	3.59
LSD (0.05)	2.8882	2.291	0.9621	0.5729

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

Table 10. Colony character of 15 isolates of *Magnaporthe oryzae triticum* (MoT) on Potato dextrose agar media

MoT Isolate	Growth pattern	Colony character	Conidial character
ME MoT13	Regular	Whitish Ash, Smooth margin, Good growth, cottony	Pyriform, hyaline to pale olive
ME MoT14	Regular	Ashy White, Smooth margin, Good growth, Cottony	Pyriform, hyaline to pale olive
ME MoT15	Regular	Ashy White, rough margin, Medium growth, Velvety	Pyriform, hyaline to pale olive
ME MoT16	Regular	Whitish, Smooth margin, Good growth, Cottony	Pyriform, hyaline to pale olive
ME MoT17	Regular	Whitish Ash, rough margin, Medium growth, cottony	Pyriform, hyaline to pale olive
ME MoT18	Regular	Ashy White, rough margin, Medium growth, cottony	Pyriform, hyaline to pale olive
MU MoT13	Regular	Whitish, Smooth margin, Good growth, Cottony	Pyriform, hyaline to pale olive
MU MoT14	Regular	Whitish, Smooth margin, Good growth, Cottony	Pyriform, hyaline to pale olive
MU MoT 15	Regular	Ashy White, Smooth margin, Medium growth, Cottony	Pyriform, hyaline to pale olive
MU MoT16	Regular	Ashy White, rough margin, Good growth, Cottony	Pyriform, hyaline to pale olive
MU MoT17	Regular	Ash color, Rough margin, Poor growth, cottony	Pyriform, hyaline to pale olive
CH MoT11	Regular	Ashy white, Smooth margin, Good growth, Velvety	Pyriform, hyaline to pale olive
CH MoT 12	Regular	Ashy white, rough margin, Poor growth, Velvety	Pyriform, hyaline to pale olive
CH MoT 13	Regular	Ashy color, Rough margin, Very poor growth, Velvety	Pyriform, hyaline to pale olive
CH MoT 14	Regular	Ashy color, Rough margin, Very poor growth, Velvety	Pyriform, hyaline to pale olive

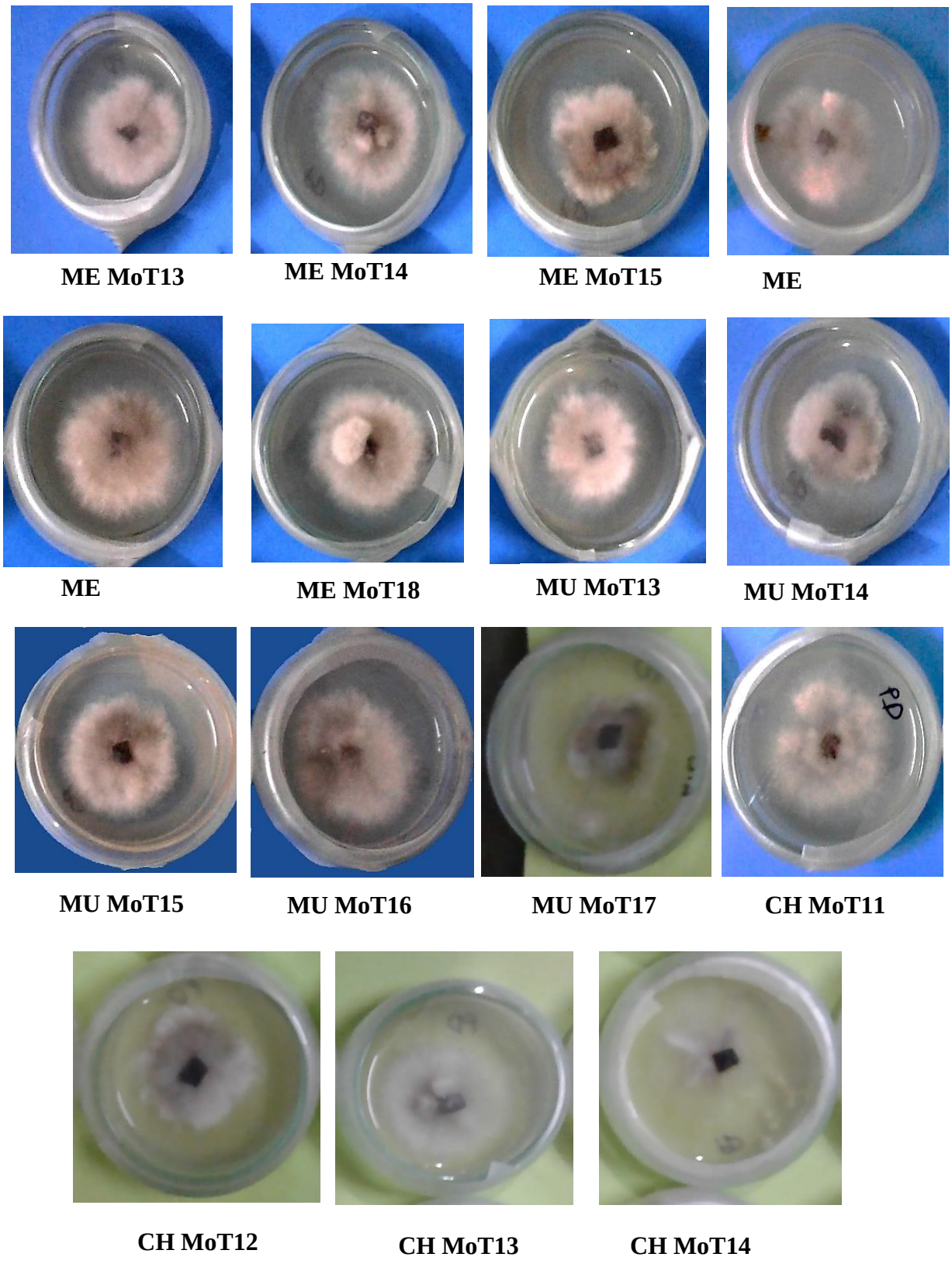

Figure 9. Radial mycelial growth of 15 isolates of *Magnaporthe oryzae triticum* on PDA media at 4 DAI (days after inoculation)

Table 11. Radial mycelial growth and Growth rate/day of 15 isolates of *Magnaporthe oryzae triticum* (MoT) on Potato Sucrose Agar (PSA) media

MoT isolate	Radial mycelial growth (mm)		Mycelial growth rate/day (mm)	
	3 DAI	4 DAI	3 DAI	4 DAI
ME MoT13	31.00 de	43.33 bcd	10.33 de	10.83 bcd
ME MoT14	35.33b	44.33 ab	11.78 ab	11.08 ab
ME MoT15	24 f	30 e	8 f	7.50 e
ME MoT16	32.33 cd	44 abc	10.78 cd	11 abc
ME MoT17	31 de	41 d	10.33 de	10.25 d
ME MoT18	38 a	46 a	12.67 a	11.50 a
MU MoT 13	33 bcd	42 bcd	11 bcd	10.50 bcd
MU MoT14	31 de	41 d	10.78 cd	10.25 d
MU MoT15	29 e	41.67 cd	9.67 e	10.42 cd
MU MoT16	31.67 cd	43 bcd	10.56 cde	10.75 bcd
MU MoT17	22.33 f	26.33 f	7.44 f	6.58 f
CH MoT11	34 bc	41 d	11.33 bc	10.25 d
CH MoT12	22 f	27.33 f	7.33 fg	6.83 f
CH MoT13	19.33 g	27.33 f	6.44 g	6.83 f
CH MoT14	12.33 h	22.33 g	4.11 h	5.58 g
CV (%)	5.60	4.28	5.82	4.28
LSD (0.05)	2.6541	2.6657	0.9224	0.6664

*DAI= Days After Inoculation.

Means with the same letter are not significantly different.

Table 12. Colony character of 15 isolates of *Magnaporthe Oryzae Triticum* (MoT) on potato sucrose agar media

MoT Isolate	Growth pattern	Colony Color	Conidial character
ME MoT13	Regular	Whitish Ash, Smooth margin, Good growth, cottony	Pyriform, hyaline to pale olive
ME MoT14	Regular	Ashy White, Smooth margin, Good growth, Cottony	Pyriform, hyaline to pale olive
ME MoT15	Regular	Ashy White, rough margin, Medium growth, Velvety	Pyriform, hyaline to pale olive
ME MoT16	Regular	Whitish, Smooth margin, Good growth, Cottony	Pyriform, hyaline to pale olive
ME MoT17	Regular	Whitish Ash, rough margin, Medium growth, cottony	Pyriform, hyaline to pale olive
ME MoT18	Regular	Ashy White, rough margin, Medium growth, cottony	Pyriform, hyaline to pale olive
MU MoT 13	Regular	Whitish, Smooth margin, Good growth, Cottony	Pyriform, hyaline to pale olive
MU MoT14	Regular	Whitish, Smooth margin, Good growth, Cottony	Pyriform, hyaline to pale olive
MU MoT15	Regular	Ashy White, Smooth margin, Medium growth, Cottony	Pyriform, hyaline to pale olive
MU MoT16	Irregular	Ashy White, rough margin, Good growth, Cottony	Pyriform, hyaline to pale olive
MU MoT17	Irregular	Ash color, Rough margin, Poor growth, cottony	Pyriform, hyaline to pale olive
CH MoT11	Regular	Ashy white, Smooth margin, Good growth, Velvety	Pyriform, hyaline to pale olive
CH MoT12	Irregular	Ashy white, rough margin, Poor growth, Velvety	Pyriform, hyaline to pale olive
CH MoT13	Regular	Ashy color, Rough margin, Very poor growth, Velvety	Pyriform, hyaline to pale olive
CH MoT14	Regular	Ashy color, Rough margin, Very poor growth, Velvety	Pyriform, hyaline to pale olive

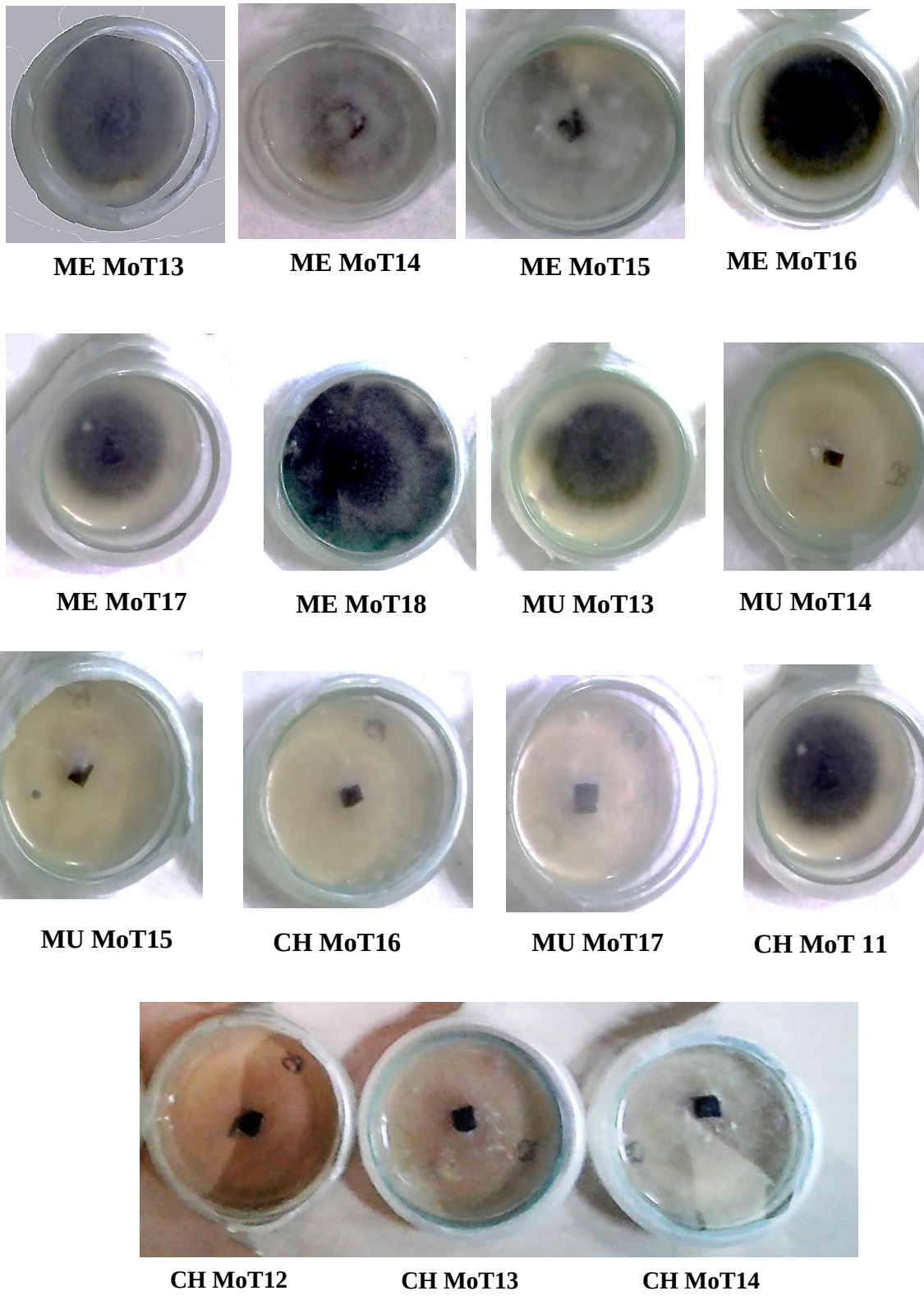

Figure 10. Radial mycelial growth of 15 isolates of *Magnaporthe oryzae triticum* on PSA media at 4 DAI (days after inoculation)

Table 13. Radial mycelial growth and growth rate of 15 isolates of *Magnaporthe oryzae triticum* (MoT) on Oat Meal Agar (OMA) media

MoT isolate	Radial mycelial growth (mm)		Mycelial growth rate/day (mm)	
	3 DAI	4 DAI	3 DAI	4 DAI
ME MoT13	34.5 bc	42.5 c	11.5 bc	10.63 c
ME MoT14	35.00 b	43.00 c	11.67 c	10.75 bc
ME MoT15	32.00 c	41.33c	10.67 c	10.33 c
ME MoT16	32.oo c	41.5 c	10.67 c	10.38 c
ME MoT17	37.00 ab	45.00 bc	12.33 ab	11.25 b
ME MoT18	36.67 ab	42 c	12.22 ab	10.5 c
MU MoT 13	39.50 a	47.67 a	13.17 a	11.91 a
MU MoT 14	39.00 a	46.5 ab	13 a	11.63 ab
MU MoT 15	37.00 ab	45.33 b	12.33 ab	11.33 ab
MU MoT16	37.67 ab	45.67 ab	12.56 ab	11.42 ab
MU MoT17	26.33 d	31.67 e	8.78 e	7.92 e
CH MoT11	36.00 b	43.00 c	12 b	10.75 bc
CH MoT12	29.67 cd	36.00 d	9.89 cd	9.00 d
CH MoT13	27.67 d	32.33 e	9.44 d	8.08 e
CH MoT14	27.67 d	33.67 e	9.44 d	8.42 de
LSD (0.05)	2.8990	2.305	0.9823	0.5848

*DAI= Days After Inoculation.

Means with the same letter are not significantly different.

Table 14. Colony character of 15 isolates of *Magnaporthe oryzae triticum* (MoT) on oat meal agar media

MoT isolates	Growth pattern	Colony Color	Conidial character
ME MoT13	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
ME MoT14	Regular	Gray, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
ME MoT15	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
ME MoT16	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
ME MoT17	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
ME MoT18	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
MU MoT 13	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
MU MoT 14	Regular	Grayish White, rough margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
MU MoT 15	Regular	Grayish Centre with whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
MU MoT16	Regular	Gray, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
MU MoT17	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
CH MoT11	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
CH MoT12	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
CH MoT13	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive
CH MoT14	Regular	Whitish, Smooth margin, Not raised, Medium growth	Pyriform, hyaline to pale olive

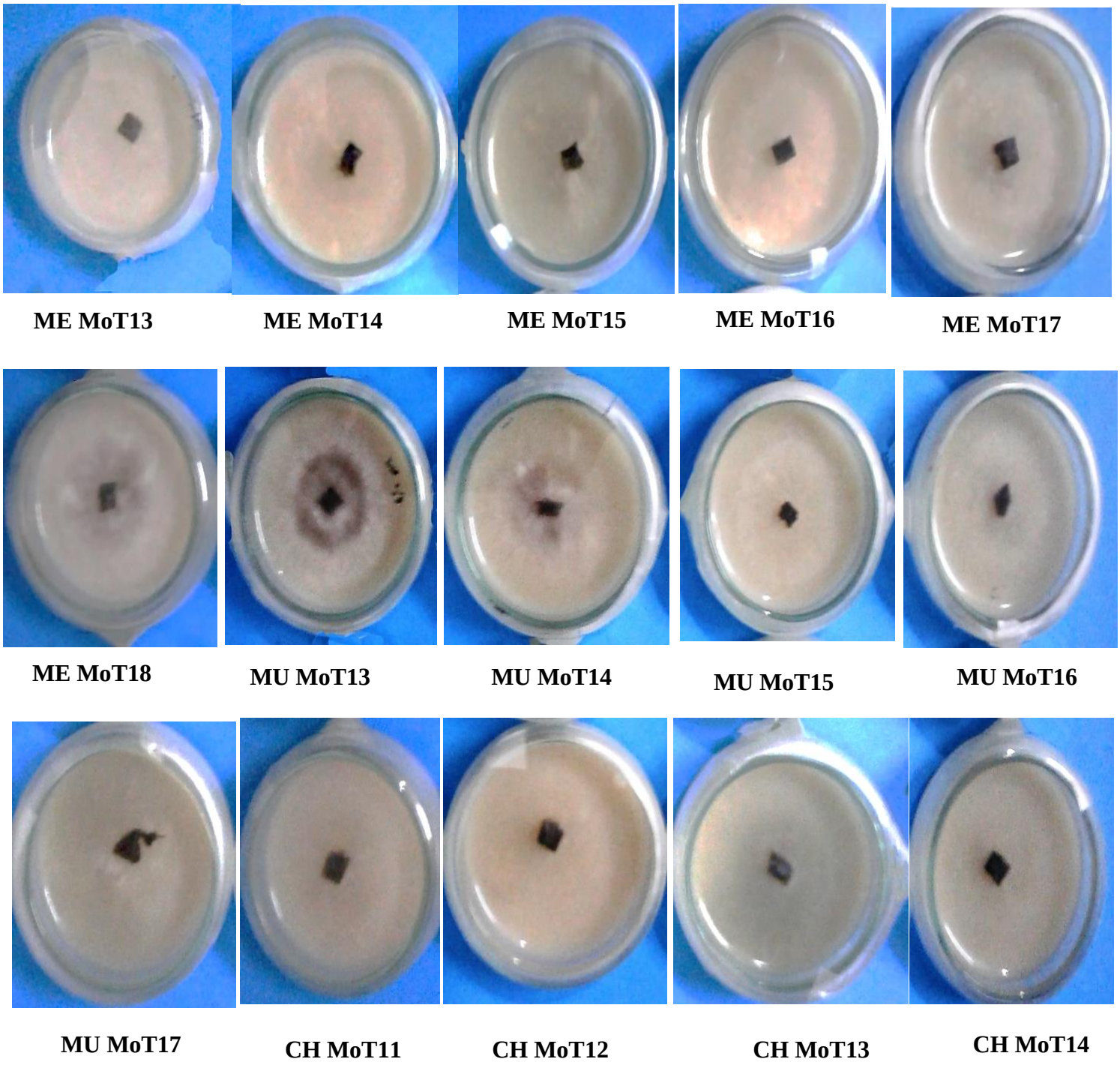

Fig. 11. Radial mycelial growth of 15 isolates of *Magnaporthe oryzae triticum* on OMA at 4 DAI (days after inoculation)

4.7. Mean mycelial diameter, growth rate/day and Growth characteristics of 10 isolates on eleven solid media

Oat meal agar

Colony color of all the isolates was usually whitish gray whereas ME MoT16 showed whitish color and MU MoT14 showed blackish white color. All isolates showed raised mycelial growth with rough margin expect ME MoT13, MU MoT13, MU MoT15, CH MoT11 isolates those showed smooth margin. Except MU MoT13 all isolates showed regular growth pattern. Highest mycelium growth was found in isolate ME MoT13(55.33mm) followed by MU MoT13 and minimum radial mycelial growth was 43.33 in isolate MU MoT16 (Table 15).

Corn meal agar

Colonies were light blackish color in all isolates. Some isolates viz. ME MoT13, ME MoT14, ME MoT15, ME MoT16, MU MoT15 had mycelia which was not raised. Others had slightly raised mycelia. Maximum mycelial growth was found in isolate MU MoT16 (21mm) at 4 DAI and in isolate CH MoT11 (41mm) at 8 DAI. Lowest mycelial growth was observed in isolate MU MoT15 (12mm) at 4 DAI and 36.00 mm was found in isolates MU MoT13, MU MoT14 and MU MoT15 at 8 DAI. (Table 16).

Prune agar

The color of the colony varied from whitish to greyish white color, Regular, Circular, raised mycelia, rough margin was observed in all isolates. Lowest radial mycelial growth and growth rate was seen in isolate ME MoT13 (20 mm) and (5mm/day) respectively at 4 DAI and highest mycelial diameter and growth rate was found about 33.33 mm and 8.33 mm/day was recorded in isolate MU MoT13 at 4 DAI. At 8 DAI, Isolate MU MoT13 showed maximum mycelial growth and growth rate (49 mm and 6.13 mm/day). Minimum growth was observed 43 mm in ME MoT14, ME MoT15 MU MoT16 isolates (Table 17).

Yeast extract agar media

Almost all isolates were colored ranges from whitish to whitish grey, having regular, ring colony except ME MoT14, MU MoT14, CH MoT11 had circular colony. These isolates had slightly raised mycelia. Some isolates such as MU MoT14, MU MoT15, MU MoT16 and CH MoT12 had mycelia that was not raised. All isolates had smooth margin except MU MoT15 isolate had rough margin. Maximum mycelial growth was found 51 mm in MU MoT13 at 4 DAI. And 70 mm was observed in ME MoT15 and MU MoT15 isolates at 8 DAI. Low growth and growth rate was observed 67 mm and 8.38 mm/day in ME MoT14 isolate at 8 DAI. And low mycelial growth and growth rate was found 46 mm and 11.50 mm/day in isolate ME MoT15 and CH MoT11 at 4 DAI (Table 18).

Table 15. Radial mycelial growth, growth rate and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on Oat Meal Agar (OMA) media

MoT isolate	Radial mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	8 DAI	
ME MoT13	55.33 a	71.67 a	13.83	8.96 a	Regular, ring, whitish grey, raised, smooth margin
ME MoT14	54.67 a	72 a	13.67	9 a	Regular, ring, whitish grey raised, rough margin
ME MoT15	53.33 a	71 ab	13.33	8.88 ab	Regular, ring, whitish grey, raised, rough margin
ME MoT16	48 b	69 b	12	8.63 b	Regular, circular, whitish, raised, rough margin
MU MoT13	49 b	55.67 d	12.25	6.96 d	Irregular, circular, whitish, raised, smooth margin
MU MoT14	48.33 b	55 d	12.08	6.88 d	Regular, concentric ring, blackish white, raised, rough margin
MU MoT15	50 b	66.33 c	12.50	8.29 c	Regular, circular, whitish grey, raised, smooth margin
MU MoT16	43.33 c	69 b	10.83	8.63 b	Regular, circular, whitish grey, raised, rough margin
CH MoT11	55 a	70 ab	13.75	8.75 ab	Regular, ring, whitish grey, raised, smooth margin
CH MoT12	54 a	69 b	9.50	8.63 b	Regular, ring, whitish grey, raised, rough margin
CV (%)	3.80	1.77	18.13	1.77	
LSD (0.05)	3.3055	2.0152	Non-significant	0.2519	

*DAI= Days After Inoculation.

Means with the same letter are not significantly different.

Table 16. Radial mycelial growth, growth rate and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on Corn meal agar (CMA)

MoT isolate	Mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME MoT13	16 c	40.33 ab	4 c	5.04 ab	Regular, light blackish, not raised, smooth margin.
ME MoT14	18 b	40 ab	4.50 b	5 ab	Regular, light blackish, not raised, smooth margin.
ME MoT15	16 c	39 bc	4 c	4.88 bc	Regular, light blackish, not raised, rough margin.
ME MoT16	19 b	36.67 abc	4.75 b	4.96 abc	Regular, light blackish, not raised, smooth margin.
MU MoT13	16 c	36 d	4 c	4.50 d	Regular, light blackish, slightly raised, rough margin.
MU MoT14	14 d	36 d	3.50 d	4.50 d	Regular, light blackish, slightly raised, smooth margin.
MU MoT15	12e	36 d	3 e	4.50 d	Regular, light blackish, not raised, smooth margin.
MU MoT16	21 a	38.33 c	5.25 a	4.79 c	Regular, light blackish, slightly raised, rough margin.
CH MoT11	14 d	41 a	3.50 d	5.13 a	Regular, light blackish, slightly raised, smooth margin.
CH MoT12	16 c	35 d	4 c	4.38 d	Regular, light blackish, slightly raised, rough margin.
CV (%)	4.78	2.49	4.78	2.47	
LSD (0.05)	1.3193	1.6158	0.3298	0.2005	

*DAI= Days After Inoculation

Means with the same letter are not significant different

Table 17. Radial mycelial growth, growth rate and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on Prune Agar

MoT solate	Mycelial growth (mm)		Growth rate/day(mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME Mo13	20 e	44.33 bc	5 e	5.54 bc	Regular, circular, whitish, raised, rough margin
ME MoT14	21 e	43 c	5.25 e	5.38 c	Regular, circular, whitish, raised, rough margin
ME MoT15	25 d	43 c	6.25 d	5.38 c	Regular, circular, whitish, raised, rough margin
ME MoT16	25 d	45 b	6.25 d	5.63 b	Regular, circular, greyish white, raised, rough margin
MU MoT13	33.33 a	49 a	8.33 a	6.13 a	Regular, circular, greyish white, raised, rough margin
MU MoT14	31 b	46 b	7.75 b	5.75 b	Regular, circular, whitish, raised, rough margin
MU MoT15	26 d	46 b	6.50 d	5.7 b	Regular, circular, greyish white, raised, rough margin
MU MoT16	29 c	43 c	7.25 c	5.38 c	Regular, circular, white, raised, rough margin
CH MoT11	26 d	48 a	6.50 d	6 a	Regular, circular, greyish white, raised, rough margin
CH MoT12	31 b	48 a	7.75 b	6 a	Regular, circular, greyish white, raised, rough margin
CV (%)	3.80	2.44	3.80	2.44	
LSD (0.05)	1.7313	1.8915	0.4328	0.2367	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

Table 18. Radial mycelial growth, growth rate/day and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on Yeast extract agar media

MoT isolate	Mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME MoT13	48 cd	68.67 a	12 cd	8.59 a	Regular, ring, whitish, slightly raised, smooth margin
ME MoT14	49.33 bc	67 b	12.33 bc	8.38 b	Regular, circular whitish, slightly raised, smooth margin
ME MoT15	46 e	70 a	11.50 e	8.75 a	Regular, ring, whitish grey, slightly raised, smooth margin
ME MoT16	49 bc	69 a	12.25 bc	8.63 a	Regular, ring, whitish grey, slightly raised, smooth margin
MU MoT13	51 a	69 a	12.75 a	8.63 a	Regular, ring, whitish grey, slightly raised, smooth margin
MU MoT14	47 de	69 a	11.75 de	8.63 a	Regular, circular, whitish grey, not raised, smooth margin
MU MoT15	48 cd	70 a	12 cd	8.75 a	Regular, ring, whitish grey, not raised, rough margin
MU MoT16	49 bc	69 a	12.25 bc	8.63 a	Regular, ring, whitish grey, not raised, smooth margin
CH MoT11	46 e	68.67 a	11.50 e	8.58 a	Regular, circular, whitish grey, raised, smooth margin
CH MoT12	50 ab	69.67 a	12.50 ab	8.71 as	Regular, ring, whitish grey, not raised, smooth margin
CV (%)	1.77	1.37	1.77	1.38	
LSD (0.05)	1.4585	1.6158	0.3646	0.2029	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

V5 media

Regular, circular, greyish, raised, rough margin colony was found in almost all isolates expect MU MoT13, MU MoT14, CH MoT11, CH MoT12 isolates showed ring and smooth margin colony. Maximum mycelial growth was 43mm and growth rate 10.75 mm/day was found in ME MoT13 at 4 DAI. Radial mycelial growth was 62 mm and 7.75 mm/day was recorded in MU MoT15, CH MoT12 isolates at 8 DAI. Minimum growth and growth rate/day were (32mm and 8mm/day) was found in ME MoT14 at 4 DAI and 8 DAI, respectively (Table 19).

Wheat straw agar

ME MoT13, ME MoT14, MU MoT16 had grayish Centre with whitish color, raised and smooth margin colony. Regular, circular colony with rough margin were found in other isolates without isolates ME MoT13, ME MoT14, MU MoT16, CH MoT12 having ring like colony. Highest mycelial growth and growth rate was seen in MU MoT16 isolate (47 mm and 11.75 mm/day) at 4 DAI and 68.67 mm and 8.59 mm/day in isolate ME MoT14 at 8 DAI. Lowest mycelial growth was 27 mm and growth rate was 6.75 mm/day observed in MU MoT14. At 8 DAI minimum growth was 51mm and growth rate was 6.38 mm/day in MU MoT15 (Table 20).

Potato dextrose agar

Colony usually showed regular, circular, grayish white to white color, raised and rough margin except ME MoT13 and CH MoT11 had smooth margin. Radial mycelial growth was maximum 54 mm and 70.34 mm in ME MoT13 isolate at 4 DAI and 8 DAI respectively. Lowest mycelial growth was recorded 19 mm in MU MoT15 and 44 mm in MU Mot16 at 4 DAI and 8 DAI, respectively (Table 21).

Potato carrot agar

Regular, circular or ring, whitish grey to blackish color, raised with rough or smooth margin was found in all isolates. Highest mycelial growth and growth rate was 48mm and 12mm/day was found in isolate ME MoT18 at 4 DAI. And mycelial growth 72 mm and growth rate 9 mm/day was seen in ME MoT13 isolate at 8 DAI. Lowest mycelial growth was 11 mm and 2.75 mm was recorded in isolate CH MoT12 at 4 DAI and 17.67 mm and 2.21 mm/day in isolate CH MoT11 at 8 DAI (Table 22).

Table 19. Radial mycelial growth, growth rate/day and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on V5 agar media

MoT isolate	Mycelial growth (mm)		Growth rate /day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME MoT13	43 a	59 bc	10.75 a	7.38 bc	Regular, circular, grayish, raised, rough margin
ME MoT14	38 c	54.67 d	9.50 c	6.83 d	Regular, circular, grayish, raised, rough margin
ME MoT15	35 e	61 ab	8.75 e	7.54 ab	Regular, circular, grayish, raised, rough margin
ME MoT16	35 e	61 ab	8.75 e	7.54 ab	Regular, circular, grayish, raised, rough margin
MU MoT13	36 de	57 cd	9 de	7.13 cd	Regular, ring, grayish, raised, smooth margin
MU MoT14	35 e	61 ab	8.75 e	7.63 ab	Regular, ring, grayish, raised, smooth margin
MU MoT15	32 f	62 a	8 f	7.75 a	Regular, circular, grayish, raised, rough margin
MU MoT16	38 c	60 ab	9.50 c	7.50 abc	Regular, circular, grayish, raised, smooth margin
CH MoT11	37 cd	59 bc	9.25 cd	7.38 abc	Regular, ring, grayish, raised, smooth margin
CH MoT12	40 b	62 a	10 b	7.75 a	Regular, ring, grayish, raised, smooth margin
CV (%)	2.57	2.61	2.57	2.96	
LSD (0.05)	1.6158	2.6568	0.4039	0.3752	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

Table 20. Mycelial growth, growth rate/day and colony character on Wheat Straw Agar media

MoT isolate	Mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME MoT13	45 b	64 c	11.25 b	8.00 c	Regular, ring, grayish Centre with whitish color, raised, smooth margin
ME MoT14	46 ab	68.67 a	11.5 ab	8.59 a	Regular, ring, grayish Centre with whitish color, raised, smooth margin
ME MoT15	46 ab	60 de	11.5 ab	7.50 d	Regular, circular, whitish, raised, smooth margin
ME MoT16	31 e	61 d	7.75 e	7.63 d	Regular, circular, blackish Centre with whitish color, raised, rough margin
MU MoT13	43 c	61 d	10.75 c	7.63 d	Regular, circular, blackish Centre with whitish color, raised, rough margin
MU MoT14	27 f	66 d	6.75 f	8.25 b	Regular, circular, blackish Centre with whitish color, raised, rough margin
MU MoT15	41 d	51 f	10.25 d	6.38 f	Regular, circular, blackish Centre with whitish color, raised, smooth margin
MU MoT16	47 a	59 e	11.75 a	7.38 e	Regular, ring, grayish Centre with whitish color, raised, smooth margin
CH MoT11	30 e	64 c	7.5 e	8.00 c	Regular, circular, blackish Centre with whitish color, raised, rough margin
CH MoT12	45 b	69 a	11.25 d	8.63 a	Regular, ring, blackish Centre with whitish color, raised, smooth margin
CV (%)	2.37	1.71	2.27	1.71	
LSD(.05)	1.61	1.8132	0.401	0.2270	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

Table 21. Radial mycelial growth, growth rate and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on PDA media

MoT isolate	Mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME MoT13	54 a	70.34 a	13.5 a	8.79 a	Regular, circular, grayish Centre with whitish color, raised, smooth margin
ME MoT14	34e	56 f	8.5 e	7.00 a	Regular, circular, whitish color, raised, rough margin
ME MoT15	52 b	66 bc	6.50 f	8.25 bc	Regular, circular, whitish Centre with whitish color, raised, rough margin
ME MoT16	45 c	58 e	11.25 c	7.25 e	Regular, ring, whitish gray color, raised, rough margin
MU MoT13	44 c	65 c	11 c	8.13 c	Regular, ring, whitish gray color, raised, rough margin
MU MoT14	51 b	67 b	12.75 b	8.38 b	Regular, circular, whitish gray color, raised, rough margin
MU MoT15	19 f	47 g	4.75 g	5.88 g	Regular, circular, whitish gray color, raised, rough margin
MU MoT16	35 e	44 h	8.75 e	5.50 h	Regular, circular, blackish Centre with whitish color, raised, rough margin
CH MoT11	41 d	63 d	10.25 d	7.88 d	Regular, circular, whitish color, raised, smooth margin
CH MoT12	45 c	69.67 a	11.25 c	8.71 a	Regular, circular, whitish gray color, raised, rough margin
CV (%)	2.61	1.54	2.69	1.54	
LSD (0.05)	1.8657	1.5856	0.4516	0.1983	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

Table 22. Radial mycelial growth, growth rate/day and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on PCA media

MoT isolate	Mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME MoT13	41 d	72 a	10.25 d	9.00 a	Regular, ring, whitish grey, very raised, smooth margin
ME MoT14	44 bc	71 ab	11 bc	8.88 ab	Regular, circular, whitish grey, very raised, rough margin
ME MoT15	41 d	67 c	10.25 d	8.38 c	Regular, circular, whitish, very raised, rough margin
ME MoT16	45.34 d	69 bc	11.33 b	8.63 bc	Regular, ring, whitish grey, raised, smooth margin
MU MoT13	37 e	69 bc	9.25 e	8.58 c	Regular, circular, whitish grey, raised, rough margin
MU MoT14	48 a	71 ab	12 a	8.88 ab	Regular, circular, whitish grey, very raised, smooth margin
MU MoT15	41 d	68.67 ab	10.25 d	8.58 c	Regular, circular, whitish grey, very raised, rough margin
MU MoT16	43 c	71 a	10.75 c	8.88 ab	Regular, circular, whitish grey, very raised, smooth margin
CH MoT11	15 f	17.67 e	3.75 f	2.21 e	Regular, circular, whitish, not raised, rough margin
CH MoT12	11 g	41 d	2.75 g	5.13 d	Regular, circular, whitish, not raised, rough margin
CV (%)	2.45	1.96	2.49	1.98	
LSD(.05)	1.554	2.0627	0.388	0.259	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

Wheat seed extract agar

Maximum isolates viz. ME MoT13, ME MoT14, ME MoT16 MU MoT15, CH MoT11 showed regular, ring, raised/slightly raised, colony with rough margin except ME MoT15, MU MoT13, MU MoT16 isolates showed smooth margin. Maximum mycelial growth was observed 39 mm in ME MoT16 and 67 mm in isolate CH MoT11, CH MoT12 at 4 DAI and 8 DAI respectively. Lowest mycelial growth was 31 mm in MU MoT14 and CH MoT11 and 61 mm was seen in MU MoT16 at 4 DAI and 8 DAI respectively. Medium mycelial growth 34.34mm was observed in ME MoT15 isolate and 64 mm was found in ME MoT15 isolate (Table 23).

Wheat flour agar

Regular, ring/circular, whitish grey to white color, not raised to slightly raised colony was found in ME MoT15, MU MoT16, CH MoT11 having rough margin and rest of the isolates had smooth margin. At 4 DAI, radial mycelial growth and growth rate was maximum in isolate MU MoT11 (49 mm and 12.25 mm) respectively. Lower mycelial growth was found in isolate ME MoT16 (45 mm). The lowest growth and growth rate were 41mm and 10.25mm/day found in ME MoT15, and MU MoT16 isolates. It was recorded at 8DAI that; maximum mycelial growth was 72mm in MU MoT15 isolate. Minimum mycelial growth was recorded in isolate ME MoT18; it was 54 mm (Table 24).

Potato sucrose agar

ME MoT13, MU MoT13 isolates showed regular, circular, whitish, very raised surface with smooth margin except others showed rough margin. Highest radial mycelial growth and growth rate per day (46.33 mm and 11.58 mm/day respectively) was found in ME MoT13 isolate at 4 DAI and (71.67 mm and 8.96 mm respectively) were observed in MU MoT14 followed by 71.33 mm was observed in ME MoT13 isolate at 8 DAI. Highest mycelial growth was found 38 mm and 46 mm in isolate ME MoT18 at 4 DAI and 8 DAI respectively. Lowest mycelial growth and growth rate per day was seen in ME MoT15, 24mm and 8mm/day at 4 DAI and 30mm and 7.50 mm/day at 8 DAI. Rest of the isolates showed sufficient mycelial growth (Table 25).

Table 23. Radial mycelial growth, growth rate and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on Wheat Seed Extract Agar (WSEA) media

MoT isolate	Mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	8 DAI	
ME MoT13	35 b	64 cd	8.75 b	8.00 bc	Regular, ring, slightly raised, rough margin
ME MoT14	36b	65.34 bc	9.00 b	8.17 abc	Regular, ring, whitish grey slightly raised, rough margin
ME MoT15	34.34 b	64 ab	8.58 b	8.00 bc	Regular, ring, whitish grey slightly raised, smooth margin
ME MoT16	39 a	65 d	9.75 a	8.13 abc	Regular, ring, whitish grey, slightly raised, smooth margin
MU MoT13	36 b	66 ab	9.00 b	8.25ab	Regular, ring, whitish grey, slightly raised, smooth margin
MU MoT14	31 c	63 e	7.75 c	7.88 c	Regular, circular, whitish grey, raised, rough margin
MU MoT15	38 a	66 a	9.50 a	8.25 ab	Regular, circular, whitish grey, raised, rough margin
MU MoT16	35 b	61 e	8.75 b	7.88 c	Regular, circular, whitish, raised, smooth margin
CH MoT11	31 c	67 a	7.75 c	8.38 a	Regular, circular, whitish black, raised, rough margin
CH MoT12	36 b	67 a	9.00 b	8.38 a	Regular, circular, whitish grey, slightly raised, smooth margin
CV (%)	2.89	1.64	2.89	2.36	
LSD (0.05)	1.7313	1.8132	0.4328	0.3267	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

Table 24. Radial mycelial growth, growth rate/day and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on Wheat Flour Agar (WFA) media

MoT isolate	Mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME MoT13	47 d	71 ab	11.75 b	8.88 ab	Regular, ring, whitish grey, slightly raised, smooth margin
ME MoT14	43 d	71.67 a	10. d75	8.96 a	Regular, ring, whitish grey, slightly raised, smooth margin
ME MoT15	41 e	54 a	10.25 e	6.75 e	Regular, circular, whitish, not raised, rough margin
ME MoT16	45 c	69.67 bc	11.50 bc	8.71 bc	Regular, circular, whitish, slightly raised, smooth margin
MU MoT13	47 b	66 d	11.75 b	8.25 d	Regular, circular, whitish, not raised, smooth margin
MU MoT14	49 a	69 c	12.25 a	8.63 c	Regular, ring, whitish, raised, smooth margin
MU MoT15	45 c	72 a	11.25 c	9.00 a	Regular, ring, white, raised, smooth margin
MU MoT16	41 e	71 ab	10.25 e	8.88 ab	Regular, circular, white, not raised, rough margin
CH MoT11	44.67 c	71.67 a	11.17 cd	8.96 a	Regular, circular, whitish grey, raised, rough margin
CH MoT12	43 d	71 ab	11.42 bc	8.88 ab	Regular, ring, whitish, raised, smooth margin
CV (%)	2.05	1.22	2.19	1.21	
LSD (0.05)	1.555 48	1.4250	0.4186	0.1772	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

Table 25. Radial mycelial growth, growth rate/day and colony character of 10 isolates of *Magnaporthe oryzae triticum* (MoT) on PSA media

MoT isolate	Mycelial growth (mm)		Growth rate/day (mm)		Colony character
	4 DAI	8 DAI	4 DAI	4 DAI	
ME MoT13	46.33 a	71.33 a	11.58 a	8.92 a	Regular, circular, whitish, very raised, smooth margin
ME MoT14	35.00 cd	71.00 a	8.75 cd	8.88 a	Regular, circular, whitish, very raised, rough margin
ME MoT15	31.00 e	39.67 e	7.75 e	4.96 e	Regular, circular, whitish, very raised, rough margin
ME MoT16	31.67 de	70.00ab	7.92 de	8.75 ab	Regular, circular, whitish, very raised, rough margin
MU MoT13	37.00 bc	71.00 a	9.25 bc	8.88 a	Regular, circular, whitish, very raised, smooth margin
MU MoT14	38.00 bc	71.67 a	9.50 dc	8.96 a	Regular, circular, whitish, very raised, rough margin
MU MoT15	39.00 e	59.00 d	9.75 b	7.38 d	Regular, circular, whitish, very raised, smooth margin
MU MoT16	35.00 cd	69.00 b	8.75 cd	8.63 b	Regular, circular, whitish, very raised, rough margin
CH MoT11	35.00 cd	65.00c	8.75 cd	8.13 c	Regular, circular, whitish, very raised, rough margin
CH MoT12	35.00 cd	70.00 ab	8.75 cd	8.75 ab	Regular, circular, whitish, very raised, rough margin
CV (%)	5.95	1.52	5.59	1.52	
LSD (0.05)	3.6793	1.7032	0.9198	0.2130	

*DAI= Days After Inoculation

Means with the same letter are not significantly different.

4.8. Comparison between 10 isolates of *M. oryzae triticum* on eleven media

10 isolates were evaluated in eleven media. The results presented in the table 26, 27, 28 and 29 revealed significant differences between isolates, media and also interaction. Maximum radial mycelial growth and growth rate were recorded in ME MoT13 isolate on OMA media at 4 DAI. (55.33 mm and 13.8 mm/day) that significantly followed by ME MoT14, ME MoT15, ME MoT16 isolates (growth was 46 mm). Mean mycelial growth was maximum on OMA (51.03 mm) followed by yeast Extract Agar media (48.33 mm) and wheat flour agar (44.57 mm). Lowest mycelial growth was found on CMA (16.2 mm) proceeded by prune agar (26.73 mm). Mycelial mean growth on PDA and wheat straw agar media was also good (40.67 mm and 40.1 mm respectively) at 4 days after inoculation (Table 26 and 28). At 8 DAI (days after inoculation), Highest mycelial growth and growth rate were recorded 72 mm and 9 mm/day in isolate ME MoT14 on OMA, ME MoT13 on PCA followed by CH MoT11(71.67 mm) on wheat flour agar and 71 mm in ME MoT14, MU MoT14,16 CH MoT12 isolates. Mean mycelial growth of 10 isolates were maximum 69 mm on yeast Extract agar medium followed by 68.70 mm in wheat flour agar medium, 66.87 mm on OMA media. Lowest mean mycelial growth of all isolates was recorded 38.13 mm on CMA, acceded by 45.53 mm on prune agar media. Others media mix. Wheat straw agar, wheat seed extract agar, PDA PSA, VS showed sufficient mycelial growth (Table 27 and 29).

Table 26. Comparative effect of culture media on radial mycelial growth of ten isolates of *Magnaporthe oryzae triticum* causing wheat blast disease in Bangladesh at 4 DAI (Days After Inoculation)

Culture media	ME Mo T13	ME Mo T14	ME Mo T15	ME Mo T16	MU Mo T13	MU Mo T14	MU Mo T15	MU Mo T16	CH Mo T11	CH Mo T12	Mean
OMA	55.3 a	54.67a	53.3 a	48 a	49 a	48.3 bc	50 a	43. 4 c	55a	54a	51.03
CMA	16 i	18 i	16 g	19 g	16 d	14 h	12 i	21h	14h	16h	16.2
Prune agar	20 h	21 h	25 f	25 f	33.33c	31 f	26 g	29g	26g	31g	26.73
Yeast Extract	48 b	49.33b	46 b	49 a	51 a	47 c	48 b	49a	46b	50b	48.33
V 5	43 e	38 e	35 d	35 d	36 c	35 e	32 f	38 e	37d	40e	36.9
Wheat straw agar	45 d	46 c	46 b	31 e	43 b	27 g	41 d	47 b	30f	45c	40.1
PDA	54 a	34 g	52 a	45 b	44 b	51 a	19 h	35 f	41c	45c	40.66
PCA	41 f	44 d	41 c	45.33b	37 c	48 bc	41 d	43 c	15h	11i	36.64
WSEA	35 g	36 f	34.33 d	39 c	36 c	31 f	38 e	35 f	31f	36f	35.14
Wheat flour agar	47 bc	43 d	41 c	45 b	47 ab	49 b	45 c	41 d	44.67b	43d	44.57
PSA	46.33cd	35 fg	31 e	31.67e	37 c	38 d	39 e	35 f	35e	35f	36.3
CV (%)	2.17	2.19	2.45	2.62	6.84	2.78	2.2 4	2.6 8	2.70	2.58	
LSD(0.05)	1.50 30	1.41 37	1.58 74	1.66 75	4.51 87	1.79 30	1.35 08	1.7 188	1.55 98	1.61 45	

Means with the same letter are not significantly different

Table 27. Comparative effect of culture media on radial mycelial growth of ten isolates of *Magnaporthe oryzae triticum* causing wheat blast disease in Bangladeshat 8 DAI

Culture media	ME MoT 13	ME MoT 14	ME Mo T15	ME MoT 16	MU MoT 13	MU MoT 14	MU MoT 15	MU MoT 16	CH MoT 11	CH MoT 12	Mean
OMA	71.67 ab	72 a	71 a	69 a	55.6 d	55 f	66.33 c	69 b	70 ab	69 b	66.86 7
CMA	40g	40 f	39 g	39.6 f	36 f	36 h	36 h	38.33 f	41 h	35 g	38.13 3
Prune agar	44.33 f	43 e	43 f	45 e	49 e	46 g	46 g	43 e	48 g	48 e	45.53 3
Yeast Extract	68.67 c	67 bc	70 a	69 a	69 a	69 b	70 b	69 b	68.67 bc	69.67 ab	691
V 5	59 e	54d	61 d	61 c	57 d	61e	62 d	60 cd	59 f	62 d	59.66 7
Wheat straw agar	64 d	68.67 b	60 d	61 c	61 c	66 c	51 f	59 d	64 e	69 b	62.36 7
PDA	70.33 b	56 d	66 b	58 d	65 b	67 c	47 g	44 e	63 e	69.67 ab	60.6
PCA	72 a	71 a	67 b	69 a	69 a	71 a	68.67 b	71 a c	17.67 i	41 f	61.73 4
WSEA	64 d	65.33 c	64 c	65 b	66 b	63 d	66 c	61 c	67 cd	67 c	64.83 3
WFA	71 ab	71.67 a	54 e	69.67 a	66 b	69 b	72 a	71 a	71.67 a	71 a	68.70 1
PSA	71.33 ab	71 a	39.6 7 g	70 a	71 a	71.67 a	59 e	69 b	65 de	70 ab	65.76 7
CV (%)	1.35	2.03	1.60	1.52	2.19	1.58	1.43	1.98	2.56	1.61	
LSD (0.05)	1.45	2.13	1.56	1.56	2.45	1.65	1.42	1.99	2.5	1.67	

Means with the same letter are not significantly different

Table 28. Comparative effect of culture media on rate of radial mycelial growth/day of ten isolates of *Magnaporthe oryzae triticum* causing wheat blast disease in Bangladesh at 4 DAI (Days After Inoculation)

Cultur media	ME MoT13	ME MoT14	ME MoT15	ME MoT16	MU MoT13	MU MoT14	MU MoT15	MU MoT16	CH MoT11	CH MoT12	Mean
OMA	13.83 a	13.75 a	13.33 a	12 a	12.25 a	12.08 bc	8.83 abc	10.8 c	13.75 a	13.50 a	12.42
CMA	4 i	4.50 i	4 g	4.75 g	4 d	3.50 h	3 e	5.25 h	3.50 h	4 g	4.05
Prune agar	5 h	5.25 h	6.25 f	6.25 f	8.33 c	7.75 f	6.50 cd	7.25 g	6.50 g	7.75 f	6.68
Yeast Extract	12 b	12.33 b	11.50 b	12.25 a	12.75 a	11.75 c	12 a bcd	12.25 a	11.50 b	12.50 b	12.08
V 5	10.75 e	9.50 e	8.75 d	8.75 d	9 c	8.75 e	8 bcd	9.50 e	9.25 d	10 d	9.22
Wheat straw agar	11.25 d	11.50 c	11.50 b	7.75 e	10.75 b	6.75 g	10.25 ab	11.75 b	7.50 f	11.25 c	10.02
PDA	13.50 a	8.50 g	6.50 f	11.25 b	11 b	12.75 a	4.75 de	8.75 f	10.25 c	11.25 c	9.85
PCA	10.25 f	11 d	10.25 c	11.33 b	9.25 c	12 bc	10.25 ab	10.75 c	3.75 h	2.75 h	9.15
WSEA	8.75 g	9f	8.58 d	9.75 c	9 c	7.75 f	9.50 abc	8.75 f	7.75 f	9 e	8.78
WFA	11.75 bc	10.75 d	10.25 c	11.50 b	11.75 ab	12.25 b	11.25 ab	10.25 d	11.17 b	11.42 c	11.23
PSA	11.58 cd	8.75 fg	7.75 e	7.92 e	9.25 c	9.50 d	9.75 abc	8.75 f	8.75 e	8.75 e	9.075
CV (%)	2.17	2.14	2.51	2.62	6.84	2.78	22.51	2.68	2.70	2.73	
LSD (0.05)	0.37	0.34	0.38	o.42	1.13	0.45	3.26	0.43	0.39	0.429	

Means with the same letter are not significantly different.

Table 29. Comparative effect of culture media on rate of radial mycelial growth/day of ten isolates of *Magnaporthe oryzae triticum* causing wheat blast disease in Bangladesh at 8 DAI (Days After Inoculation)

Culture media	ME MoT 13	ME MoT 14	ME MoT 15	ME MoT 16	MU MoT 13	MU MoT 14	MU MoT 15	MU MoT 16	CH MoT 11	CH MoT 12
OMA	8.96 ab	9.00 a	8.88 a	8.63 a	6.96 e	6.88 f	8.29 c	8.63 a	8.75 ab	8.63 b
CMA	5.04 g	5.00 f	4.88 g	4.96 f	4.50 g	4.50 h	4.50 h	4.79 e	5.13 h	4.38 g
Prune agar	5.54 f	5.38 e	5.38 f	5.63 a	6.13 f	5.75 g	5.75 g	5.38 d	6.00 g	6.00 e
Yeast Extract	8.59 c	8.38 bc	8.75 a	8.63 a	8.63 ab	8.63 b	8.75 b	8.63 a	8.58 bc	8.71 ab
V 5	7.46 e	6.83 d	7.54 d	7.54 c	7.13 e	7.63 e	7.75 d	7.33 c	7.38 f	7.75 d
Wheat straw agar	8.00 d	8.59 b	7.50 d	7.63 c	7.63 d	8.25 c	6.38 f	7.38 c	8.00 e	8.63 b
PDA	8.79 b	7.00 d	8.25 b	7.25 d	8.13 c	8.38 c	5.88 g	5.50 d	7.88 e	8.71 ab
PCA	9.00 a	8.88 a	8.38 b	8.63 a	8.58 b	8.88 a	8.58 b	8.88 a	2.21 i	5.13 f
WSEA	8.00 d	8.17 c	8.00 c	8.13 b	8.25 c	7.88 d	8.25 c	7.88 b	8.38 cd	8.38 c
WFA	8.88 ab	8.96 a	6.75 e	8.71 a	8.25 c	8.63 b	9.00 a	8.88 a	8.96 a	8.88 a
PSA	8.92 ab	8.88 a	4.96 g	8.75 a	8.88 a	8.96 a	7.38 e	8.63 a	8.13 de	8.75 ab
CV (%)	1.28	2.03	1.87	1.77	2.21	1.58	1.43	2.52	2.55	1.62
LSD (0.05)	0.17	0.12	0.23	0.23	0.28	0.21	0.18	0.32	0.32	0.23

Means with the same letter are not significantly different.

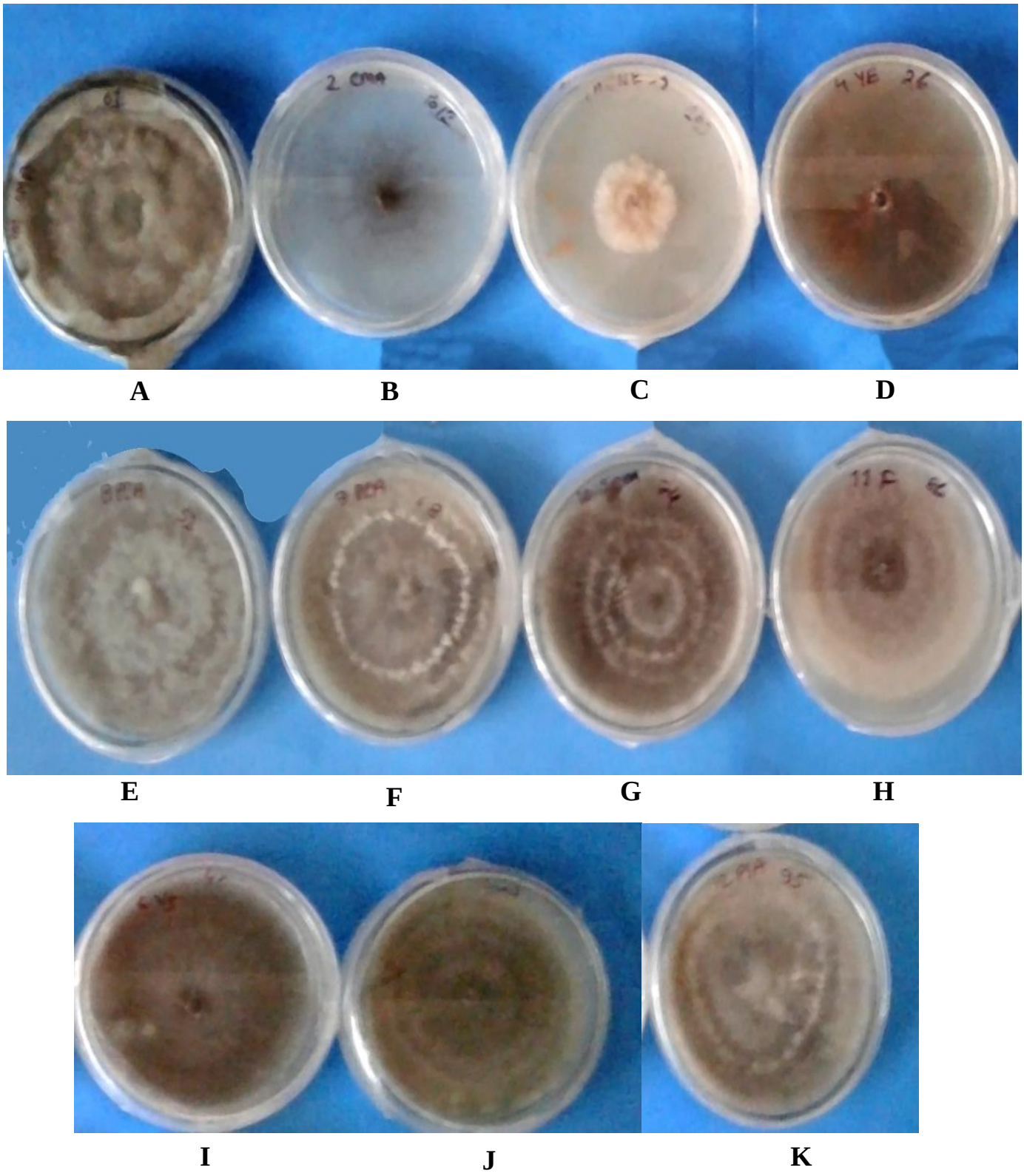

Figure 12. Mycelial growth of *M. oryzae triticum* isolate on eleven different culture media (A) OMA (B) CMA (CPrune agar (D) Yeast extract (E) PDA (F) PCA (G) Wheat seed polished agar (H) WFA (I) V5 agar (J) Wheat straw agar (K) PSA

DISCUSSION

In the present study, wheat blast prevalence and intensity varied among survey locations. Highest prevalence was recorded in Meherpur sadar upazilla (25%), then in Mujibnagar upazilla (15%) followed by Chuadanga sadar upazilla (5%). That results indicated that wheat blast disease was occurred in all survey sites. But the occurrence of wheat blast was varied from sites to sites. This may be due to geographic regional difference and difference in weather parameters such as temperature, relative humidity and light intensity. Cardoso *et al.* (2008) reported that the development of wheat blast disease is favored by higher temperature (18-30°C) coupled with high humidity (wetting of plants for at least 10 h due to rain or dew fall). Kohli *et al.* (2011) found that the disease may become an epidemic and devastate wheat crop within a week under most conducive temperature ranges of 8-30°C and at >80% RH during ear emergence or grain filling. Miah *et al*. (2014) showed that average winter temperatures were ranged from 18.5 ± 0.7°C in December to 21.0 ± 0.9°C in February in Bangladesh (from 1950 to 1998) favored rapid development of bleached spikes. In this study, variation on blast intensity was also found among cultivars that may be due to the difference of genetic makeup of the cultivar cultivated. Difference in wheat varietal reaction to spike blast is very common in the field condition. From the present study, it has been found that Prodip (BARI Gom 24) was highly susceptible to wheat blast in some villages but interestingly it has also been found that this variety is free from spike blast in some other villages though weather condition was similar in both of the villages. Soil edaphic factors, microclimate or presence or absence of isolates of different degrees of pathogenicity might be responsible for this different reaction in same variety in different locations. Islam *et al*. (2016) found the same results where they isolated 10 strains of wheat blast pathogen from infected samples. Among them only two strains such as BTJP 3-1 and BTJP 4-1 produced characteristic symptoms on artificially inoculated plants five days after inoculation. Though the blast incidence and severity in this study ranged from 22 to 100 and 28 to 100, Respectively, there were many wheat fields not infected by wheat blast in the same season. Considering all wheat fields in the survey region the prevalence of wheat blast is 5 to 25%. The reason behind this, that was probably weather condition, micro climate, soil factors, wind velocity and direction for

dissemination of MoT conidia or presence or absence of weed or alternate or collateral hosts in the field. Maciel *et al*. (2013) reported that conidia are considered the primary means for spread and infection of the peduncle and rachis. Proposed sources of MoT conidia include secondary hosts and crop residues. Castruagudin *et al*. (2016) reported that more than 50 species of poaceous plants serve as alternate host of MoT conidia. Wheat grain yield of healthy and blast panicle was also varied from location to location. Although BARI Gom 24 (Prodip) was more susceptible to blast pathogen than the variety BARI Gom 26 and BARI Gom 28, but it was found in this study that number of seed per panicle, seed weight per panicle and 1000 seed weight was higher in healthy panicle of BARI Gom 24 (Prodip) than blast affected panicle. This result is similar to Martinez *et al.* (2018). They showed that 1000 seed weight was significantly reduced by wheat blast. BARI Gom 26 and BARI Gom 28 produced less yield compared to BARI Gom 24. Similar study was conducted by Islam *et al*. (2018), they used fourteen varieties including eight old varieties (Sonalika, Kanchan, Sourav, Gourab, Shatabdi, Sufi, Bijoy, Prodip) and six newly released varieties (BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29 and BARI Gom 30) for evaluation. The higher grain yield was obtained due to the higher grains spike-1 (45 no.) and 1000 grain weight (36.6 g) and lower blast disease incidence. Among the varieties, the variety Gourab produced the highest yield (3395 kg ha-1) which was similar to the newly released variety BARI Gom 28 (3196 kg ha-1) and BARI Gom 30 (3134 kg ha-1). In this study, 15 MoT isolates identified and were morphologically characterized on three different growth media such as PDA, OMA and PSA. Later on , 10 isolates were characterized on eleven growth media such as OMA, CMA, Prune agar, Yeast extract agar, v5 agar, Wheat straw agar, PDA, PCA, Wheat seed polished agar, Wheat flour agar, PSA. From this study it was found that mycelial growth rate different significantly among isolates and among growth media used. ME MoT13 showed highest mycelial growth (55.3 mm) and MU MoT16 showed lowest mycelial growth (43.4 mm) on OMA at 4 DAI. On wheat flour agar, isolate ME MoT13 and ME MoT13 showed maximum mycelial growth (47 mm). Isolate ME MoT15 and MU MoT16 showed lowest mycelial growth (41.00 mm) at 4 DAI. Similarly, all isolates show different mycelial growth on different media. Isolates also showed different colony characteristics on different

media. Colony differences was also observed among the isolates on same media. Similarly, all isolates show different mycelial growth on same media. This may be due to different strains of the isolates, pathogenic variation of MoT, nutrient content in different media, temperature effect on media etc. Castruagudin *et al.* (2016) examined a representative subgroup of 30 isolates were grown on Corn Meal Agar (CMA), Malt Extract Agar (MEA), Oatmeal Agar (OA), Potato Dextrose Agar (PDA), and Synthetic Nutrient-poor Agar (SNA). They showed maximum mycelial growth was recorded on PDA. But in this study, other media including PDA and CMA were used. It was found highest mycelial growth on OMA. Similar results were also reported by Asfaha *et al.* (2015) that the OMA was suitable for growth of *Pyricularia oryzae*. Similarly, Kulkarni (1973) reported that among the solid and liquid medias, OMA was found to be good for the *P. oryzae* isolates. At 8 DAI, Mean mycelial growth of 10 isolates were maximum (69 mm) on yeast Extract agar media followed by 68.70 mm in wheat flour agar media, 66.87 mm on OMA media. It was observed in this study that growth of *Magnaporthe oryzae triticum* was maximum on yeast extract agar at 8 DAI. It was also observed that sufficient growth (68.70 mm) was occurred on wheat flour agar, which was not used previously by others.

CHAPTER 5
SUMMARY AND CONCLUSION

Wheat is the basis of important staple food grains in both developing and developed countries around the world. About 80% people of the word depend on wheat after rice. But now a day's wheat production is under threat dew to many diseases.

Wheat blast is relatively a new disease of wheat that first appeared in Brazil in 1985**.** Wheat blast, caused by a host genus- specific subgroup (*Triticum* pathotype or *Triticum* isolates) of *Magnaporthe oryzae*, is a devastating disease of wheat. It causes yield losses upto 100% in severe case.

The primary objectives of this study was to determine the wheat blast disease prevalence, incidence and severity on different wheat cultivars in the South western region of Bangladesh such as Meherpur, Mijibnagar and Chuadanga sadar upazilla and to characterize the wheat blast pathogen isolates collected from various fields of those areas. The results of this study will provide useful information for developing strategies for controlling wheat blast disease in field conditions in the future. This will have direct implications for food security in Bangladesh as well as South Asia.

The experiment was conducted in two different sections, Field experiment and lab study. The lab experiment was conducted in Plant Pathology Laboratory of Sher-e-Bangla Agricultural University during February 2018 to April 2019. The lab experiment was laid out in Completely Randomized Design (CRD) with three replications.

Initially, a survey was conducted in the southwestern wheat cultivators of Bangladesh during March 2019.The infected samples of wheat plants were collected from different agro climatic zones of Meherpur (Meherpur & Mujibnagar) and Chuadanga districts of Bangladesh. The highest incidence and severity was scored in BARI Gom-24 (Prodip). In some fields, yield losses were caused upto 100%. Some fields were burned which were cultivated Prodib. The lowest incidence and severity were found in BARI Gom 26 and Bari Gom 28. Lowest severity was 28% and incidence 26% in case of BARI Gom 28. In case of BARI Gom 26, minimum incidence and severity were 29% and 40%, respectively.

In vitro experiment was carried out in two different sections. At first section, 15 isolates were evaluated on three media viz. Potato dextrose agar, Potato sucrose agar and Oat meal agar. PDA showed ashy to white color, smooth to rough margin, good to medium growth, cottony surface mycelia. Highest mycelial growth in ME MoT18 and lowest growth was found in ME MoT15. On PSA, isolates were blackish color with medium growth, some isolates show smooth margin and cottony surface. Others showed rough margin and velvety surface. Most of the isolates on OMA showed whitish to grey color colony with smooth margin, medium growth. Highest mycelial growth and growth rate was recorded 39.50 mm and 13.17 mm at 3 DAI and 47.67 mm and 11.91 mm at 4 DAI in isolate MU MoT13. Low radial mycelial growth was found 31.67 mm and growth rate was 10.75 mm was observed in MU MoT17 at 4 DAI on OMA media. ME MoT13, ME MoT14, MU MoT 14, MU MoT15, CH MoT11 showed sufficient mycelial growth at both 3 DAI and 4 DAI. With regard to cultural variation, oat meal agar media supported maximum mycelial growth. There was no significant variation in shape of conidia. All the conidia were pyriform shape, hyaline to olive color. All the conidia of isolates were three celled and had two septation.

Later on, the different culture media was evaluated based on colony diameter to get suitable media for the growth of the *Magnaporthe oryzae triticum* isolate. Eleven culture media for evaluation of 10 isolates were used. Mean mycelial growth of isolates was maximum on OMA, it was 51.03mm followed by Yeast Extract Agar (44.57 mm) at 4DAI (days after inoculation). Lowest mean mycelial growth was found on CMA (16.2 mm) proceeded by Prune Agar (26.73 mm). PDA and Wheat Straw Agar media also showed good growth of mycelia, 40.47 mm and 40.1 mm, respectively at 4 DAI (days after inoculation).

At 8 DAI (days after inoculation), highest mycelial growth was recorded in ME MoT14 (72mm) on OMA and lowest mycelial growth was observed on CMA media. Mean mycelial growth and growth rate was also low in CMA media (38.13mm) proceeded by prune agar medium (45.53mm). The maximum mean value of radial mycelial growth and growth rate was found on Oat meal agar followed by Yeast extract agar and Wheat flour agar media.

Further studies have to be conducted to evaluate their resistance under green house and natural environmental conditions. To increase wheat production in the southwestern part of Bangladesh, blast disease can be managed by using resistant cultivars. Farmers and development agents should be trained in the management of wheat blast disease using resistant varieties. In order to further investigation, the differences among *Magnaporthe oryzae triticum*, more isolates should be collected from other geographical locations. In addition, the field research should also be expanded and linked to research on wheat blast management.

CHAPTER 6

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APPENDIX

Appendix-I: Map showing the sample collected region under study

Appendix-II (A): ANOVA for radial mycelial growth of 15 isolates on PDA at 3 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	22.59	16.32	53.36	0.0000
Error	30	9.1795	9.1795		
Total	44	237.7			

Appendix-II (B): ANOVA for radial mycelial growth of 15 isolates on PDA at 4DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	171.9	12.28	76.92	0.0000
Error	30	4.7917	0.1597		
Total	44	176.7861			

Appendix-III (A): ANOVA for radial mycelial growth rate of 15 isolates on PDA at 3 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	1914.80	136.77	45.59	0.0000
Error	30	90.0	3.00		
Total	44	2004.80			

Appendix-III (B): ANOVA for radial mycelial growth rate of 15 isolates on PDA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	1695.64	121.11	64.12	0.0000
Error	30	56.6667	1.8889		
Total	44	1752.3111			

Appendix-III (C): ANOVA for radial mycelial growth of 15 isolates on PSA at 3 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	212.68	15.1920	45.64	0.0000
Error	30	9.98	0.3329		
Total	44	222.67			

Appendix-III (D): ANOVA for radial mycelial growth of 15 isolates on PSA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	105.9778	7.5698	64.12	0.0000
Error	30	3.5417	0.1181		
Total	44	109.51			

Appendix-IV (A): ANOVA for radial mycelial growth rate of 15 isolates on PSA at 3 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	2309.244	164.946	3.54	0.0018
Error	30	1399.3333	46.6444		
Total	44	3708.5778			

Appendix-IV (B): ANOVA for radial mycelial growth rate of 15 isolates on PSA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	2509.4667	179.2476	4.08	0.0006
Error	30	1319.3333	43.9778		
Total	44	3828.8000			

Appendix-IV (C): ANOVA for radial mycelial growth of 15 isolates on OMA at 3 (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	255.0070	18.2148	3.39	0.0025
Error	30	161.3883	5.3796		
Total	44	416.3953			

Appendix-IV (D): ANOVA for radial mycelial growth of 15 isolates on OMA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	168.0361	12.0026	4.55	0.0002
Error	30	79.1250	2.6375		
Total	44	247.1611			

Appendix-IV (E): ANOVA for radial mycelial growth rate of 15 isolates on OMA at 3 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	14	168.0361	12.0026	4.55	0.0002
Error	30	79.1250	2.6375		
Total	44	247.1611			

Appendix-V (A): ANOVA for radial mycelial growth rate of 15 isolates on OMA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	427.3667	47.4852	12.61	0.0000
Error	20	75.3333	3.7667		
Total	29	502.7000			

Appendix-V (B): ANOVA for radial mycelial growth of 10 isolates of MoT on OMA at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	1069.4667	84.88	84.88	0.0000
Error	20	28.0000	1.4000		
Total	29	1097.4667			

Appendix-V (C): ANOVA for radial mycelial growth of 10 isolates of MoT on OMA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	52.5104	5.8345	1.16	0.3709
Error	20	100.7083	5.0354		
Total	29	153.2188			

Appendix-V (D): ANOVA for radial mycelial growth rate of 10 isolates of MoT on OMA at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	16.6775	1.8531	84.69	0.0000
Error	20	0.4376	0.0219		
Total	29	17.1151			

Appendix-VI (A): ANOVA for radial mycelial growth rate of 10 isolates of MoT on OMA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	184.8000	20.5333	34.22	0.0000
Error	20	12.0000	0.6000		
Total	29	196.8000			

Appendix-VI (B): ANOVA for radial mycelial growth of 10 isolates of MoT on CMA at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	129.4667	14.3852	15.98	0.0000
Error	20	18.0000	0.9000		
Total	29	147.4667			

Appendix-VI (C): ANOVA for radial mycelial growth growth of 10 isolates of MoT on CMA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	11.5500	1.2833	34.22	0.0000
Error	20	0.7500	0.0375		
Total	29	12.3000			

Appendix-VI (D): ANOVA for radial mycelial growth rate of 10 isolates of MoT on CMA at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	2.0201	0.2245	16.19	0.0000
Error	20	0.2772	0.0139		
Total	29	2.2973			

Appendix-VII (A): ANOVA for radial mycelial growth rate of 10 isolates of MoT on CMA at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	511.2000	56.8000	54.97	0.0000
Error	20	20.6667	1.0333		
Total	29	531.8667			

Appendix-VII (B): ANOVA for radial mycelial growth of 10 isolates of MoT on Prune agar at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	136.8000	15.2000	12.32	0.0000
Error	20	24.6667	1.2333		
Total	29	161.4667			

Appendix-VII (C): ANOVA for radial mycelial growth of 10 isolates of MoT on Prune agar at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	31.9500	3.5500	54.97	0.0000
Error	20	1.2917	0.0646		
Total	29	33.2417			

Appendix-VII (D): ANOVA for radial mycelial growth rate of 10 isolates of MoT on Prune agar at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	2.1387	0.2376	12.30	0.0000
Error	20	1.2917	0.0646		
Total	29	2.5251			

Appendix-VIII (A): ANOVA for radial mycelial growth rate of 10 isolates of MoT on Prune agar at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	74.0000	8.2222	11.21	0.0000
Error	20	14.6667	0.7333		
Total	29	88.6667			

Appendix-VIII (B): ANOVA for radial mycelial growth of 10 isolates of MoT on yeast extract agar at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	20.0000	2.2222	2.47	0.0443
Error	20	18.0000	0.9000		
Total	29	38.0000			

Appendix-VIII (C): ANOVA for radial mycelial growth of 10 isolates of MoT on yeast extract agar at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	4.6250	0.5139	11.21	0.0000
Error	20	0.9167	0.0458		
Total	29	5.5417			

Appendix-VIII (D): ANOVA for radial mycelial growth rate of 10 isolates of MoT on yeast extract agar at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	0.3100	0.0344	2.43	0.0473
Error	20	0.2839	0.0142		
Total	29	0.5939			

Appendix-IX (A): ANOVA for radial mycelial growth rate of 10 isolates of MoT on yeast extract agar at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	254.7000	28.3000	31.44	0.0000
Error	20	18.0000	0.9000		
Total	29	272.7000			

Appendix-IX (B): ANOVA for radial mycelial growth of 10 isolates of MoT on V5 agar at 8 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	148.0000	16.4444	6.76	0.0002
Error	20	48.6667	2.4333		
Total	29	196.6667			

Appendix-IX (C): ANOVA for radial mycelial growth of 10 isolates of MoT on V5 agar at 4 DAI (Days After Inoculation)

Source	Degree of Freedom	Sum of Square	Mean Square	F Value	P Value (> F)
treat	9	15.9187	1.7687	31.44	0.0000
Error	20	1.1250	0.0562		
Total	29	17.0437			