SURVEY ON WHEAT BLAST AND MORPHOLOGICAL VARIABILITY OF *MAGNAPORTHE ORYZAE TRITICUM*

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SURVEY ON WHEAT BLAST AND MORPHOLOGICAL VARIABILITY OF *MAGNAPORTHE ORYZAE TRITICUM*

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CERTIFICATE

*This is to certify the thesis entitled "SURVEY ON WHEAT BLAST AND MORPHOLOGICAL VARIABILITY OF MAGNAPORTHE ORYZAE TRITICUM" submitted to the department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (MS) in PLANT PATHOLOGY**, embodies the result of a piece of bona fide research work carried out by Tanjina Akter, Registration No. 12-05031 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation have duly acknowledged by author.

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SURVEY ON WHEAT BLAST AND MORPHOLOGICAL VARIABILITY OF *MAGNAPORTHE ORYZAE TRITICUM*

ABSTRACT

Wheat blast caused by *Magnaporthe oryzae triticum is* a serious disease of wheat causing high yield losses which has become a critical problem for wheat production in Bangladesh. A survey was conducted in eight sites (village) of four upazila under two districts of southwestern region of Bangladesh during November 2017 to March 2018. A total of 500 bleached spike samples were collected. Disease incidence and severity varied among collection sites. The highest disease incidence (43.00%) was recorded in Chithlia of Mirpur and the lowest disese incidence (15.00%) was recorded in Bollobpur of Mujibnagar. The highest disease severity (20.00%) was recorded in Dhanakkhula of Gangni and the lowest disease severity (10.00%) was recorded in Chithlia and Amla of Mirpur, Bollobpur and Doriapur of Mujibnagar. Diseased and bleached spikes were collected, dried and kept in brown paper envelope and maintained in 4°C temperature for the study. *Magnaporthe oryzae triticum* isolates were isolated on Potato dextrose agar and Oat meal agar media and identified based on 3 celled pyriform conidia and cultural and microscopic characteristics. Eight isolates has been isolated from eight sites and cultured on Potato dextrose agar and Oat meal agar. Cultural characteristics of eight isolates differed among the collection sites. Mycelial growth rate/day of eight isolates ranged from 2.17 mm to 3.58 mm on PDA and 1.70 mm to 5.99 mm on OMA. Collected Isolates varied based on their sporulation from poor to excellent in microscopic field. Sporulation of *Magnaporthe oryzae triticum* isolates on PDA media is rare.

CHAPTER I

Introduction

Wheat (*Triticum aestivum*) is the second most important cereal crop after rice in Bangladesh. It has optimistic impact on the national economy. It provides 20% of the total food calories and is staple food for nearly 40% of the world population. It produces 93% of total cereal production in Bangladesh (BBS, 2014). However, during the last decade, wheat consumption has been increasing rapidly and it was almost doubled to six million MT in 2016. Fungal diseases outbreak has become a great threat to world food security (Fisher *et al.*, 2012). For example, blast disease of rice, wheat and other grasses can destroy enough food supply (Fisher *et al.*, 2012; Pennisi, 2010; Liu *et al.*, 2014).

In South America, wheat blast is caused by isolates of *Magnaporthe oryzae* (synonymn *Pyricularia grise*a) known as (Urashima *et al.*, 1993; Kato *et al.*, 2000; Tosa *et al.*, 2006). The rice infecting isolates of *Magnaporthe oryzae* are genetically different from wheat infecting isolates and generaly do not infect each other (Prabhu *et al.*, 1992; Urashima *et al.*, 1993; Urashima *et al.*, 1994; Farman 2002; Faivre Rampant *et al.*,2008).

Wheat blast was first reported in Parana province of Brazil in 1985 (Igarashi *et al.*, 1986). But first epidemic was reported in the year of 1996 in Bolivia (Barea and Toledo, 1996) and then in Paraguay, then in Argentina in 2002 and 2007. It has caused about 70–80% wheat production loss (Alberione *et al.*, 2008; Viedma and Morel, 2002).

There are still some regions in South America where wheat is not cultivated because of the potential threat of this disease (Callaway, 2016). As mentioned above, no case was reported outside of South America except one in the experimental field in USA in 2011 (Callaway, 2016).

But unfortunately, wheat blast disease was spotted in Bangladesh in 2016 and this was the first occurrence in the Asia outside America (Callaway, 2016).

Recent outbreak proved the predictions of International Maize and Wheat Improvement Center (CIMMYT) experts that wheat blast can be spread to Asia and Africa from disease existing countries because of similar climatic conditions in these regions (CIMMYT, 2016). India, Pakistan, and China rank third, seventh, second in the world wheat production respectively (Index Mundi, 2016). Plant pathologists from Wheat Research Center (WRC) of Bangladesh also warned that this disease has the chance to spread to India, Pakistan, and China due to favourable environment for disease development. But in early 2016, this prediction has become true. Wheat blast disease appeared for the first time in the middle of February of 2016 in Chuadanga, Meherpur and Kustia districts and rapidly spread to adjacent four districts within fifteen days (http://en.prothom-alo.com/bangladesh/ news/102091/).

The recent report also indicated the high risk of wheat production throughout the Bangladesh and in neighbor countries. Because blast disease also found in other region which is quite far from the first spotted place (Barisal and Bhola districts) (Islam *et al.*, 2016).

Morphological characters *viz*., size and shape of conidia are pyrifom, almost hyaline to pale olive, 2-septate and 3 celled (Hossain, 2000).

The present research work has conducted with the following objectives:

1) To survey on incidence and severity of wheat blast in some selected sites of southwestern wheat growing region of Bangladesh.

2) To study the morphological variation and cultural characteristics of *Magnaporthe oryzae triticum* grown on two different media.

CHAPTER II

Review of Literature

In 1985, a blast pathogen which was a major fungal foe of rice was identified in Parana state of Brazil on wheat. Wheat blast causes enormous loss to wheat production. Further it has also been reported from Uruguay, Paraguay, some parts of Argentina. Slowly it has spread to other South American countries. The biggest shock for wheat cultivation was occurrence of blast on wheat in Bangladesh during February 2016 outside of America. It is an alarming for wheat cultivation in tropical and sub-tropical areas in the world.

2.1. Survey on disease incidence and severity and collection of different isolates of *Magnaporthe oryzae triticum*

Department of Agricultural Extension (DAE) informed that the infected area was about 15,000 ha, which correspond to 3.5% of total wheat fields in Bangladesh. Wheat blast was observed in eight southwestern districts, viz., Pabna, Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal and Bhola. The severity of wheat blast disease varied among districts.

Malaker *et al.* (2016) reported that the highest percentage of infected wheat blast fields was observed in Meherpur (70 %) followed by Chuadanga (44 %), Jessore (37 %), Jhenaidah (8 %), Bhola (5 %), Kushtia (2 %), Barisal (1 %) and Pabna (0.2 %). The infected wheat fields were burned, which causes 15% decrease in wheat production of the eight infected districts.

Islam *et al.*, (2016) stated that yield losses in different affected districts varied. The highest yield loss was reported in Jhenaidah (51%) followed by Chuadanga (36 %), Meherpur (30 %), Jessore (25 %), Barisal (21 %), Pabna (18 %), Kushtia (10 %) and Bhola (5 %).

A disease surveillance program on wheat blast was organized in collaboration with CIMMYT and CU, USA in mid February 2017. Out of 103 surveyed sites, 33 sites were found infected with wheat blast. Overall disease incidence in 2017 was comparatively lower than the previous season with low disease severity (5-10%). (https://www.globalrust.org/content/wheat-disease-surveillance-and-Monitoring-bangladesh).

2.2. Symptomatology

Prabhu *et al*. (1992) stated that the typical symptom of wheat blast is the head infection, which is common and destructive in the field. It consists of blighting of immature spikelets. Infection of the rachis blocks the translocation of photosynthates to the part of the spike above the point of necrosis, resulting in partial or total sterility of the spike. The kernels of the affected spikelets are often shrivelled.

Urashima and Kato (1994) reported that the affected spikelets above the infection point exhibit a bleached straw color, which can be easily distinguished from normal green color of the healthy spikelets. On the leaves, the symptoms of wheat blast are elliptical to elongate lesions with light to dark green centers and yellow borders. However, the occurrence of disease symptoms on the leaves or stems before the heading stage is rare.

2.3. Isolation

Padmanabhan *et al.* (1970) isolated *Magnaporthe grisea triticum* from samples of diseased leaves, necks, and nodes of the infected rice plant on oat meal agar (OMA) with traces of biotin and thiamine. Single spore isolates were grown and multiplied on OMA + Biotin and Thiamine at 25^{o}c.

Xia *et al.* (1993) stated that collected panicles with the symptoms of blast was washed once with sterile distilled water, and placed on Moist filter paper in Petridishes at room temperature to induce sporulation. Conidia from the lesion surface of rachis were spread onto 3% water agar with a sterile loop and incubated overnight. Single conidium was isolated from culture and transferred to potato dextrose agar.

2.4. Morphological Studies

The causal agent of rice blast in Japan and Taiwan have been described by Nishikado (1926), as follows : “Mycellium in cultures aerial or submerged, hyaline or olivaceous, 1.5 – 6.0 µm in width, septate branched, conidiophores 1 to many, fasciculate, simple or rarely branched, 2 – 4 septate, not or slightly constricted at septa; at first Monosporic, then pleurogenous on sympodium, olivaceous to fuliginous, base swollen, dark coloured and becoming lighter colour towards the apex”.

Conidia variable in size and shape, terminal, pyriform to obelavate, base rounded, apex narrowed: 2 septate, rarely 1–3 septate not or slightly constricted at septa, almost hyaline to pale olive, 14–40 x 6–13 µm in size, usually 19–23 x 7–9 µm, with small basal appendage. Basal appendage 1.6 – 2.4 µm (2 µm) in width; basal cell 4.8–10 µm (6.3µm), middle cell 4.8 – 12.8 µm (7.8 µm), apical cell 4.8 – 10.7 µm (7.4µm). Conidia germinate from apical or basal cell and less frequently from middle cell. Germinating hyphae hyaline, not or constricted at septa, branched, 3 – 5 µm in width”.

Nishikado (1917) described morphology of *P. grisea* spores which measured 16 – 33 x 5 – 9 µm. Usually 22 –27 x 7 – 8 µm with a small basal appendage, other dimensions were, basal appendage 1.2 – 1.8 (1.6) µm in width, basal cell 4.8 – 11.5 (7.8µm), middle cell 1.8 – 11.5 (6.6 µm), apical cell 6 – 14 µm in length. The *Pyricularia* forms on other plants do not differ distinctly from the rice fungus in size of conidia.

Nishikado (1926) described the conidia of the *Pyricularia* on crab grass as somewhat slender. He classified 39 isolates into nine forms on the basis of cultural characteristics. On steamed rice straw, the conidia of the isolates belonging to four forms were short, the mean value ranged from 19.3 to 22.8µm. The conidia of other five forms were long, the mean value ranged from 26.8 to

29.9µm. All isolates from the affected spikes or glumes of rice plants were of the long conidium type, while Most isolates from the nodes were of the short conidium type. This suggests considerable difference in the length of conidia among the isolates of *Pyricularia* on rice.

Aoki (1955) measured 16 isolates in potato dextrose agar culture and showed that, the average length of the isolate ranged from 21.2 to 28.4µm, and the average width from 7.3 to 9.0µm. The size of conidia of *P. grisea* varied with the culture media also.

Hossain (2000) observed mycelium in cultures was first hyaline in colour, then changed to olivaceous, 1 – 5.2 µm in width, septate and branched. The spore measurements were 15 – 22 µm x 4 – 7 µm (Average, 17.4 µm x 5.2µm).

CHAPTER III

Materials and Methods

The laboratory experiment was conducted in Plant Pathology laboratory of Sher-e-bangla Agricultural University. The details of the materials and methodology adopted during this investigation are described here under.

3.1. Survey Area

A survey was conducted in southwestern wheat growing regions *viz.* Kustia (Doulatpur, Mirpur) at latitude 23^{0}55'N, longitude 89^{0}13'E and Meherpur (Gangni, Mujibnagar) at latitude 23^{0}45'N and longitude 88^{0}42'E of Bangladesh. (Figure 1).

3.2. Survey period

Survey was made to the selected area for the collection of wheat blast infected sample during November 2017 to March 2018.

3.3. Experimental site

The experiment was conducted in the Laboratory of the Department of Plant Pathology, Sher-E-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh.

3.4. Collection of wheat blast samples

Wheat blast samples were collected from different wheat growing regions of Kustia (Doulatpur, Mirpur) and Meherpur (Gangni, Mujibnagar). The spikes, stems and leaves were collected wherever the symptoms were seen. In each field, three random spots of ($2×2m^2$) were selected for survey and sampling (Meena, 2005). After collection, samples were sundried and kept in envelop and taken to the laboratory. These samples were kept in 4^{o}ctemperature in the refrigerator.

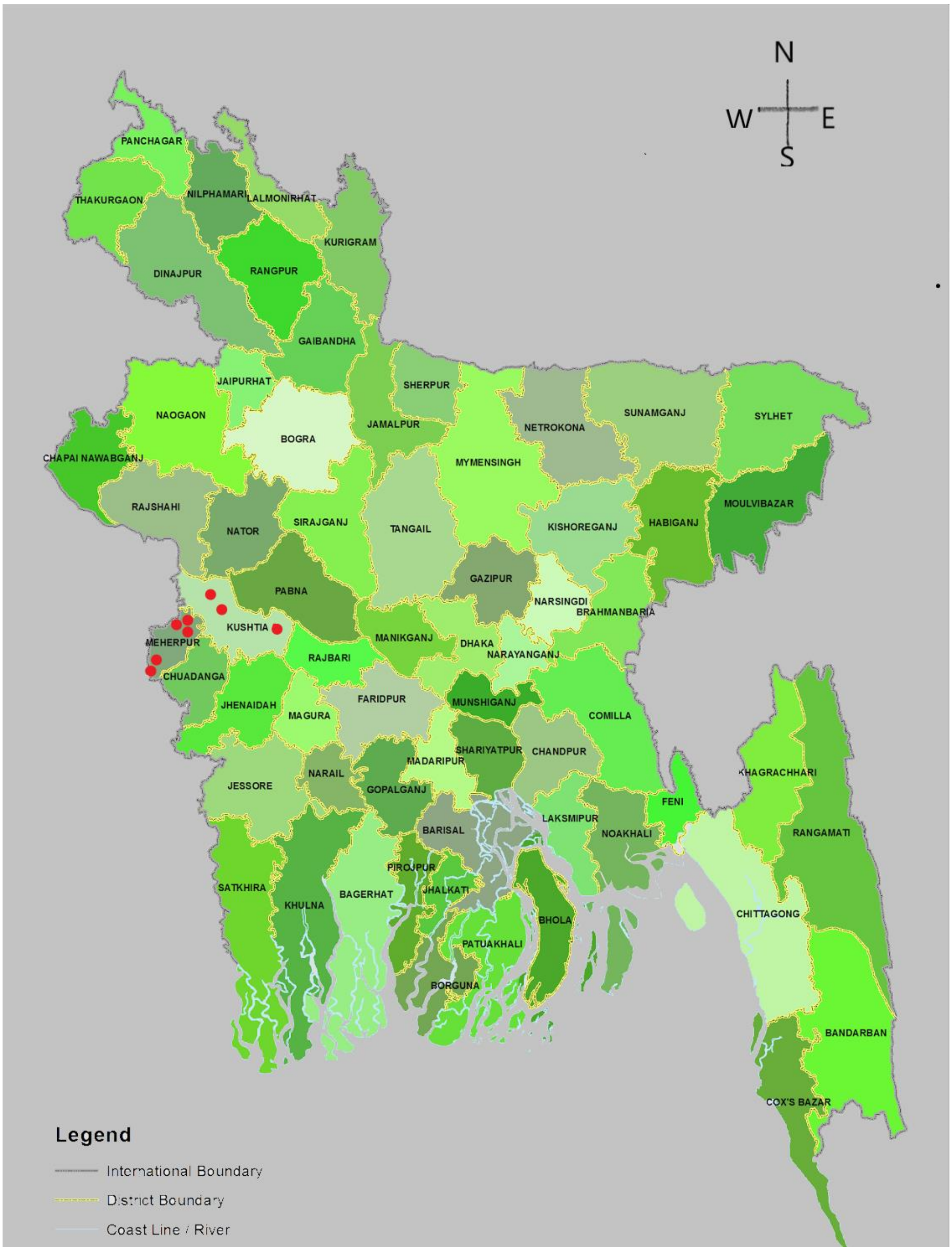

Figure 1. Survey area(●)of wheat blast in Bangladesh

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

3.5. Determination of disease incidence, severity, yield loss and economic loss

Disease incidence was assessed using the following formula of Waller *et al.*, 2002.

$$\text{Disease incidence (\%DI)} = \frac{\text{Total number of infected plant in field}}{\text{Total number of plants assessed}} \times 100$$

A common formula as follows is generally used to calculate disease severity.

$$\text{\% spike area diseased} = \frac{\text{Spike area Diseased}}{\text{Total area}} \times 100$$

Yield and economic loss were estimated initially by randomly throwing standard ($2\times2m^2$) quadrat in each varietal plot maintaining three replicates and harvesting all the plants within the quadrat. Later on, the data was transformed into kg ha-1. Yield loss and economic loss assessment was calculated by comparing the grain yield and market price difference of grain yield of each variety in diseased and disease free plots.

3.6. Isolation of *Magnaporthe oryzae triticum*

The cultures of *Magnaporthe oryzae triticum* used throughout the investigation were isolated from blast infected plant parts of different varieties collected from the farmer's field of different agro climatic regions of Kustia (Doulatpur, Mirpur) and Meherpur (Gangni, Mujibnagar) of Bangladesh during November 2017 to March 2018. The infected leaves, neck and nodal portion were cut longitudinally with the help of sterilized knife and washed well with running tap water. These bits were surface sterilized with 1:1000 mercuric chloride ($Hgcl_2$) solution for 30 sec and washed three times in distilled water before transferring them to oat meal agar plates.

The composition and procedures for preparation of the media used in this experiment were followed as explained by Tuite (1969). In oat meal agar (Oat flakes 30g, Agar – agar 20.0 g, Distilled water (to make up) 1000.00 ml). First oat flakes were boiled with 500 ml of distilled water for 30 min. and filtered through muslin cloth. Agar was melted in 500 ml water separately. Both the solutions were mixed thoroughly and sterilized. They were incubated at 28 ± 1^{o}c for two weeks.

In Potato dextrose agar (Potato 200 g, Dextrose 20 g, Agar – Agar 20.0 g, distilled water (to make up) 1000 ml, autoclaved at 121^{o}c, 15 PSI, 15 min). First, potatoes were peeled off and cut into small pieces. Then they were boiled and extract was filtered through muslin cloth. The dextrose was dissolved in the solution and sterilised. Eight isolates were grown on oat meal agar and colony morphology was observed.

3.7. Monoconidial isolation

The bleached wheat spikes were surface sterilized with mercuric chloride ($Hgcl_2$) and then washed serially with sterile double distilled water and allowed for sporulation on sterilized glass slides by incubating in a moist chamber at 28^{o}C for 48 h. Well sporulated spikes were placed in the test tubes containing in sterile distilled water and vortexed for 1 min. About 1 ml of spore suspension was poured to sterilized plates and 2% agar was added. Single spore was located and picked up microscopically. Each spore was eventually transferred to potato dextrose agar media. The slants were incubated at 28^{o}C for 2 days.

3.8. Pathogenecity test

Inoculation with eight *Magnaporthe oryzae triticum* isolates was individually performed using the three leaves stage of wheat seedlings (3 weeks old). After harvesting conidia from fungal cultures on oat meal agar using sterile distilled water, the concentration was adjusted to 1×10^{5} conidia / ml. Three seedlings were sprayed with 20 ml of conidial suspension using sprayer and covered by plastic bags to keep 100% relative humidity. The inoculated seedlings were kept in a growth chamber at 25^{o}C, 16 hr/ 8 hr light / dark and 80 % relative humidity. Lesion types reflecting disease severity were assessed 10 days after inoculation.

3.9. Maintenance of isolates *Magnaporthe oryzae triticum*

The fungus was subcultured on Oat meal agar media and kept at 26±1^{O}C for 15 days. Subsequent, subculturing of isolates was done at an interval of 20 days. Such isolates were stored in a refrigerator at 5^{0}C and revived monthly.

3.10. Cultural characterisation

The *Magnaporthe oryzae triticum* isolates were grown on PDA and OMA. Radial mycelial growth and cultural characteristics such as colony color and colony shape were determined on 15 days after inoculation.

3.11. Morphological characterisation of conidia

A microscopic slide was prepared from the infected host tissue and was mounted in lactophenol on a clean slide. Spores were mixed with lactophenol, a uniform spread is obtained and then a cover slip was placed over it. No. of spore/ microscopic field was counted. Photographs were taken to show the typical spore morphology of the pathogen.

Sporulation was determined by a rating system described by Meena (2005).

Sporulation Type	No. of spores/ microscopic field	Index
Excellent	>30	4
Good	20-30	3
Fair	10-20	2
Poor	<10	1
Nil	0	0

3.12. *In vitro* experimental design

The experiment was conducted *invitro* in the plant pathology lab, SAU. Experiment was laid out in Completely Randomized Design (CRD) with 3 replications.

3.13. Statistical analysis of data

The data was analyzed by using the “R” Software (R Core Team, 2018). The mean value was compared according to LSD range test at 5% level of significance.

CHAPTER IV

Results and Discussion

4.1. Survey on incidence and severity and collection of bleached spikes of wheat

A survey was conducted during November 2017 to March 2018, for the incidence of blast disease in different wheat growing regions of Kustia (Doulatpur, Mirpur) and Meherpur (Gangni, Mujibnagar) of Bangladesh (Table 1). The spikes, stems and leaves blast infected samples were collected wherever the incidence was seen. Survey was conducted in 8 sites of 4 upazila belong to 2 districts namely Kustia and Meherpur of northwestern wheat growing region of Bangladesh. During survey three wheat varieties *viz*. BARI GOM 21 (Prodip), BARI Gom 26 and BARI Gom 28 were found to be infected by wheat blast. A total of 470 samples were collected for isolation of *Magnaporthe oryzae triticum.*

4.2. Symptomatology

Leaf blast symptoms

Magnaporthe oryzae triticum pathotype created lesions and dark grey spots on a mature leaf. Typical eye-shaped lesions with light grey centres, and dark brown margin was observed on a severely blast infected wheat leaf.

Head symptoms

Blast affected wheat spikes had typical bleached head symptom from the point of infection. Most of the spikes become completely bleached and grey color from blast infection at the neck of the spike (Figure 2).

Table 1. Survey area and wheat blast infected land area of eight sites of southwestern wheat growing region of Bangladesh

District	Upazila	Village	Land (Decimals)	Plant part	Variety	No. of sample collected
Kustia	Doulat Pur	Kalidas pur	40.00	Spike, Stem, Leaf	BARI Gom-21(Pradip)	50
Kustia	Mirpur	Chithlia	80.00	Spike, Leaf, Stem	BARI Gom-21(Pradip)	70
Kustia	Mirpur	Amla	120	Spike, Leaf, Stem	BARI Gom-28	75
Meherpur	Gangni	Sahar Bati	80.00	Spike, Leaf, Stem	BARI Gom-26	60
Meherpur	Gangni	Dhanak Khula	80.00	Spike, leaf, stem	BARI Gom-28	50
Meherpur	Gangni	Tentul Baria	40.00	Spike, Leaf, Stem	BARI Gom-28	50
Meherpur	Mujib Nagar	Bollob Pur	120	Spike, Leaf, Stem	BARI Gom-28	55
Meherpur	Mujib Nagar	Doriapur	80.00	Spike, Stem	BARI Gom-28	60
2	4	8	640		3	470

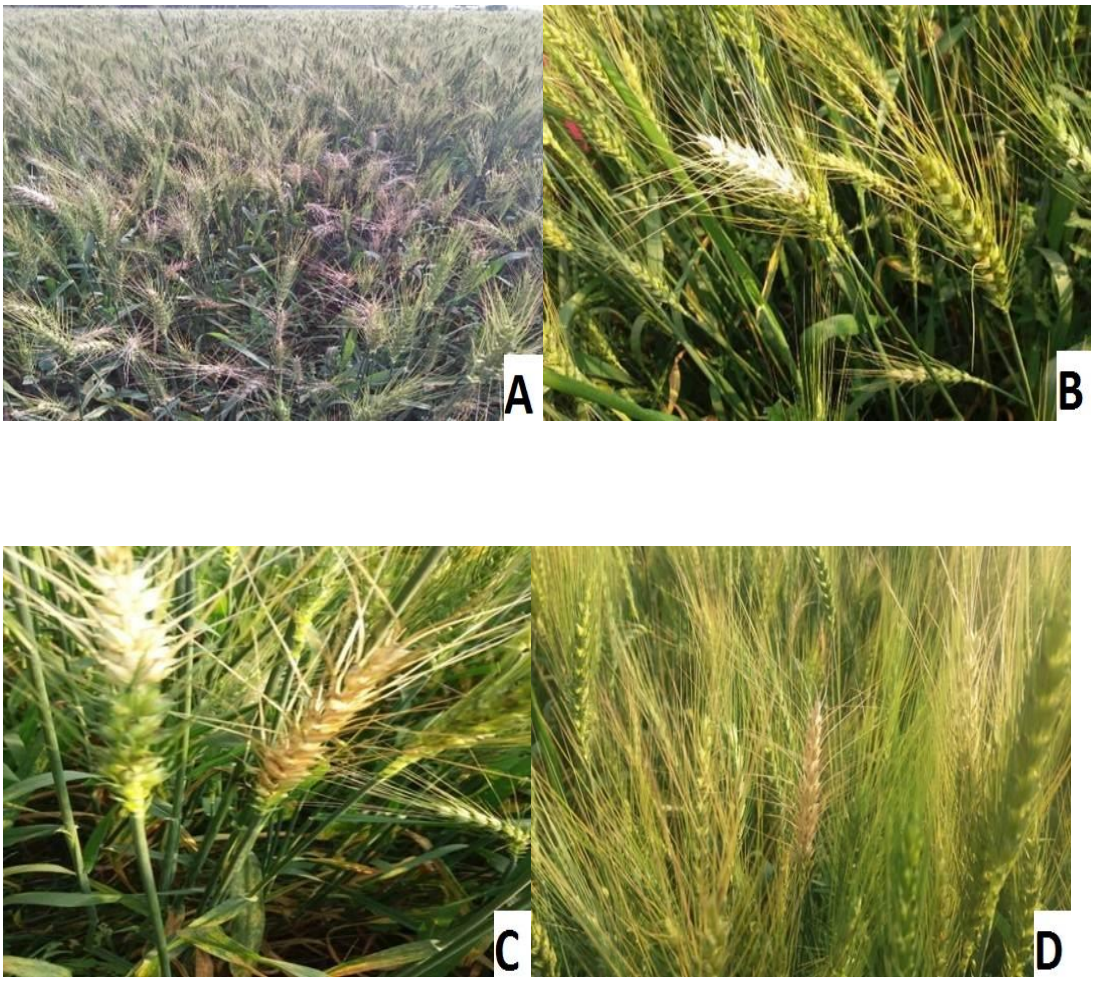

Figure2. Wheat blast Symptoms; Infected field (A) and Bleached spike of spikelets (B, C and D).

Figure 3. Typical eye shaped lesions on stem (A), Infected rachis (B), Infected glumes (C) and Infected seeds (D).

Blast incidence among collection sites ranged from 15% to 43% where the highest blast incidence (43.00%) was recorded in Chithlia (Mirpur, Kustia) and lowest disease incidence (15.00%) was recorded in Bollobpur (Mujibnagar, Meherpur) (Figure 4).

The highest disease severity (20.00%) was observed in Dhanakkhula (Gangni, Meherpur) and the lowest disease severity (10.00%) was observed in Chithlia, Amla (Mirpur, Kustia) and Bollobpur, Doriapur (Mujibnagar, Meherpur) (Figure 5).

Yield loss varied from location to location where the highest yield loss (66.66%) was recorded in Dhanakkhula (Gangni, Meherpur) and the lowest yield loss (30.55%) was recorded in Bollobpur (Mujibnagar, Meherpur) (Figure 6).

The highest economic loss (63.85%) was observed in Dhanakkhula (Gangni, Meherpur) and the lowest economic loss (25.75%) was recorded in Bollobpur (Mujibnagar, Meherpur) (Figure 7).

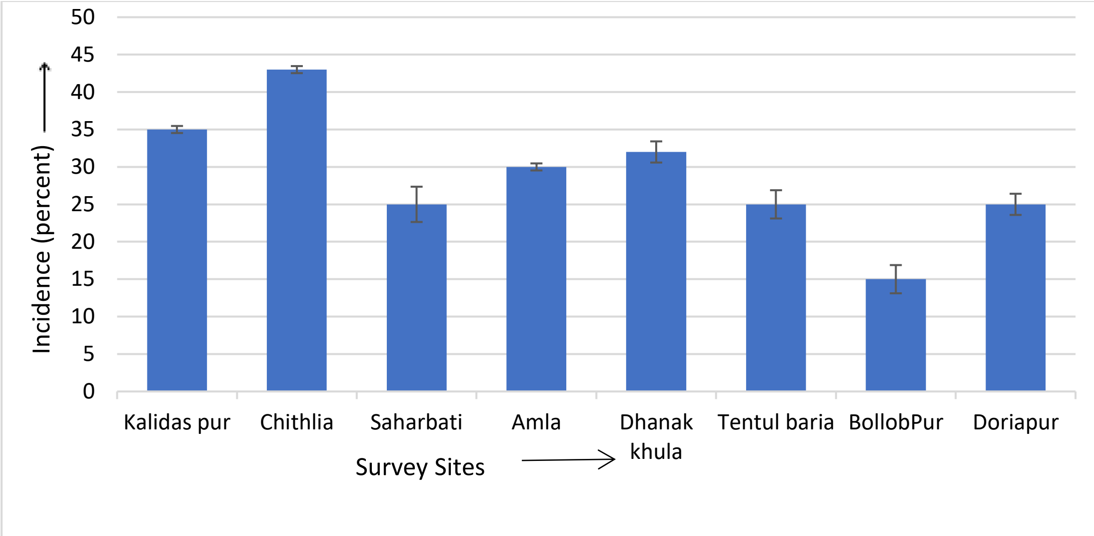

Figure 4. Incidence of wheat blast in eight sites of Bangladesh. a. Kalidaspur, Doulatpur, Kustia; b. Chithlia, Mirpur, Kustia; c. Amla, Mirpur, Kustia; d. Saharbati, Gangni, Meherpur; e. Dhanakkhula, Gangni, Meherpur; f. Tentulbaria, Gangni, Meherpur; g. Bollobpur, Mujibnagar, Meherpur; h. Doriapur, Mujibnagar, Meherpur.

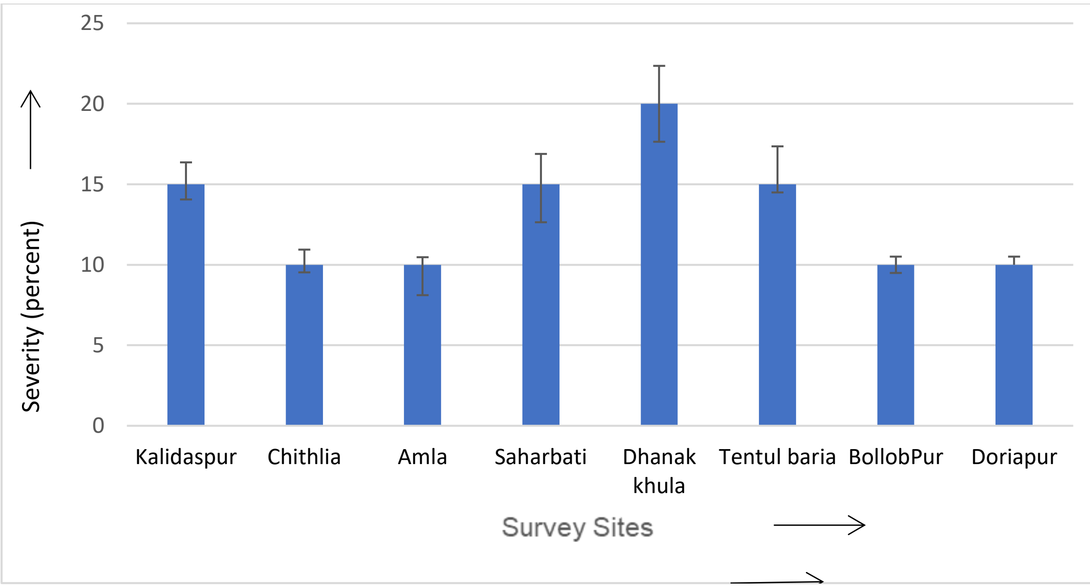

Figure 5. Severity of wheat blast in eight sites of Bangladesh. a. Kalidaspur, Doulatpur, Kustia; b. Chithlia, Mirpur, Kustia; c. Amla, Mirpur, Kustia; d. Saharbati, Gangni, Meherpur; e. Dhanakkhula, Gangni, Meherpur; f. Tentulbaria, Gangni, Meherpur; g. Bollobpur, Mujibnagar, Meherpur; h. Doriapur, Mujibnagar, Meherpur.

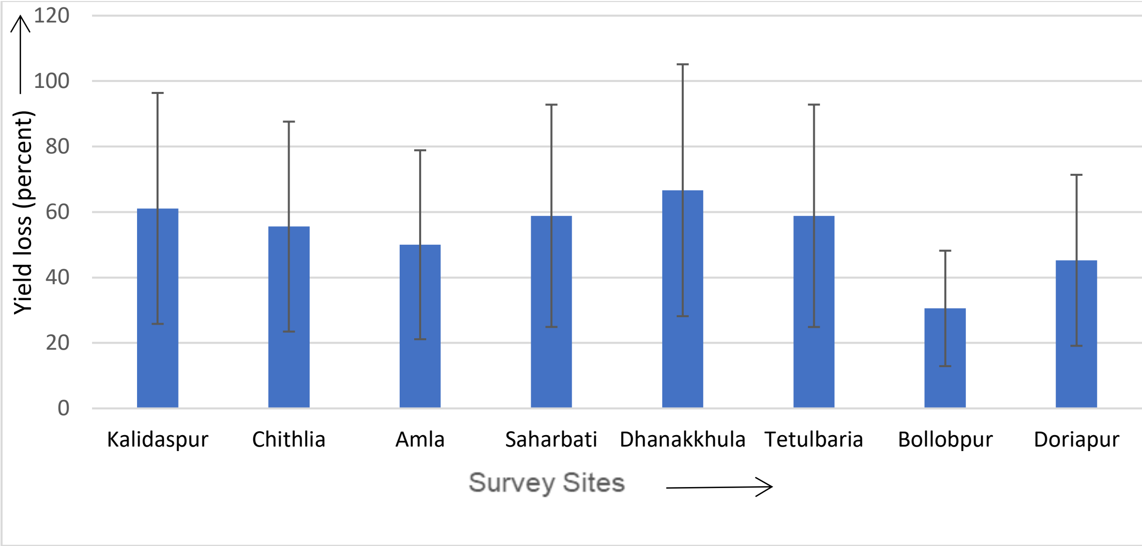

Figure 6. Yield loss of wheat blast in eight sites of Bangladesh. a. Kalidaspur, Doulatpur, Kustia; b. Chithlia, Mirpur, Kustia; c. Amla, Mirpur, Kustia; d. Saharbati, Gangni, Meherpur; e. Dhanakkhula, Gangni, Meherpur; f. Tentulbaria, Gangni, Meherpur; g. Bollobpur, Mujibnagar, Meherpur; h. Doriapur, Mujibnagar, Meherpur.

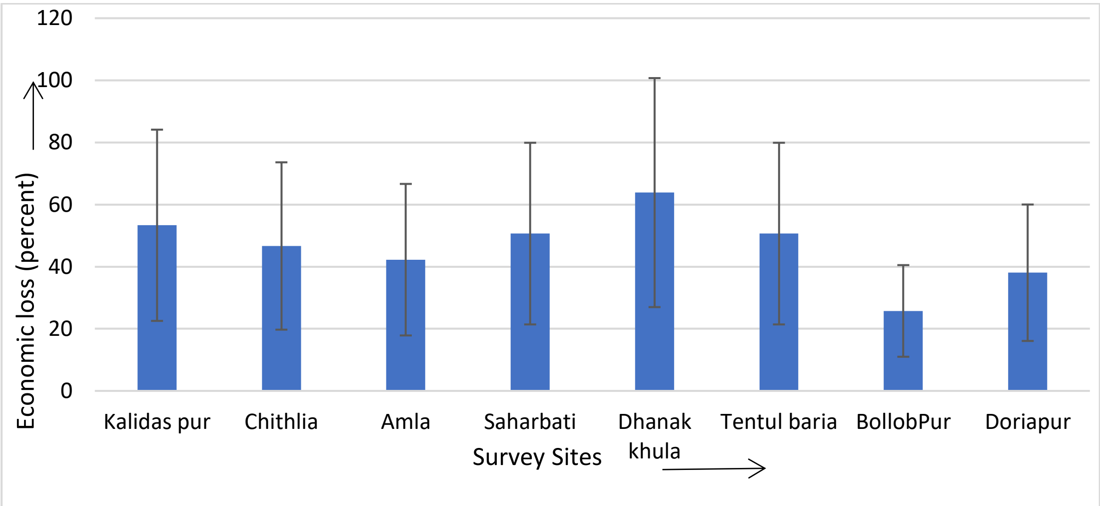

Figure 7. Economic loss of wheat blast in eight sites namely a. Kalidaspur, Doulatpur, Kustia; b. Chithlia, Mirpur, Kustia; c. Amla, Mirpur, Kustia; d. Saharbati, Gangni, Meherpur; e. Dhanakkhula, Gangni, Meherpur; f. Tentulbaria, Gangni, Meherpur; g. Bollobpur, Mujibnagar, Meherpur; h. Doriapur, Mujibnagar, Meherpur.

4.3. Cultural characteristics of *Magnaporthe oryzae triticum* isolates

Magnaporthe oryzae triticum was cultured on Potato dextrose agar and Oat meal agar and the result on mycelial growth and colony characters were shown presented in Table 2.

4.3.1. Mycelial growth on oat meal agar

Mycelial growth on OMA ranged from 15.2 mm to 25.1 mm at 15 days interval where the highest mycelial growth was recorded in MoT 2 isolate. The lowest mycelial growth was recorded in MoT 4 isolate. Colony colour of all the isolates was usually Ash, coloured with good growth whereas, MoT 1 and MoT 8 showed greyish black colour irregular whitish margin (Plate1).

4.3.2. Mycelial growth on potato dextrose agar

Mycelial growth on PDA ranged from 25.6 mm to 89.90 mm where the highest mycelial growth was recorded in MoT 5 isolate. The lowest mycelial growth was recorded in MoT 8 isolate. Colonies were greyish black colour with irregular margin. The isolates MoT 1, MoT 3, MoT 5, MoT 7, MoT 8 showed irregular colony margin whereas other isolates were with smooth colony margin (Plate 2).

4.4. Morphological characteristics of conidia

In all isolates, the shape of the conidia was pyriform. Base rounded, apex narrowed, almost hyaline to pale olive, 2 – septate, 3 celled, middle cell was broader than others in all isolates (Table 3 and Plate 3). Sporulation ability of 8 isolates of *Magnaporthe oryzae triticum* are shown in Table 4. The isolates of MoT 3 and MoT 4 yielded poor sporulation whereas isolates MoT 8 yielded excellent sporulation with 35 spore/ microscopic field. No sporulation was found in the isolate of MoT 6.

Table 2. Cultural characteristics of eight isolates of *Magnaporthe oryzae triticum* on Potato dextrose agar and oat meal agar media

Isolates of *Magnaporthe Oryzae triticum*	Radial mycelial growth (mm)				Cultural characters	
	OMA 7 DAI	Growth rate /day	PDA 15 DAI	Growth rate/day	OMA	PDA
MoT 1	**22.5ab**	**3.21ab**	**85.1b**	**5.67a**	Ash colour, smooth margin	Greyish Black colour with concentric ring, irregular whitish margin
MoT 2	**25.1a**	**3.58a**	**89.6a**	**5.97a**	Ash colour, irregular margin, concentric ring pattern	Greyish black colour, irregular margin, velvety
MoT 3	**19.6bc**	**2.8bc**	**74.9c**	**4.9b**	Greyish Black colour, Irregular margin	Greyish Black colour, irregular margin, velvety
MoT 4	**15.2d**	**2.17d**	**89.3ab**	**5.36a**	Ash colour, smooth margin, raised mycelial growth	Greyish Black colour, irregular margin, velvety
MoT 5	**16.6cd**	**2.37cd**	**89.9a**	**5.99a**	Ash colour, smooth margin	Greyish Black colour, irregular margin, velvety
MoT 6	**15.5d**	**2.21d**	**89.8a**	**5.98a**	Yellowish colour, sMooth margin	Greyish Black colour, irregular margin, velvety
MoT 7	**18.4cd**	**2.62cd**	**85.2b**	**5.68a**	Greyish colour, Irregular margin	Greyish Black colour irregular margin, velvety
MoT 8	**16.3d**	**2.32d**	**25.60d**	**1.70c**	Ash colour, irregular margin, concentric ring pattern	Greyish Black colour, irregular whitish, velvety margin

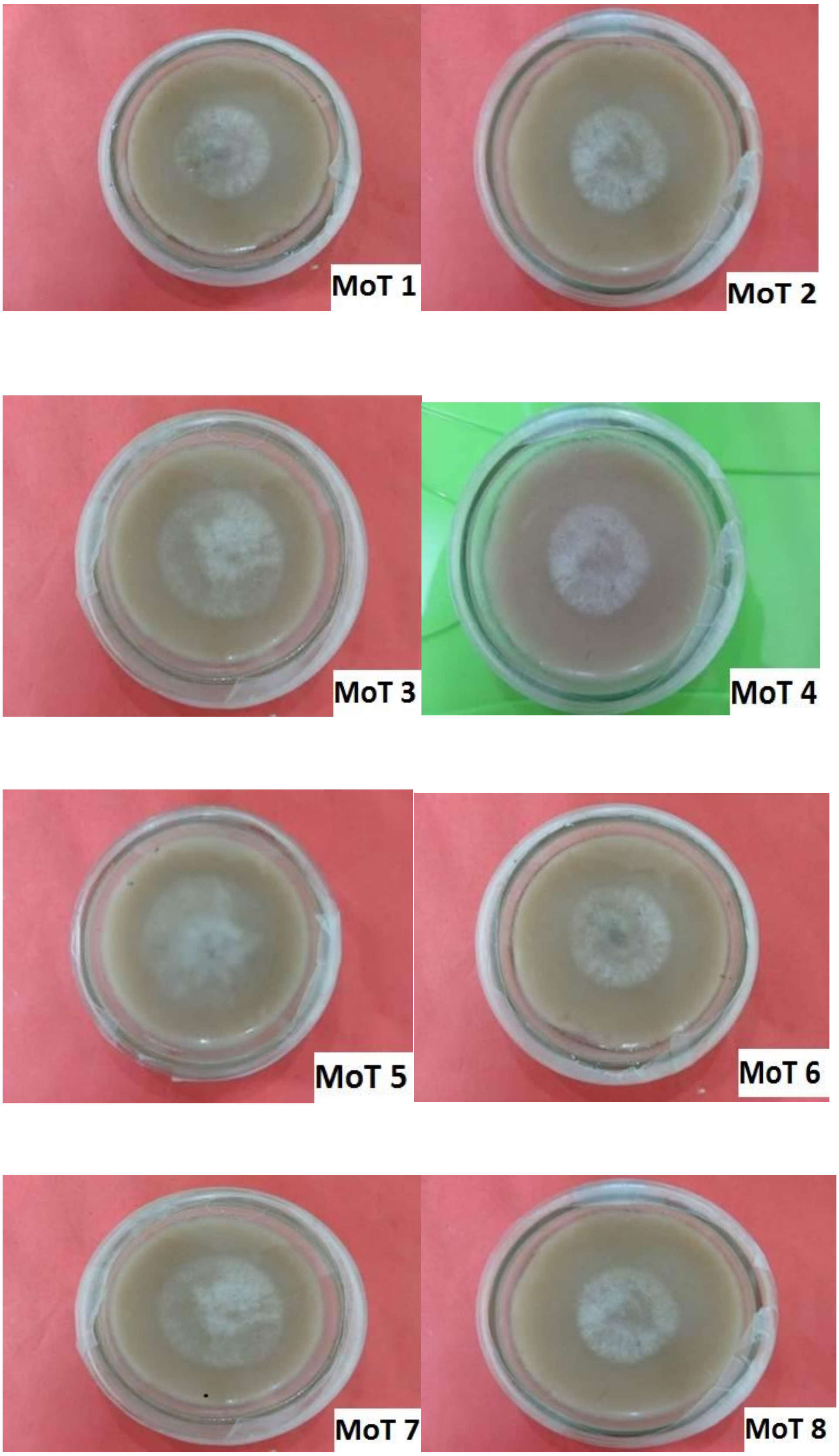

Plate 1. Colony growth of eight isolates of *Magnaporthe* oryzae *triticum* on oat meal agar media at an interval of 7 days after inoculation.

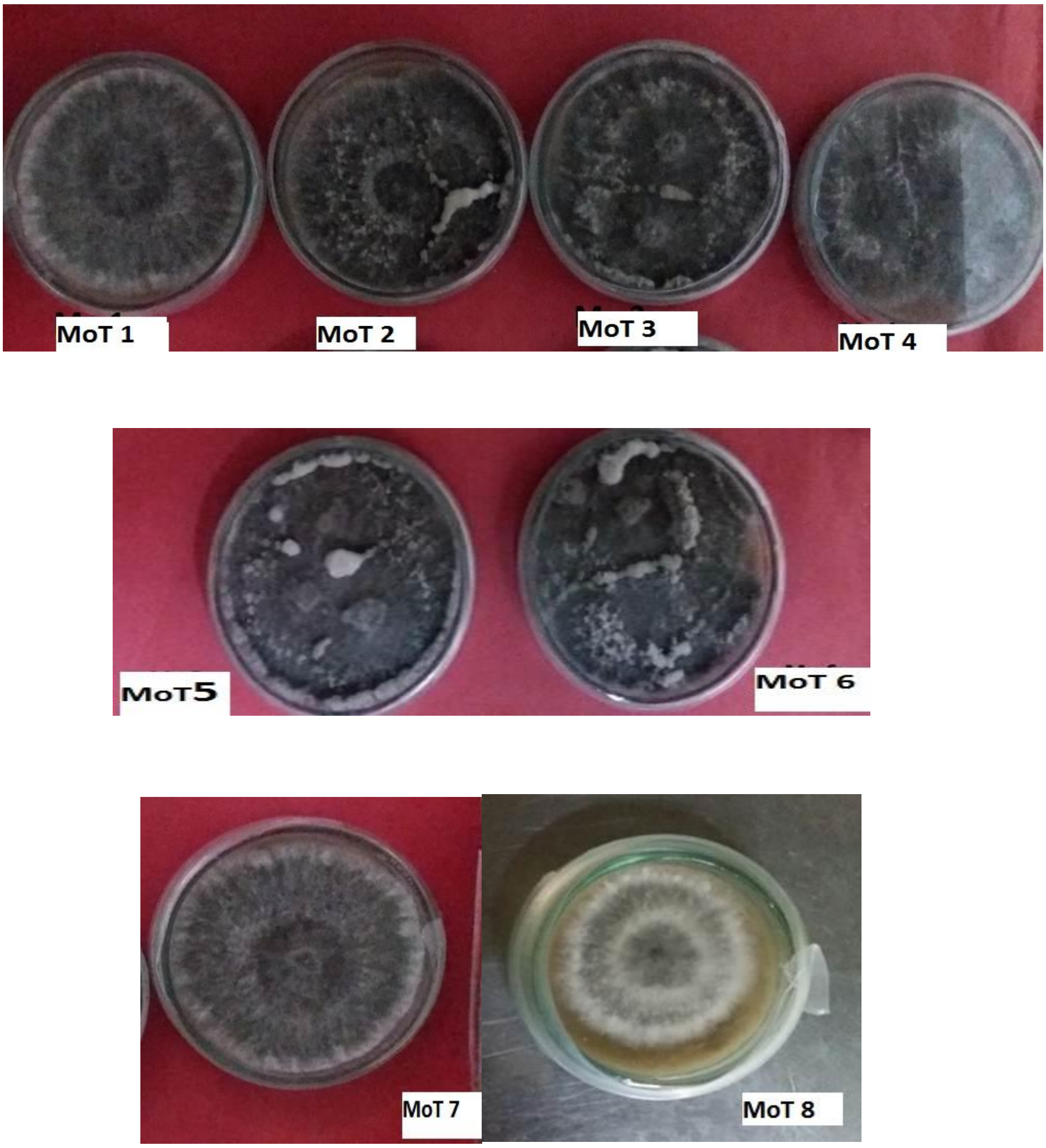

Plate 2. Morphological growth characterisation of eight isolates of *Magnaporthe oryzae triticum* on PDA media at an interval of 15 days after inoculation.

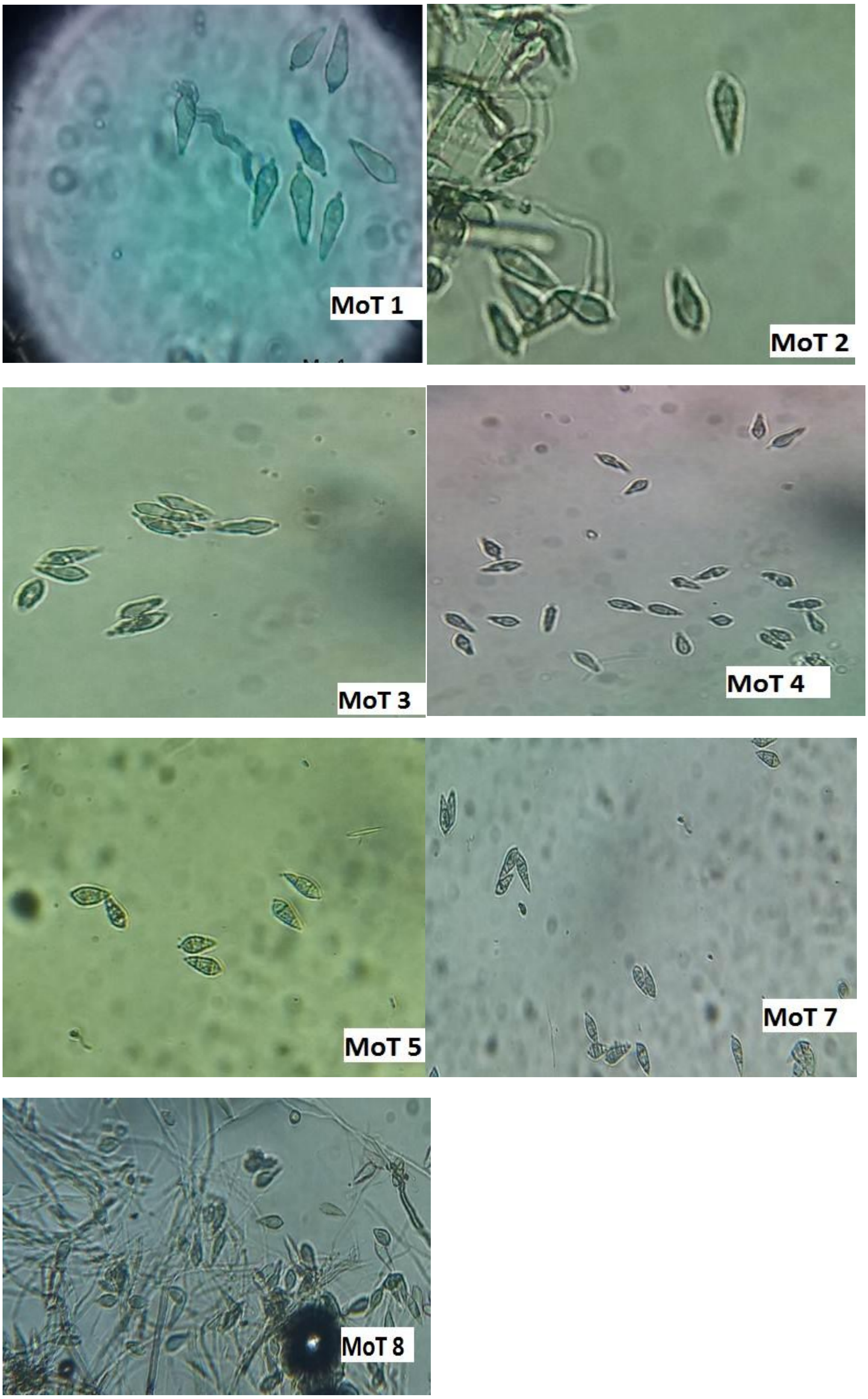

Plate 3. Spore morphology of seven isolates of *Magnaporthe oryzae triticum* (×400)

Table 3. Characteristics of conidia of seven isolates of *Magnaporthe oryzae triticum* (MoT)

Isolates	Shape	Septation	Cell	Color
MoT 1	Pyriform	2	3	Hyaline
MoT 2	Pyriform	2	3	Hyaline
MoT 3	Pyriform	2	3	Pale olive
MoT 4	Pyriform	2	3	Pale olive
MoT 5	Pyriform	2	3	Hyaline
MoT 7	Pyriform	2	3	Hyaline
MoT 8	Pyriform	2	3	Hyaline

Table 4. Sporulation ability of eight isolates of *Magnaporthe oryzae triticum* (MoT)

Isolates	No of spore/ Microscopic field	Index	Sporulation
MoT 1	8	1	Poor
MoT 2	15	2	Fair
MoT 3	9	1	Poor
MoT 4	25	3	Good
MoT 5	6	1	Poor
MoT 6	0	0	Nil
MoT 7	15	2	Fair
MoT 8	35	4	Excellent

In the present study it has been found that wheat blast incidence and severity varied from location to location. Waller *et al.* (2002) used a formula to identify disease incidence which is used here. Disease incidence and severity vary from location to due to temperature and relative humidity. From November 2017 to March 2018 temperature was recorded around 25-30^{o}c and relative humidity was 90% may favour disease development. The climate has a strong influence on the appearance of blast epidemics (Suzuki, 1975). Atkins *et al.* (1966) demonstrated that the weather is an important factor in the variability of disease development. When there are no fluctuations in relative humidity and temperature, there appears to be no change in the number of diseases. Wei (2015) found that ear bleaching was more severe at temperatures above 26°C compared to lower temperatures and spike wetness significantly contributed to disease severity.

In the present study, the highest yield loss (66.66%) and the highest economic loss (63.85%) was observed in Dhanakkhula (Gangni, Meherpur) and the lowest yield loss (30.55%) and the lowest economic loss (25.75%) was observed in Bollobpur (Mujibnagar, Meherpur). Prabhu *et al*. (1992) has reported the typical symptom of wheat blast is the head infection. This result partial or total sterility of the spikelets. Infection of the rachis blocks the translocation of photosynthates to the part of the spike above the point of necrosis, resulting partial or total sterility of the spike. Kernels of the affected spikelets are often shrivelled.

Cultural and morphological studies were carried on PDA and OMA to find out the variation for the growth and sporulation of isolates. Cultural characters of each of the isolate on two different solid media *viz*., Oat meal agar, Potato dextrose agar at the room temperature (27 ± 1^{o}c) showed the variation among the isolates of *Magnaporthe oryzae triticum.* Meena (2005) used four different types of solid media *viz*. Host extract+ 2% sucrose agar, OMA, PDA and Richard's agar and found 51.50 mm to 90.00 mm growth on OMA and 50.30 mm to 89.90 mm growth on PDA.

Cultural characteristics studies on PDA media showed the variation among the 8 isolates of *Magnaporthe oryzae triticum* with respect to colony character like type of growth, color of colony and colony margin. Colour varied from Ash, yellowish to greyish black colour, smooth to irregular margin, medium to good growth of colony was observed. The highest mycelial growth (89.90 mm) was found in MoT 5 collected from Dhanakkhula (Gangni, Meherpur) and the least mycelial growth (74.90 mm) was found in MoT 3 collected from Amla (Mirpur, Kustia).

On OMA, colony showed greyish Black colour irregular margin MoT 1 and MoT 8 showed irregular whitish margin. MoT 1 also showed concentric ring. OMA showed good mycelial growth. Meena (2005) observed concentric ring growth pattern on OMA media.

However, no variation with respect to conidial shape was found. Where conidia were pyriform, almost hyaline to pale olive, 2-septate, 3-celled. These characters are in an agreement with Arunkumar and Singh (1995).

The isolates varied in their ability to grow and rarely sporulate on PDA media. On oat meal agar isolate was able to sporulate. Among 8 isolates MoT 8 showed excellent, MoT 4 good, MoT 2, MoT 7 fair, MoT 1, MoT 3, MoT 5 poor sporulation and MoT 6 failed to sporulate. Hence, Meena (2005) variation observed in cultural characters are important from the point of view of the biology of the pathogen it occurs in nature, it is linked with the question of physiological races of pathogen.

CHAPTER V

Summary and Conclusion

The study aimed to determine disease incidence, disease severity of wheat blast and morphological variation of conidia of blast isolates of different sites. The result from this research would provide useful information for the development of strategies for the future control of wheat blast under field conditions.

Initially, a survey program was carried out in south western wheat growing region of Bangladesh. Infected samples of wheat plants were collected from different agro climatic regions of Kustia (Doulatpur, Mirpur) and Meherpur (Gangni, Mujibnagar) of Bangladesh during November 2017 to March 2018. The highest disease incidence (43.00%) was observed in Chithlia (Mirpur, Kustia). The lowest disease incidence (15.00%) was recorded in Bollobpur (Mujibnagar, Meherpur). The highest disease severity (20.00%) was observed in Dhanakkhula (Gangni, Meherpur) and the lowest disease severity (10.00%) was observed in Chithlia, Amla (Mirpur, Kustia) and Bollobpur, Doriapur (Mujibnagar, Meherpur).

Cultural characteristics of eight isolates were recorded *in vitro*. The differences in colony morphology of isolates were demonstrated on different media *viz*. Oat meal agar, Potato dextrose agar. PDA showed Ash, yellowish to greyish black colour, smooth to irregular margin, medium to good growth. On OMA, colony showed greyish black colour irregular margin with good growth. MoT 1 and MoT 8 showed irregular whitish margin. With regard to cultural variation, oat meal agar media supported maximum sporulation and potato dextrose agar media failed to sporulate. MoT 8 showed excellent sporulation. There is no significant variation in shape of conidia. All the conidia were pyriform shape. All the conidia of isolates were 3 celled and had 2 septation.

In order to further investigate the differences among *Magnaporthe oryzae triticum,* more isolates should be collected from other geographical locations. In addition, research should also be expanded to field conditions and associated with the study of wheat blast management.

CHAPTER VI

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