SURVEY ON WHEAT BLAST AND MORPHOLOGICAL CHARACTERIZATION AND *IN-VITRO* MANAGEMENT OF *MAGNAPORTHE ORYZAE TRITICUM* THROUGH BOTANICALS

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This is to certify that thesis entitled, ***"Survey on wheat blast and morphological characterization and in-vitro management of Magnaporthe oryzae triticum through botanicals"*** *submitted to the Faculty of AGRICULTURE, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in PLANT PATHOLOGY,*** *embodies the result of a piece of bona fide research work carried out by* ***MST. REHENA KHATUN*** *bearing Registration No.* ***18-09044*** *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 has duly been acknowledged.

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ABSTRACT

Wheat blast disease caused by *Magnaporthe oryzae triticum* (MoT) has become a serious constrain in increasing the wheat area of infection and decreasing the cultivable area of the crops. A survey was conducted in 30 villages of blast infected South-Western wheat growing region of Bangladesh during January to April, 2019. During the survey at Meherpur Sadar, Mujibnagar and Chuadanga Sadar Upazilla, the highest incidence and severity was recorded in cultivar BARI Gom-24 (Pradip) at Kutubpur, Monkhali and Parkrisnapur village and the lowest incidence and severity was found in BARI Gom-26 and BARI Gom-28 at Pirojpur, Charulia and Bollolpur village. Thirty-five MoT isolates were isolated, identified and tested their pathogenicity. Growth response and cultural characteristics of the MoT isolates were done on PDA. In the present study, the highest radial mycelial growth observed on the isolate CHMoT 09 (25.67 mm) on the 7^{th}days, CHMoT 06 (56.33 mm), CHMoT 07 (55.83 mm), CHMoT 08 (56.33 mm) and CHMoT 09 (57.00 mm) on the 14^{th} days incubation and CHMoT 08 (75.50 mm) on the 30^{th} days incubation. Based on mycelial growth per day, isolates of *M. oryzae triticum* were classified into three cluster groups, cluster I, cluster II, cluster III that indicates the presence of a morphologically diversified group of pathogens. *Aloe vera* (Allovera leaf) extracts and *Nigella sativa* (Black cumin seeds) extracts @ (1:1 w/v) concentration were found the most effective botanicals to reduce mycelial growth of *M. oryzae triticum* under *in-vitro* condition. However, this experiment with more plant extracts needs to be coined out to assess the field efficacy of these botanical extracts with different concentrations and frequencies in controlling blast disease of wheat.

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ABBREVIATIONS

FULL WORD	ABBREVIATION
Agricultural	Agril.
Agriculture	Agric
American	Am.
And	&
And others	*et al.*
As for example	e.g.
At the rate of	@
Bangladesh Agricultural university	BAU
Bangladesh Agricultural Research Institute	BARI
Bangladesh Bureau of Statistics	BBS
Biology	Biol.
Biotechnology	Biotech
Continued	Cont'd
Centimeter	cm
Days after inoculation	DAI
Degree Celsius	^{0}C
Degree of freedom	df.
Deoxyribonucleic acid	DNA
Distilled deionized water	ddH_20
Genetics	*Genet.*
Government	Govt.
Gram	g
Gram per Litre	g/L
International	*Int.*
Journal	J.

ABBREVIATIONS (Cont'd)

FULL WORD	ABBREVIATION
Mililitre	ml
Milimetre	mm
Milimole	mM
Molecular	Mol
Namely	*viz.*
Negative logarithm of hydrogen ion concentration (-log[H+])	pH
Number	No.
Percentage	%
Polymerase chain reaction	PCR
Polymorphic information content	PIC
Research	*Res.*
Science	*Sci*.
Sodium chloride	NaCl
Species	Spp.
That is	i.e
Ultra Violet	UV
Namely	Viz.
Volume	Vol.
Water agar	WA

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most important cereal crops in the world. Globally, it is a common human food grain and created optimistic impact on the national economy. It provides 20% of the world food calories and it is staple food for nearly 40% of the world population (Wiese *et al.,* 1987). Wheat is originated from the Levant region of the Near East but now cultivated worldwide. Wheat is grown on more than 701.5 million hectares, thus being larger than any other crop (The Statistics Portal, 2014). In 2017, world production of wheat was 771.7 million tons, making it the third most-produced cereal (FAOSTAT, 2017). It is the primary staple food in North Africa and the Middle East, and is growing in popularity in Asia. The four largest producers of wheat in 2017 in the world were China (134.3 million tons), India (98.5 million tons), Russia (85.9 million tons) and USA (47.3 million tons), (FAOSTAT, 2017).

Wheat plants are suffering from several diseases and wheat blast caused by *Magnaporthe oryzae* pathotype *triticum* (MoT) reported as a devastating disease of wheat recently (Kihoro *et al.*, 2013). Wheat blast, or 'brusone', is caused by the haploid, filamentous, ascomycetous fungus *Magnaporthe oryzae triticum* B. Couch (Anamorph *Pyricularia oryzae* Cavara) (Couch and Kohn 2002; Zhang *et. al.,* 2016a). Blast has emerged as an explosive threat to wheat production that can cause up to 100% yield losses under the favourable environmental conditions. Wheat blast is caused by a subpopulation within *M. oryzae triticum*. The *M. oryzae triticum* pathotype (MoT) that is distinct from sub populations infecting rice (the *Oryza* pathotype, MoT); finger millet (the *Eleusine* pathotype); Italian or foxtail millet (the *Setaria* pathotype); and turf grasses (the *Lolium* pathotype, MoL); among others (Zhang *et. al.,* 2016a).

Typical symptoms of wheat blast on spikes are premature bleaching of spikelet's and entire heads. Severely infected wheat heads can be killed, resulting in severe yield losses. The disease is generally spread by infected seeds and airborne spores, and the fungus can survive in infected crop residues and seeds (Urashima *et al.,* 1999). Little information is known about the physiology and genetics of the wheat blast pathogen, and our understanding of the molecular interactions of this pathogen with wheat remains limited.

Since its first report in Paraná, the disease has spread to the most important wheat producing regions of Brazil (Dos Anjos *et al.,* 1996; Goulart *et al.,* 1990; Igarashi, 1990; Picinini and Fernandes, 1990; Goulart and Paiva, 2000), as well as to Bolivia (Barea and Toledo, 1996) and Paraguay (Viedma, 2005). In 2007, it was reported for the first time in northeastern Argentina (Cabrera and Gutierrez, 2007).

The *M. oryzae* pathotype *triticum* is considered the causal agent of wheat blast in South America and has also been associated with blast disease on barley, rye, triticale, and signal grass (Urochloa sp., ex Brachiaria sp.) in central-western and southern Brazil (Verzignassi *et al.,* 2012). Due to the lack of resistant cultivars and effective fungicides for disease management, wheat blast is widely distributed across all the wheat-cropping areas in Brazil, causing crop losses from 40–100 % (Silva *et al.,* 2009, Maciel 2011, Castroagudín *et al.,* 2015). Wheat blast also occurs in Bolivia, Argentina, and Paraguay (Duveiller *et al.,* 2010). The disease was not found outside South America (Maciel 2011) until a recent outbreak reported in Bangladesh (Callaway 2016), though wheat blast is considered a major quarantine disease and a threat to wheat crops in the United States (Duveiller *et al.,* 2007, Kohli *et al.,* 2011).

The pathogen *M. oryzae triticum* was isolated and by comparative analysis of sequenced whole genomes, it was concluded that this strain was more similar to native strains isolated from U.S. *Lolium* than to *Triticum* isolates from South America (Farman, Pedley, and Valent, unpublished). *M. oryzae triticum* has also been previously reported in wheat interplanted with ryegrass in Louisiana where no serious losses were reported (Rush and Carver, 1973). The origin of the wheat blast pathogens is still unknown. However, it has

been suggested that host shifts may account for their recent emergence in Brazil, the U.S. and Japan (Khang and Valent, 2010). Wheat blast is today considered a major disease affecting wheat production in Brazil (Urashima *et al.*, 2009). The economic importance of this disease derives from the fact that the fungus can reduce yield and the quality of the wheat grain (Goulart, 2005). Infected grains from highly susceptible cultivars are usually small, wrinkled, deformed, and have low-test weight (Goulart, 2005). The highest yield losses occur when infections start during flowering or grain formation (Goulart, 2005). Reported yield losses in Brazil on susceptible cultivars vary from 10.5 up to 100% (Goulart *et al.*, 1992; Goulart and Paiva, 2000).

In 2016, a wheat blast outbreak was reported for the first time outside of South America, in the districts of Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola, Magura, Narail, and Faridpur (Malaker *et al.*, 2016). Bangladesh is an agricultural country where different types of agricultural crops are grown. After rice, wheat is the third most cultivated grain in Bangladesh. The production of wheat is increasing day by day in this country. The total area for wheat cultivation now extends to about 1.78 lakh ha and the annual production is about 10 lakh m tons (BBS, 2017). But this first incidence of wheat blast affected approximately 15% of Bangladesh's total wheat area. Wheat blast is caused by *Magnaporthe oryzae* pathotype *triticum* (MoT), a devastating disease of wheat was spotted in Bangladesh for the first time, the first case in Asia and confirmed with genome sequencing by Dr. Sophien Kamoun, Sinsbury Laboratory, UK (Malaker *et al.*, 2016 and Islam *et al.*, 2016).

Wheat blast is a new disease in this area, indicating the higher possibility spreading of this pathogen spreading throughout the Asia, the world's largest wheat producing area. Occurrence of this disease caused ~3.5% reduction of the total wheat fields in Bangladesh. Its economic effect on the Bangladesh wheat market was little because wheat contributes to 3% of total cereal consumption, among which ~70% have been imported from other countries. However, as a long-term perspective, much greater losses

will occur when this disease spreads to other major wheat producing areas of Bangladesh, India, and Pakistan due to the existing favorable condition for the blast pathogen.

Wheat blast was observed in the year 2016 at eight south-western districts viz., Pabna, Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal and Bhola in Bangladesh (Malaker *et al*.,2016). The severity of wheat blast and associated yield losses varied among districts. The highest percentage of infected wheat fields was observed in Meherpur (70%). Yield losses in different affected districts also varied. The highest average yield loss was recorded in Jhenaidah (51%). Although the average yield loss was lower than 51% across districts, yield losses in individual fields were as high as 100% (Islam *et al.*, 2016).

Wheat blast symptoms and its causal agent were not previously reported in Bangladesh. A great deal of controversy is how this disease was invaded in Bangladesh. Three probable reasons might have caused wheat blast invasion in Bangladesh. Firstly, virulent strains of *M. oryzae tritici* have been introduced with a seed-transmitted pathogen escaping quarantine regulations from South America. Secondly, virulent strains of *M. oryzae triticum* have evolved from pre-existing avirulent strains in Bangladesh. Finally, strains of minor cereals blast fungus (*Pyricularia oryzae*) that is already diverse and widespread in Bangladesh become pathogenic to wheat under changing climate conditions (Tiedemann *et al*., 2016).

Infected wheat seeds may not have symptoms and they may constitute a source of primary inoculum of the disease in the field, generating epidemics by providing initial inoculum to new areas (Coelho *et al.*, 2016), including for the fields of seed production, with serious consequences. Seed planting with *M. oryzae* t*riticum* was the probable cause for the dissemination of the fungus in wheat from Paraná to Mato Grosso do Sul (Urashima *et al.*, 2007; Silva *et al.*, 2009); and in triticale from Paraná to São Paulo (Medina *et al.*, 2009). Furthermore, with regard to seed quality, the presence of the pathogen may be related to the low germination and low vigor. Studies on the effect of *M. oryzae triticum* on germination are not well known (Urashima *et al.*, 2009).

The infected wheat fields were burned, which contributes to 15% decrease in wheat production of the nine infected districts (Islam *et al.,* 2016; Malaker *et al.,* 2016; Aman, 2016). In spite of such decrease, total wheat production in Bangladesh increased a little (35,000 metric ton [MT], 2.7%) in 2016 compared to that of 2015. Increasing of total harvested areas (420,000–425,000 ha) and yields (3.10–3.14 MT/ha) contributed to the total wheat production in 2016 (USDA, 2019)

Importantly, 100% of government owned Bangladesh Agricultural Development Corporation (BADC) seed multiplications farm in the affected districts (355ha) were completely burned to destroy pathogen inoculum by the decision of the Ministry of Agriculture. Farmer wheat fields that were severely affected (up to 100%) were also burned (Islam *et al.,* 2016).

Some fungicides can be used for controlling wheat blast. The frequent use of fungicides on crops may cause hazards to human beings, plant health and beneficial micro-organisms and develop fungicide resistance into the pathogens and residual toxicity in plant parts. On the other hand, some botanical pesticides and bio-control agents have proved to be most secure and have no adverse impact on environment (Iftikhar *et al.,* 2010; Babar and Khan, 1999). Use of chemical fungicides for controlling this disease might have health hazard for human being and animals.

Therefore, environment-friendly management of this pathogen with botanical extracts will be very effective until the development of resistance cultivars against this pathogen in Bangladesh. As no work for controlling wheat blast disease by using plant extracts, and due to this work is a new effort for non-chemical as well as environment friendly management of wheat blast in Bangladesh. The present research was undertaken to find out the antifungal effect of some botanical extracts on MoT and to determine the effect of the plant extracts on disease reduction and yield contributing parameters of wheat.

In view of above facts, the present research work was undertaken with the following objectives

Objectives

- To determine incidence and severity of wheat blast in selected South Western wheat growing regions of Bangladesh.
- To determine a morphological diversity, cultural characterization and pathogenicity of different isolates of *Magnaporthe oryzae triticum.*
- To evaluate the efficacy of ethanol extracts of botanicals against mycelial growth of *Magnaporthe oryzae triticum in-vitro.*

CHAPTER II

REVIEW OF LITERATURE

The available literature of work done on blast disease of wheat and its management strategies have been reviewed in this chapter. The review of literature is presented by the following headings and sub-headings.

2.1. Importance of wheat

Wheat (*Triticum aestivum*) is one of the most important cereal crops in the world. Wheat accounts for a fifth of humanity's food and is second only to rice as a source of calories in the diets of consumers in developing countries and is first as a source of protein (Braun *et al.* 2010). Wheat is an especially critical foodstuff for 1.2 billion people classified as 'wheat-dependent'; 2.5 billion are classified as 'wheat-consuming' and live on, US$2 day21. There are also 30 million poor wheat producers and their families for whom wheat is the staple crop (FAOSTAT 2012). Demand for wheat in the alone, the most conservative climate change projections suggest a minimum decline across South Asia of between 4 and 10 %. In Bangladesh, rice production could fall by 8 % and wheat production by 32 % as early as 2050 (Anonymous 2008).

Anonymous (2013) reported among the cereals, wheat is second to rice in economic and consumption importance. It occupies 4 % of the total cropped area and 11 % of the area cropped in Rabi (winter crops starting from November to February), and contributes 7 % to the total output of food cereals. By collecting 52 years of data from (Index Mundi 2012b) showed the trend of area, production and growth rate of wheat in Bangladesh from 1960 to 2011.

Wheat provides 19% of the world's dietary energy supply, while rice supplies 20% and maize 5%. During 2012-13 and 2013-14, the world production has increased by 1% (from 472 Million Tonnes to 476 Million Tonnes), trade by 8% (from 38 Million Tonnes

to 41 Million Tonnes) and consumption by 3% (from 469 Million Tonnes to 481 Million Tonnes) (Commodity profile for rice - January 2015). Wheat is the second major cereal crop (3% of total cereal consumption) after rice (93% of total cereal production) in Bangladesh (BBS, 2014).

Singh *et al.,* (2015) showed that scarce availability of healthy seeds is one of the major impediments for achieving high yields in Bangladesh. Farmers get wheat seeds for cultivation only from the government agencies (Bangladesh Agricultural Development Corporation (BADC and DAE). Although, the seed requirement for wheat cultivation has increased constantly (BADC, 2015), the government agencies fulfilled only 40–50% of the total seed requirement of the country (Jaim and Akter, 2012).

The country requires about 40 lakh m tons of wheat seed annually. About 20,000 m tons of seed is supplied from the public sector and the rest (80,000 m tons) comes from the farmers. The Bangladesh government imported wheat from other countries to fulfill the domestic requirement, because the wheat production stayed around one million MT for last ten years. This might be one of the reasons that the Bangladesh government allows to increase wheat import from diverse sources including Brazil and Argentina. After the outbreak, domestic consumption suddenly increased to 16.4% where last three years (2012–2015) average increasing rate was 11.2% (Department of Agricultural Extension, Bangladesh).

In proportion to this, the amount of wheat imports increased and this made the wheat price stable in Bangladesh (Index Mundi, 2016). The Bangladesh government should take strategies to increase wheat production as a long-term goal which will reduce food dependency of Bangladesh. Compared to rice, wheat has much less economic impact on Bangladesh. And the government might not consider the wheat blast seriously if it does not occur next year. Our diagnosis leads to suggestions of some strategies to sustain the wheat cultivation in Bangladesh.

However, during the last decade, wheat consumption has been increasing gradually and it was almost doubled to six million MT in 2016. Bangladesh is also an agricultural country

where different types of agricultural crops are grown. Cereal crops are main cultivated crops here. After rice, wheat is the second most cultivated grain in Bangladesh. The production of wheat is increasing day by day in this country. The total area for wheat cultivation now extends to about- 1.78 lakh ha and the annual production is about 10 lakh m tons (BBS, 2017).

2.2. Significance of blast disease of wheat

According to (Couch and Kohn, 2002; Zhang *et al.,* 2016) stated that, the wheat blast pathogen belongs to the *Magnaporthe oryzae* (syn. *Pyricularia oryzae*) species complex. Choi *et al.,* (2013) also showed that, the members of this species complex cause blast disease on more than a hundred of species in the Poaceae family including rice, wheat, barley and rye. Several phylogenetic species (e.g., pathotypes) are proposed by cladistic analyses based on the multi-gene sequence and the host specificity (Choi *et al.,* 2013; Hirata, 2007; Kato *et al.,* 2000; Tosa *et al.,* 2004).

Goulart *et al.,* (2007) reported that, wheat blast is considered a major disease affecting wheat production. The economic importance of this disease derives from the fact that the fungus can reduce yield and grain quality. Grains from blast-infected spikes from highly susceptible cultivars are often small, shriveled and deformed, with low test weight. Highest yield losses occur when spike infections begin during flowering or early grain formation. Urashima *et al.,* (2009) also found that, these grains are often discarded during the post-harvest process of threshing or winnowing. Goulart and Paiva (1992, 2000) also reported that, the yield losses up to 100% due to the cultivation of susceptible cultivars.

Urashima *et al.,* (2009) experimented that, wheat blast is today considered a major disease affecting wheat production in Brazil. Goulart (2005) also experimented the economic importance of this disease derives from the fact that the fungus can reduce yield and the quality of the wheat grain. Infected grains from highly susceptible cultivars are usually small, wrinkled, deformed, and have low-test weight. The highest yield losses occur when infections start during flowering or grain formation. Goulart *et al.,* (1992);

Goulart and Paiva (2000) reported yield losses in Brazil on susceptible cultivars vary from 10.5 up to 100%.

Fisher *et al., (*2012) showed that, the outbreaks caused by fungal diseases have increased in frequency and are a recurrent threat to global food security. Fisher *et al.,* (2012); Pennisi (2010); Liu *et al.,* (2014) also showed that, one example is blast, a fungal disease of rice, wheat and other grasses, that can destroy enough food supply to sustain millions of people. Until the 1980s, the blast disease was not known to affect wheat, a main staple crop critical to ensuring global food security.

World wheat production is now under threat due to the wheat blast outbreak in Bangladesh in early March 2016. Islam *et al.,* and Malaker *et al.,* (2016) reported that is a new disease in this area, indicating the higher possibility of this pathogen spreading throughout the Asia, the world's largest wheat producing area. Occurrence of this disease caused ~3.5% reduction of the total wheat fields in Bangladesh. Its economic effect on the Bangladesh wheat market was little because wheat contributes to 3% of total cereal consumption, among which ~70% have been imported from other countries.

Officials from the Department of Agricultural Extension (DAE) informed that the infected area was estimated about 15,000 ha, which correspond to ~3.5% of total wheat fields in Bangladesh. Islam *et al.,* and Malaker *et al.,* (2016) also reported that, the infected wheat fields were burned, which contributes to 15% decrease in wheat production of the nine infected districts. In spite of such decrease, total wheat production in Bangladesh increased a little (35,000 metric ton [MT], 2.7%) in 2016 compared to that of 2015. Increasing of total harvested areas (420,000–425,000 ha) and yields (3.10–3.14 MT/ha) contributed to the total wheat production in 2016.

The blast has shown high potential to cause reductions in wheat productivity in the tropical regions of the country. In Mato Grosso do Sul, Brazil, there is a record of losses in productivity of up to 74% (Goulart *et al.,* 2007). In Minas Gerais, Brazil, fourteen wheat genotypes had a reduction in productivity as a function of the incidence of blast in two different sowing periods (Coelho *et al.,* 2016). Saharan *et al.,* (2016) stated that

researches claim that losses in wheat production caused by blast may vary and may reach up to 100%.

Urashima *et al.,* (2007); Debona *et al.,* (2016) pointed in addition to the wind, the conidia of the fungus can also be efficiently dispersed through the seeds. Coelho *et al.,* (2016) showed that, the infected wheat seeds may not have symptoms and they may constitute a source of primary inoculum of the disease in the field, generating epidemics by providing initial inoculum to new areas including for the fields of seed production, with serious consequences.

Duveiller *et al.,* (2016) reported that, the losses due to disease depend on weather conditions and level of resistance against wheat blast in a variety. The losses due to wheat blast were estimated in the range of 10 to 100% in recent years in South American countries. The disease may leave the farmers to feel deceived since it affects the spike and grains badly and its spread on susceptible wheat cultivars is quite fast (2-3 weeks) thus leaving little time to farmers to prepares and cope up with the situation.

Gomes *et al.,* (2017) also reported that, the infected spikes had lower productivity and yielded seeds with reduced physiological quality as compared to uninoculated spikes. The seed-borne inoculum of *M. oryzae* pathotype *Triticum* infected the wheat seeds developed on the mother plant. The seeds harvested from fields with blast incidence from 20% on spikes were therefore not recommended for seed purposes. The genotypes confining the low infection on the leaves with little infection on the spike are preferred.

2.3. Nature and disease symptoms

Igarashi (1990); Islam *et al.,* (2016); Malaker *et al.,* (2016); Urashima *et al.,* (2010) observed that, the infected field has dry and bleached spikes while the leaves may be still green. The symptoms appear on seed, leaf, peduncle, and spike. On leaves, initial symptoms may be gray-green and water-soaked lesions with dark green borders which become light tan with necrotic borders, once they have completely expanded with typical eye shaped necrotic lesions with grey centres. In the field, the symptoms on leaves may,

however, be difficult to identify due to mixed infection of spot blotch. The symptoms on spikes are most prominent and easily visible. The pathogen infects the rachis and develops dark brown discoloration at the point of infection with or without dark brown mycelial growth. The spikes may be completely or partially bleached.

Urashima *et al.,* (2010) observed that head infections during the flowering stage resulted in no grain production, whereas infection at the grain filling stage resulted in small, shriveled, light in weight, and discolored (pale) grains. Igarashi (1990); Urashima (2010) also observed that, the typical symptoms of wheat blast on spikes are premature bleaching of spikelet's and entire heads. Severely infected wheat heads can be killed, resulting in severe yield losses. The disease is generally spread by infected seeds and air borne spores and the fungus can survive in infected crop residues and seeds (Urashima *et al.,* 1999).

Maleker *et al.,* (2016) reported that, symptoms from Bangladesh in 2016 the pathogen attacked the base or upper part of the rachis, severely affecting spikelet formation above the point of infection. Complete or partial bleaching of the spike above the point of infection with either no grain or shriveled grain was common in all areas affected by wheat blast. We commonly observed bleached heads with traces of gray, indicative of fungal sporulation at the point of infection.

2.4. Occurrence of wheat blast disease

Igarashi *et al.,* (1986), Anjos *et al.,* (1996) reported wheat blast was first reported in Paraná State, Brazil in 1985. Silva *et al.,* (2009); Maciel (2011); Castroagudín *et al.,* (2015) reported due to the lack of resistant cultivars and effective fungicides for disease management, wheat blast is widely distributed across all the wheat-cropping areas in Brazil, causing crop losses from 40–100 %. Wheat blast disease also occurs in Bolivia, Argentina, and Paraguay (Duveiller *et al.,* 2010). The disease was not found outside South America (Maciel *et al.,*2011) until a recent outbreak reported in Bangladesh (Callaway *et al.,* 2016), though wheat blast is considered a major quarantine disease and a threat to wheat crops in the United States (Duveiller *et al.,* 2007, Kohli *et al.,* 2011).

In 1986 blast caused by *M. oryzae triticum* emerged as a new field disease of wheat in Brazil causing considerable yield losses (Urashima *et al.,* 1993, 2004). Compared with blast of rice (Caracuel-Rios & Talbot, 2007; Ribot *et al.,* 2008) studies of the wheat–*Magnaporthe* interaction are limited.

In 1996, blast was reported for the first time outside of Brazil, in Bolivia's most important region for wheat production, the Santa Cruz Department (Barea and Toledo 1996). Wheat blast reached Itapúa and Alto Paraná Departments of Paraguay in 2002 (Viedma 2005), and the province of Formosa in northeastern Argentina in 2007 (Cabrera and Gutiérrez 2007).

Callaway, 2016 showed terrifying blast disease of wheat (*Triticum aestivum*) was spotted in Bangladesh and this was the first occurrence in the Asia. CIMMYT (2016) also showed 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. Plant pathologists from Wheat Research Center (WRC) of Bangladesh also warned that this disease has the chance to spread to India, Pakistan, and China which ranks third, seventh, second in the world wheat production, respectively (Index Mundi, 2016).

Islam *et al.,* (2016) reported that, the wheat blast symptoms appeared first in the middle of February of 2016 in Chuadanga and Meherpur districts and rapidly spread to adjacent four districts within two weeks. 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).

2.5. Distribution of wheat blast disease

Tosa *et al.,* (2004) and Tosa *et al.,* (2016) stated that, this disease also occurs in Japan, presumably introduced through movement of perennial ryegrass seed from the U.S. In the U.S. in 2011, *M. oryzae triticum* was isolated from a single diseased wheat spike in a University of Kentucky wheat trial plot in Princeton, Kentucky.

Kohli *et al.,* (2011) reported wheat blast has remained restricted to South American countries, Brazil, Bolivia, Paraguay, Argentina and Uruguay. Callaway (2016); Malaker *et al.,* (2016) also reported in April 2016, it was observed in Bangladesh. Malaker *et al.,* (2016) showed in 15% of wheat area in Bangladesh. Areas in the districts of Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola and several others. There were reported that wheat production was affected in 15000 hectares with resultant fall in production by 20%. Standing wheat crop was burnt in some areas. Malaker *et al.,* (2016) stated that, based on the molecular characteristics, wheat blast absolute from Bangladesh was found similar to that of Brazil.

Farman *et al.,* (2017); Malaker *et al.,* (2016) also reported this first incidence of wheat blast affected approximately 15% of Bangladesh's total wheat area. Comparative genome analyses showed that fungal isolates from diverse wheat regions in Bangladesh appeared clonal and were closely related to highly aggressive MoT isolates from South America. Islam *et al*., (2016) also reported an independent path genomics analysis confirmed that the Bangladeshi wheat blast fungus was most likely moved in from South America.

2.6. *Magnaporthe oryzae triticum*

Valent *et al.,* (1986) and Zeigler (1998) observed that, *M. oryzae triticum* is a filamentous, heterothallic ascomycete that has potential for sexual and asexual reproduction; however, there is evidence that sexual fertility has been lost in some populations. The genus *Pyricularia* was first described by (Saccardo 1980) and later illustrated by other authors (Barret and Hunter 1998; Henry and Andresen, 1948). Barnett and Hunter (1998) also observed that, originally from a leaf of the grass Setaria, it was

characterized as a fungus with long, slender, mostly simple conidiophores; 2- to 3-celled, obpyriform to nearly ellipsoid hyaline conidia attached at the broader end. Conidia are approximately 8-9 x 20-26 m (Henry and Andersen, 1948).

Klaubauf *et al.,* (2014) examined infected plant samples using a light microscope. A hallmark of blast fungi is the production of asexual spores that have a specific morphology consisting of three-celled pyriform conidia. Microscopic analyses revealed that gray colored lesions observed on both spikes and leaves which produce a large number of three-celled pyriform conidia from aerial conidiophores.

Zhang *et al.*, (2014) also showed fully fertile strains are self-sterile hermaphrodites, with compatibility for mating governed by alternate alleles of the mating type locus MAT1. At ~20 °C with light, highly fertile hermaphroditic strains mate as a female (contributing cytoplasm and producing the perithecium) and as a male in crosses with hermaphroditic strains of opposite mating Phagophore-like anamorph in which small, crescent-shaped microconidia are produced from phialides (These microconidia germinate at low levels and infect plants through wounds, but their role in nature is unknown.

USDA (2015) and Subramanian (1968) experimented the colonies of MoT appeared as white, light gray, or dark gray in color after 5 to 7 days. The conidiophores were single or clustered, simple, infrequently branched and exhibit sympodial growth. At the tip of the conidiophore, conidia form at points that ascend sympodial and in succession, narrowed toward the tip, pyriform to obclavate, rounded at the base, 2septate, rarely 1- or 3-septate, hyaline to pale olive, primarily 19-23 × 7-9 µm with a distinct bulging basal hilum. *M. oryzae triticum* culture may be stored at 4 to -20°C in desiccated form on a range of media without much losing of viability or pathogenicity for 20 years.

Cruz *et al.*, (2015) reported that *M. oryzae triticum* saprophytic growth and conidiation on basal senescent leaves coincide with spike emergence under greenhouse and field conditions. MoT sporulation in the field was significantly greater on a susceptible cultivar (Atlax) than on more resistant cultivars. Based on the evidence, (Cruz *et al.*,

2015a) proposed that the lower canopy of certain wheat cultivars could play an important role in the initial development of wheat head blast epidemics.

2.7. Climatic conditions favorable to disease development

According to (Kohli *et al.,* 2011) most severe blast years coincide with wet years (El Nino phenomenon) characterized by several days of continuous rains and average temperatures between 18-20^{0}C during the flowering stage of the crop followed by sunny host and humid days. (Cardoso *et al.,* 2008) observed under controlled conditions highest blast intensity at 30^{0}C which increased with duration of wetting period and lowest at 25^{0}C with a wetting period of less than 10hrs. However, with increasing wetting period of 40hrs. at 25^{0}C blast intensity of 85% was observed.

CABI (2017) observed the disease may become an epidemic and devastate wheat crop within a week under most conducive temperature range of 8-30°C and at >80% RH during ear emergence or grain filling. Kohli *et al.,* (2011) also observed the wet years; warm temperatures and high humidity were found associated with wheat blast epidemics. The rains for several days and average temperatures ranging from 18-25°C during flowering, followed by sunny, hot, humid days is favorable for the epidemic of the wheat blast.

Ha *et al.,* (2017) showed the disease is seed-borne and able to survive on alternative hosts and in situations like changing the climate and lack of satisfactory genetic resistance against wheat blast, more research is needed to generate data to forecast epidemics and protect other regions and cropping systems from this menace. Cardoso *et al.,* (2008) also showed the lowest blast intensity was reported at 15°C and the maximum between 25°C and 30°C with a wetting period of at least 10 hrs. MoT requires tropical and subtropical temperatures to sporulate and survive.

2.8. Seed and secondary hosts as source of primary inoculum

Goulart and Paiva (1990) studied seed has been shown as a primary/initial source of inoculum; however, it plays a minor role in epidemiology. Prabhu *et al.,* (1992) and Urashima *et al.,* (1993) also studied majority of the spike infection comes from the air borne conidia from various secondary hosts. Mehta *et al.,* (2006) showed infection on *Pyricularia* on triticale was reported by and black oats.

Prabhu *et al.,* (1992); Urashima *et al.,* (1993) observed seed infection seems to play only a minor role in the epidemiology of the disease because spike infection comes from the air-borne conidia mainly from several secondary hosts. Mehta *et al.,* (2006) reported by first infections on triticales and recently, blast infection in commercially grown black oats (*Avena strigosa*) has been added to this list.

2.9. Disease cycle and spread

Wheat and rye grass isolates of *M. oryzae triticum* exhibit almost same disease cycle as that of rice. The characteristic feature of this disease is the pyriform conidia that give rise. Two septate pyriform conidia of *Magnaporthe* on wheat to the disease. Conidium has three cells, with each having single nucleus per cell. Isolates can be purified through single conidium. Presence of melanin in the appressoria, arms the conidia to build up a very high pressure essential to puncture the outer plant surface and gain access to the host tissues. Presence of a free film of water is essential during the infection process. After entry into host, the fungus colonizes for 4 days without any visible appearance. Thereafter, conidia are released and process is facilitated by water. In other words, disease outbreak is hard to predict as by the time first visible symptoms, appear, blast is already established (Anonymous, 2013). Urashima *et al.,* (2007) showed young and expanding leaves are more susceptible to spores of wheat blast fungus. Conidia have been detected up to 1000 meter away from the fields. Seed is the primary sources of inoculum to new areas. However, possibility of a grass strain jumping to wheat can also cause wheat blast (Callaway, 2016).

2.10. Assessment of blast disease intensity

Trindade *et al.,* (2006) stated the spike blight incidence is recorded in percent spikes infected. The scale proposed by is 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%, for 75% and for 100% length of spike affected.

Maciel *et al.,* (2013) showed the infected spikes. The blast severity was ascertained by using resources of the software ImageJ. A diagrammatic scale was prepared and the disease severity values were 3.7, 7.5, 21.4, 30.5, 43.8, 57.3, 68.1, 86.0, and 100.0%.

2.11. Isolation of *Magnaporthe oryzae triticum*

Standard blotter method ISTA, (1996) was used for the isolation of this MoT pathogen from infected spikes. In this blotter method, 10 spikes were placed on blotter paper (moistened with water drop) in plastic Petri dishes. Then the Petri dishes were incubated at the incubation room at 25^{O}C. After 3-4 days of incubation pathogenic structures (mycelia) on spikes and blotter paper were observed under stereo binocular microscope. Then the fungal structures were transferred on Potato Dextrose Agar (PDA) medium for 10-12 days in the incubation room to allow the fungus to grow. The concern pathogen was detected by preparing slide and comparing the morphological character as pear shaped conidia.

Meena, (2005) studied the four culture media viz. oat meal agar media (40 g of rolled oats, 5 g of sucrose, 16 g of agar and 1000 ml of distilled water), Sucrose agar media (200g of peeled potatoes, 20 g of sucrose, 20g of agar and 1000 ml of distilled water), malt extract agar media (35.5 g of malt extract agar, and 1000 ml of distilled water) and potato dextrose agar media (200g of peeled potatoes, 20 g of dextrose, 20 g of agar and 1000 ml of distilled water) were used to compare the growth rate of *M. oryzae triticum* isolates after 10 days inoculation.

Priya Vanaraj *et al.,* (2013) Blast infected spike were surface sterilized with 0.1% mercuric chloride for 1 minute and placed over clean glass slides kept in sterile Petri dishes padded with moist cotton. The Petri dishes were incubated for 48 hours at room temperature (28±2°C). Single conidia were identified from the sporulating lesions using a stereomicroscope and aseptically transferred to potato dextrose agar (PDA) slants for maintenance. The causal organism was identified as *Pyricularia oryae* based on the spore morphology.

2.12. Sporulation of the pathogen

Meena, (2005) showed colony color of all the wheat blast (*M. oryzae triticum*) isolates was usually buff with good growth on oat meal agar, greyish black with medium growth on host seed extract + 2% sucrose agar, the raised mycelial growth with smooth colony margin on potato dextrose agar and raised mycelium with concentric ring pattern on Richard's agar medium. On host seed extract + 2% sucrose agar all the blast pathogenic isolates showed black to greyish black color with smooth colony margin and good growth.

Srivastava *et al.,* (2014) observed blast fungal isolates produced ring like, circular, irregular colonies with rough and smooth margins on oat meal agar media having buff color, greyish black to black color. The colony diameters of different groups ranged from 67.40 to 82.50 mm and the conidial shape of the different groups was pyriform (pear-shaped) with rounded base and narrowed towards the tip which is pointed or blunt. Gashaw *et al.,* (2014) also observed on oat meal agar, colony color of all the isolates was usually grey with good growth. All the isolates showed raised mycelial growth with smooth colony margin.

2.13. Morphological characters of the pathogen

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. (Ono and Nakazato 1958) observed that, the size of conidia of *M. oryzae*

triticum varied with the culture media also (Mijan 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 × 4 – 7 µm (Average, 17.4 µm × 5.2µm). (Veeraraghavan and Padmanabhan1965) also measured the dimensions of conidia produced by *P. oryzae* ranged from 17.6 to 24.0 µm in length and 8.0 to 9.6 µm in width.

Meena, (2005) also studied from the margin of actively growing of *M. oryzae triticum* isolates; 6 mm diameter mycelia discs of the 14-day old cultures of different *M. oryzae triticum* isolates were inoculated on the middle of the Petri plates and three replications were maintained for each media. The inoculated Petri plates were kept at 30°C. The colony diameter of the growth of each isolate was measured after 10th day of the incubation period and the growth was calculated in mm with the help of a scale. The different colony characters like pigmentation, color of mycelia, surface texture, margin, mycelial growth, sporulation and size, shape and septation of conidia were recorded in all four media by visual and microscopic observations.

2.14. Cultural characterization of the pathogen

2.14.1. Growth of the pathogen in different media

Mijan Hossain, (2000) observed that among the non-synthetic media, potato dextrose agar produced maximum radial growth (85.00 mm), next was host extract + 2 per cent sucrose agar medium (80.33 mm) followed by oat meal agar (75.00 mm). Cruz *et al.,* (2009) observed the higher sporulation on wheat meal culture medium in alternate light, dark regime.

Mahdieh *et al.,* (2013) reported that OMA culture medium could provide the best medium for *M. oryzae triticum* vegetative growth, regardless of light condition. However, *M. oryzae triticum* could sporulate when light was provided either continuously or at intervals. A combination of 16/8 hrs. light/darkness intervals and adding rice materials to culture media could induce *M. oryzae triticum* for a better sporulation.

Ravindramalviya, (2014) used four culture media for the study of mycelial growth of *M. oryzae triticum* under *in-vitro*. Among them PDA media produced maximum mycelial growth followed by Richard's Agar medium after 168 hrs. of incubation. Then sporulation of *M. oryzae triticum* was observed in traces in Potato dextrose agar medium and Richard's Agar medium after 168 hrs. of incubation.

2.15. *In-vitro* evaluation botanical against *Magnaporthe oryzae triticum*

Ankri and Mirelman, (1999); Harris *et al.,* (2001); Borlinghaus *et al.,* (2014) observed based on the findings of the present study it may be concluded that garlic clove extract was most effective under *in-vitro* as it completely inhibited mycelial growth up to 93.33% and exhibited minimum disease incidence and severity and highest yield contributing parameters at 1:10 dilution. Ghazanfar *et al.,* (2011); Satya *et al.,* (2007) also observed the literature it is clear that garlic extract contains antifungal compounds Allicin and this compound might also inhibit the vegetative and reproductive growth of MoT studied here. Moreover, garlic extract might also trigger the genes which are involved with the induced resistance in the host plants.

Tripathi *et al.,* (2004) observed that spraying of plant products Wanis, Achook and Neem gold along with standard chemical fungicide shows an effective reduction in leaf blast severity in rice. Pandey *et al.,* (2015) tested four leaf extract and observed that *A. indica* leaf extract was found most effective in suppressing mycelial growth of blast pathogen *in-vitro*.

Iftikhar *et al.,* (2010); Babar and Khan, (1999) studied on the other hand, some botanical pesticides and bio-control agents have proved to be most secure and have no adverse impact on the environment. So, finding out of eco-friendly and non-toxic approaches for wheat blast management is the main aspect of the present research work.

Borlinghaus *et al.,* (2012) investigated two essential oils and one oleoresin and found that pepper oil was effective against *P. oryzae* pathogen. Plant extract of rue (*Rutagra veolens*) has potentiality to suppress rice blast *in-vitro,* as well as in greenhouse

conditions without damaging cell wall and plasma membrane of the fungus (Reis *et al.,* 2015). Sireesha, (2013) observed that Neem seed kernel extract, was considered second best after *Pseudomonas flourescens* in controlling leaf blast and enhancing grain yield in rice.

Manjappa, (2013) reported these findings are in agreement with the findings reported by other researchers. Eupatorium (*Chromolaena odorata* L.) an obnoxious weed which can inhibit the growth of *Pyricularia oryzae* when eupatorium extract extracted with acetone (91.3%) followed by methanol (85.6%), distilled water (74.5%) and petroleum ether (53.9%).

Suriani *et al.,* (2015) also showed there is an alternative measure to control rice blast disease by using leaf extract of *Piper caninum* blume. Antifungal activity of *P. caninum* against *M. Oryzae triticum* was done under laboratory condition on potato dextrose agar (PDA) medium and the leaf extract of *P. caninum* significantly ($P<0.05$) inhibited the fungal radial growth, spore's formation, and biomass formation.

Pandey, (2015) studied aqueous leaf extract of *Azadirachta indica*, *Emblica officinalis, Pongamia glabra* and *Acacia nilotca* inhibit the mycelial growth of *Magnaporthe oryzae triticum* causing leaf blast and *Bipolaris oryzae* causing brown spot in rice under laboratory condition.

Hubert *et al.,* (2015) examined roots of *Chloranthus japonica* and stem of *Paulownia coreana* were effective in the management of rice blast. Treatments with *P. guineense* and Carbendazim had comparable for leaf blast suppression (Choi *et al.,* 2004).

Jantasorn *et al.,* (2016) showed this result is in conformity with the findings where they described that at the 10,000-ppm concentration of *H. anthelminthicus* fruit extracts exhibited antifungal potential to growth inhibition, and recorded 100% growth inhibition against *Pyricularia oryzae, P. palmivora and R. solani* followed by *S. rolfsii* at 96.33% when compared with water control. *X. lanceatum* fruit extract logged excellent inhibitory activity against *P. oryzae*.

The use of plant derived products with azadiractin as principal constituent was tested to be successful in controlling rice leaf blast (Amadioha *et al.*, 2000, Sireesha *et al.*, 2013, Govindaraju *et al.*, 2016, Kumar *et al.*, 2017). Spraying of commercial neem-based biopesticide increases height, number of tillers per plant and yield contribution factors such as percentage of productive spikelet's, 100 grains of weight and grains yield per plant as compared to chemical pesticide (Kumar *et al.*, 2017).

CHAPTER III

MATERIALS AND METHODS

Wheat blast disease caused by *Magnaporthe oryzae triticum* of the genus *Magnaporthe* of the family Pyriculariaceae is one of the most devastating diseases in the world and can cause up to 100% yield losses under the favorable environmental conditions. The present investigations were carried out under field as well as laboratory condition during January-July, 2019 to ascertain the incidence, severity of wheat blast and in-vitro evaluation of botanicals against *Magnaporthe oryzae triticum* in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, and Dhaka. The material used and techniques adopted during the investigation are being summarized under here.

3.1. Survey period and survey site

A survey was conducted on the farmers' field affected by wheat blast disease at 10 village of Meherpur and 10 village of Chuadanga districts and at 10 village of Mujibnagar Upazila of Meherpur Bangladesh during January-April, 2019. (**Table 3**). *In-vitro* experiment was conducted in the Plant Pathology laboratory, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh, during the period of January-July, 2019. (**Fig. 1**)

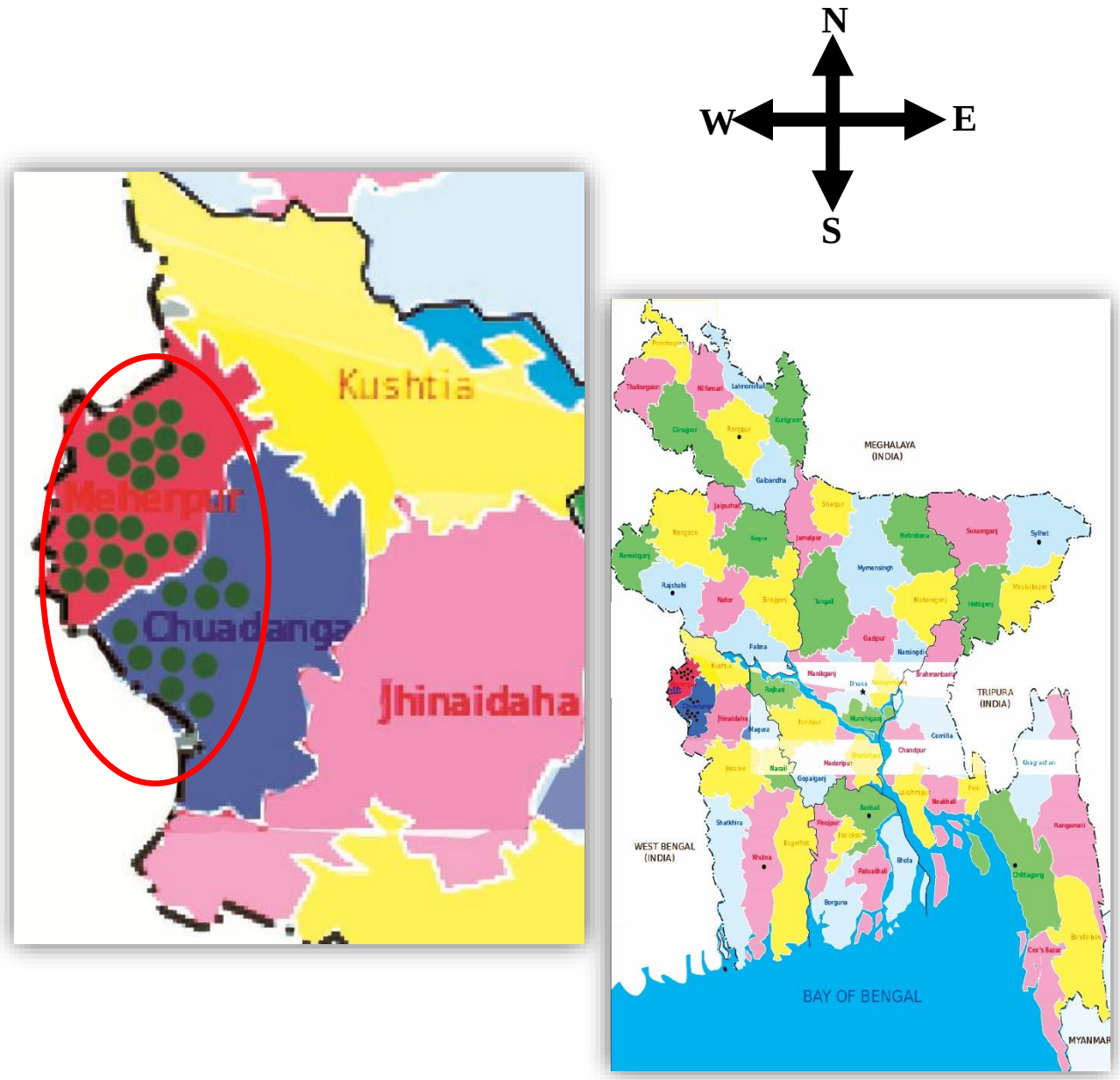


Fig 1. Survey sites of wheat blast in south-western wheat growing regions of Bangladesh(https://en.wikipedia.org/wiki/Districts_of_Bangladesh#/media/File:BD_Map_admin.svg)

3.2. Survey on incidence and severity of wheat blast at farmers' field

Visual symptoms of disease were assessed. The survey was conducted using simple random sampling method, within at 2-3 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 $1m^2$ quadrants and used to calculate the average incidence and severity of wheat blast (Asfaha *et. al.* 2015). 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. The incidence of the disease was calculated using the number of infected panicles affected by the disease divided by the total number of panicles assessed and expressed in percentage (Maciel *et. al*. 2013).

Disease incidence (%) = Pi/Pt ×100

Where, Pi =Number of panicle infected, and Pt =Total number of panicles.

Blast disease severity was determined using the scale proposed by Trindade *et al.*, (2006). It was based according to the point at which the pathogen had penetrated the rachis and affected the length of the spike. The score 0 referred to no 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**).

Table 1. Scale used for severity measurement of wheat blast disease

Scale	Description
0	No visual symptoms
1	25 % of the spike area affected
2	50% of the spike area affected
3	75% of the spike area affected
4	100% of the spike area affected

3.3. Diseased plant sample collection

Blast infected ears of wheat were collected from infected farmers' fields. Infected ears were cut from the mother plant field dried and placed in brown paper envelopes, which were labeled with all necessary information's including the name of the region, district, localities, cultivars and date of collection. Samples were kept in refrigerator at 4^0C until the surveys in all the districts were finalized. Then samples were transported to Plant Pathology Laboratory, SAU for pathogen identification and characterization.

3.4. Isolation and identification and purification of wheat blast isolates

The water agar (Agar 20 g with 1000 ml distilled water), and potato dextrose agar media (200g of peeled potatoes, 20 g of dextrose, and 20 g of agar and 1000 ml of distilled water) were used for the isolation of blast pathogen. Diseased spikes of wheat cultivars infected with pathogen were cut into suitable size (15-20 cm in size) around the area showing the blast lesion and were surface sterilized with 1% sodium hypochlorite for 1 minute followed by 3 times washes with sterile distilled water. Then the plant pieces were placed in Petri dishes lined with moist filter papers and it was incubated at 26 ± 1^0C for 24 hours to encourag sporulation. After incubation, these infected spike pieces were examined under stereo-dissecting microscope. Abundant sporulation was observed from in and around the lesions with grey, dense and bushy appearance. A sterile moistened needle was used to pick out single conidia by the needle across the sporulating lesion. The conidia were placed on water agar. After 12 hours, mycelium was visible in petri dish and it then hyphal tip was placed in potato dextrose agar media plates containing Streptomycin (40 mg/L) and pure culture of *M. oryzae triticum* was prepared by incubating there in 26 ± 1^0C. The marginal mycelial growth that developed subsequently was picked-up aseptically for sub-culturing until pure culture of *M. oryzae triticum* was obtained. The pure culture was maintained by sub culturing at an interval every 15 days and preserved at low temperature (4^0C) in refrigerator.

For sporulation, oat meal agar (40g of oats, 5g of sucrose, 20g of agar and 1000ml of distilled water) plates were used. After placing the block of mycelium of *M. oryzae*

triticum, plates were incubated at 26±1°C for about 10 to 15 days with alternate 12 hours. darkness and 12 hours light for sporulation. After conidia production in OMA plates, the conidia of *M. oryzae tricicum* were checked under compound microscope. Identification of the pathogen was carried out according to the cultural and morphological characteristics as described by (Agrawal *et al.,* 1989 and Mew *et al.,* 2002).

3.5. Designation of collected isolates

Collected isolates were designated based on location. For example, on isolate designated by MEMoTO1 means it was collected from Meherpur, MoT means *Magnaporthe oryzae triticum* on 01 denotes serial number.

3.6. Pathogenicity tests for *M. oryzae triticum* isolates

The pathogenicity test of the isolates was done for further confirmation of pathogenic isolates of wheat blast pathogen. The pathogenicity of all 35 purified isolates of *M. oryzae triticum* was confirmed by Koch's postulates using the method of Chevalier *et al.,* 1991. The pot was prepared for this test using sterilized soil. The soil was collected from near field. Disinfected viable seeds of BARI Gom-24 (Pradip) susceptible to wheat blast variety were sown in pots with 6-7 seeds per pot. The plants were inoculated after germination, at the age of 3-4 leaves and the seedlings in each pot was sprayed with 40–50 ml of spore suspension adjusted to 10^5 spores/ml with the help of hemocytometer. Atomizer sprayer was rinsed with 95% Ethanol and then washed with sterile distilled water and used for spraying (Hans *et al.*, 2003). The conidial suspensions were sprayed on to the wheat seedlings until runoff while water was used for spraying the control treatment. Inoculated pots were covered with polythene bags. The plants were placed inside the dew chamber at 26±^{0}C for 7 days. Periodical observations were made for the development of symptoms on the leaves starting 7 days after inoculation. Experiments were done with three replications. The fungus was re-isolated from the artificially inoculated wheat seedlings leaves showing typical blast symptom.

3.7. Morphological diversity and cultural characteristics of *Magnaporthe oryzae triticum*

Different isolates of MoTs were grown on Potato Dextrose Agar (PDA) for 30 days. Data on radial mycelia growth (mm), growth rate (mm/day) and cultural characteristics were determined at 7, 14, 30 days after inoculation. Morphological diversity of the collected isolates was determined and expressed by cluster dendrogram analysis.

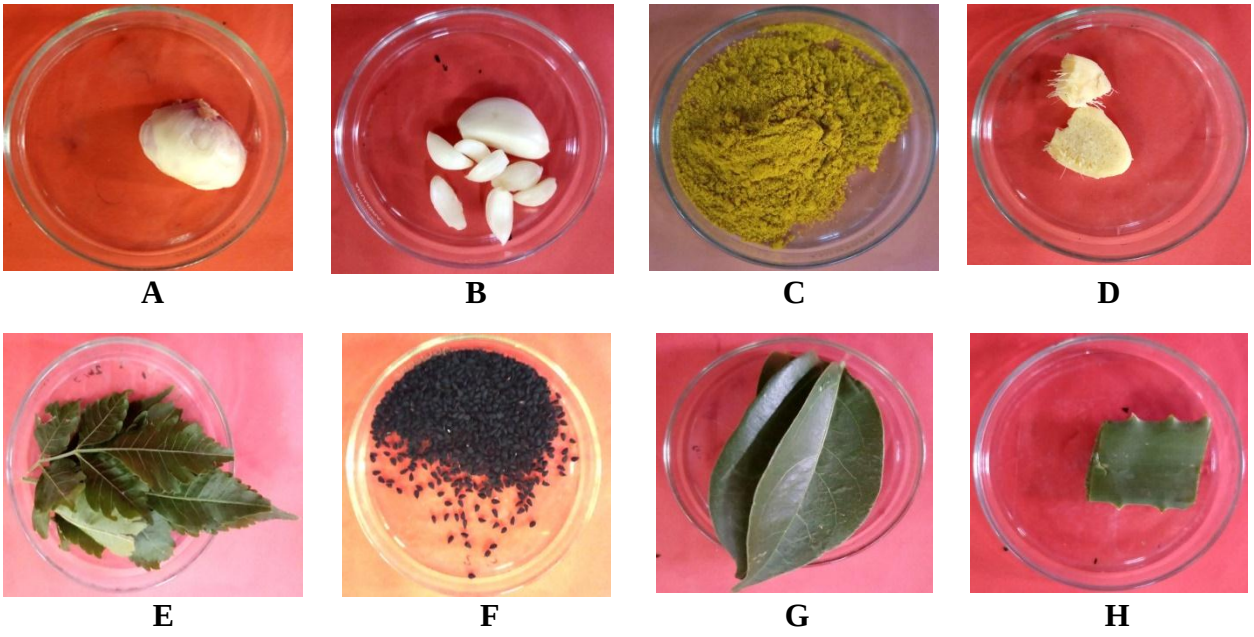
3.8. Evaluation of ethanol extracts of botanicals against *Magnaporthe oryzae triticum in-vitro*

3.8.1. Preparation of botanical extracts

Fresh plant parts namely *Azadirachta indica* (Neem leaf), *Allium cepa* (Onion bulb), *Allium sativum* (Garlic), *Curcuma longa (*Turmeric), *Zingiber officinale* (Ginger), *Allamanda cathartica* (Allamanda leaf), *Nigella sativa* (Black cumin) and *Aloe vera* (Allovera) were used as treatments (**Table 2 and Plate 1**). Solvent, ethanol (95%) was used for the phytochemical extraction of various plant parts. Three concentration 1:1 (w/v), 1:0.50 (w/v) and 1:0.25 (w/v) of ethanol was used for botanical extraction. For 1:1 (w/v) concentration extraction with ethanol, 100g of plant materials was dissolved in 100ml ethanol (**Plate 2**) The mixture was kept undisturbed at room temperature for 24 hrs. in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath until 100ml extract was left in the container. For 1:0.50 (w/v) and 1:0.25 (w/v) 100g of plant materials were dissolved in 50ml and 25ml ethanol, respectively. Ethanolic extract thus obtained were immediately evaluated for antifungal activities using poisoned food technique (Daferera *et al.*, 2000).

Table 2. Botanicals used in controlling mycelia growth of *Magnaporthe oryzae triticum in-vitro*

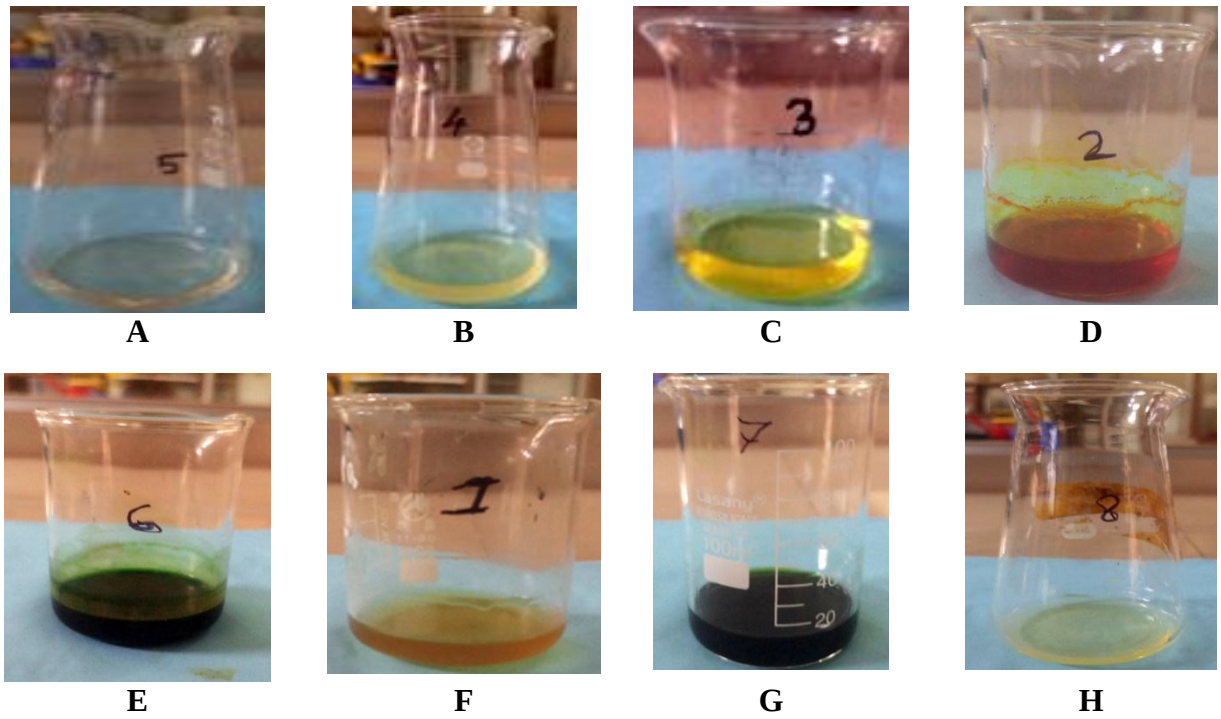
Sl. No.	Name of botanicals	Plant parts used	Concentration used
1	*Allium cepa* (0nion)	Bulb	1:0.25 (w/v)
			1:0.50 (w/v)
			1:1 (w/v)
2	*Allium sativum* (Garlic)	Clove	1:0.25 (w/v)
			1:0.50 (w/v)
			1:1 (w/v)
3	*Curcuma longa (*Turmeric)	Rhizome	1:0.25 (w/v)
			1:0.50 (w/v)
			1:1 (w/v)
4	*Zingiber officinale* (Ginger)	Rhizome	1:0.25 (w/v)
			1:0.50 (w/v)
			1:1 (w/v)
5	*Azadirachta indica* (Neem)	Leaf	1:0.25 (w/v)
			1:0.50 (w/v)
			1:1 (w/v)
6	*Nigella sativa* (Black cumin)	Seed	1:0.25 (w/v)
			1:0.50 (w/v)
			1:1 (w/v)
7	*Allamanda cathartica* (Allamanda)	Leaf	1:0.25 (w/v)
			1:0.50 (w/v)
			1:1 (w/v)
8	*Aloe vera* (Allovera)	Leaf	1:0.25 (w/v)
			1:0.50 (w/v)
			1:1 (w/v)
9	Control	No botanical extracts	0nly PDA



A **B** **C** **D**

E **F** **G** **H**

Plate 1. Botanicals used in controlling mycelial growth of *Magnaporthe oryzae triticum in-vitro.* A. *Allium cepa,* B. *Allium sativum,* C. *Curcuma longa,* D. *Zingiber officinale,* E. *Azadirachta indica,* F. *Nigella sativa,* G. *Allamanda cathartica,* H. *Aloe vera*



A **B** **C** **D**

E **F** **G** **H**

Plate 2. Botanicals extracts used in controlling mycelial growth of *Magnaporthe oryzae triticum in-vitro.* Extracts of A. *Allium cepa,* B. *Allium sativum,* C. *Curcuma longa,* D. *Zingiber officinale,* E. *Azadirachta indica,* F. *Nigella sativa,* G. *Allamanda cathartica,* H. *Aloe vera*

3.8.2. *In-vitro* efficacy of botanical extracts against *Magnaporthe oryzae triticum*

PDA plates were amended with different concentration (1:1 w/v, 1:0.50 w/v, 1:0.25 w/v) of botanicals extracts separately. The plates were inoculated with 5mm fungal blocks of *M. oryzae triticum* and these blocks were transferred to the center of the petri plates with the help of sterilized needle. Five days after inoculation, the mycelial growth of *M. oryzae triticum* was recorded by measuring the average of two diameters at right angles to one another. Three replications were maintained for each of the treatment and the mean radial mycelial growth was considered for each of the treatment. Then the effect of plant extract was calculated as percent growth inhibition using the following formula as adopted by (Satish *et al.*, 2007 and Dubey *et al.*, 2009).

3.9. Statistical analysis

Analysis of data of different parameters was subjected to perform by statistical analysis using R software version 3.6.0 (R Core Team, 2019). Cluster analyses with Ward's hierarchical method were used to classify percent mycelial growth data with respect to blast isolates into groups. Comparison of means were conducted with Duncan's multiple range tests at 5% statistical probability level to examine mean statistical differences among treatments.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Assessment of blast disease intensity

During the survey, the results of the assessment revealed that the intensity (incidence and severity) of the disease vary from slight to high intensity depending on the survey sites agro-ecological zones and cultivars. A cultivar with awns on the spikes was chosen to elaborate the diagrammatic scale because most wheat cultivars used in Brazil have this characteristic. However, to obtain the severity values shown in the scale, the awns were not considered as the part of the spikes **(Fig 2**.). This convention was used for both the scale elaboration

Fig 2. Diagrammatic scale for assessing blast severity caused by *Magnaporthe oryzae triticum* on wheat spikes. The awns were not considered in the determination of severity on the spikes

and the validation procedures and was adopted by considering that, in general, the infection process caused by the fungus *M. oryzae triticum* does not reach this segment of the spike. Minimum and maximum blast severity levels found for the collected spikes were 25% and 100%, respectively. These values were considered lower and upper limits, respectively, in relation to the symptomatic spikes of the diagrammatic scale (Figure 1). In total, 6 spikes formed the diagrammatic scale, one of which is asymptomatic (0% no blast, i.e., the first one in the scale) and the other four shown different blast severity values.

4.2. Symptomatology

Leaf blast symptoms

Magnaporthe oryzae triticum created lesions on leaves, initial symptoms gray-green and water-soaked lesions with dark green borders which become lighten with necrotic borders, once they have completely expanded with typical eye shaped necrotic lesions with grey centers and dark brown margin was observed on a severely blast infected wheat leaf.

Head symptoms

Magnaporthe oryzae triticum affected wheat spikes was typical bleached head symptom from the point of infection. The symptoms on spikes are most prominent and easily visible. The pathogen infects the rachis and develops dark brown discoloration at the point of infection with or without dark brown mycelial growth. The spikes may be completely or partially bleached. (**Plate 3)**

4.3. Incidence and severity of wheat blast across wheat cultivars

The assessment of incidence and severity of blast disease varied depends on cultivars. The most dominant cultivar BARI Gom-24 (Pradip) grown by the farmers named but the other cultivars like BARI Gom-26, BARI Gom-28 etc. were recently introduced which were grown in few farmer fields in Meherpur Sadar, Mujibnagar and Chuadanga Sadar

upazilla. During this assessment, cultivar such as BARI Gom-24 (Pradip) was scored the highest incidence and severity level of blast disease whereas the cultivars such as BARI Gom-26, BARI Gom-28 were scored the lowest incidence and severity (**Table 3)**.

Table 3. Severity of spike blast (*Magnaporthe oryzae triticum*) disease of wheat at different locations in Bangladesh in Rabi season, 2019

Name of districts	Name of upazilla	Village Name	Name of variety	Blast disease		
				Incidence (%)	Severity %	Degree of severity
Meherpur	Meherpur Sadar	Kathalpara	BARI Gom-28	21	25	1
		Amdoho	BARI Gom-24 (Pradip)	65	71	3
		Kathalpota	BARI Gom-24 (Pradip)	52	53	2
		Amjhupi	BARI Gom-24 (Pradip)	89	97	4
		Kutubpur	BARI Gom-24 (Pradip)	100	100	4
		Pirojpur	BARI Gom-26	19	23	1
		Buripota	BARI Gom-24 (Pradip)	63	68	3
		Rajapur	BARI Gom-28	23	24	1
		Sonapur	BARI Gom-24 (Pradip)	87	92	4
		Chanbil	BARI Gom-26	22	25	1
Meherpur	Mujibnagar	Darajpur	BARI Gom-24 (Pradip)	69	77	3
		Monkhali	BARI Gom-24 (Pradip)	95	99	4
		Baguan	BARI Gom-24 (Pradip)	51	54	2
		Charulia	BARI Gom-26	17	19	1
		Joterpur	BARI Gom-24 (Pradip)	92	94	4
		Komurpur	BARI Gom 28	22	25	1
		Mohajonpur	BARI Gom-24 (Pradip)	67	76	3
		Hogoldanga	BARI Gom-24 (Pradip)	46	50	2
		Ibrahimpur	BARI Gom-28	21	24	1
		Korimpur	BARI Gom-26	23	25	1
Chuadanga	Chuadanga Sadar	Parkrisnapur	BARI Gom-24 (Pradip)	96	100	4
		Hajrahati	BARI Gom-24 (Pradip)	40	48	2
		Begumpur	BARI Gom-26	25	25	1
		Muminpur	BARI Gom-24 (Pradip)	42	47	3
		Jotarpur	BARI Gom-24 (Pradip)	87	92	4
		Komorpur	BARI Gom-28	21	24	1
		Shibpur	BARI Gom-24 (Pradip)	73	75	3
		Muminpur	BARI Gom-28	71	75	3
		Bollolpur	BARI Gom-26	20	23	1
		Maniknagar	BARI Gom-24 (Pradip)	49	53	2

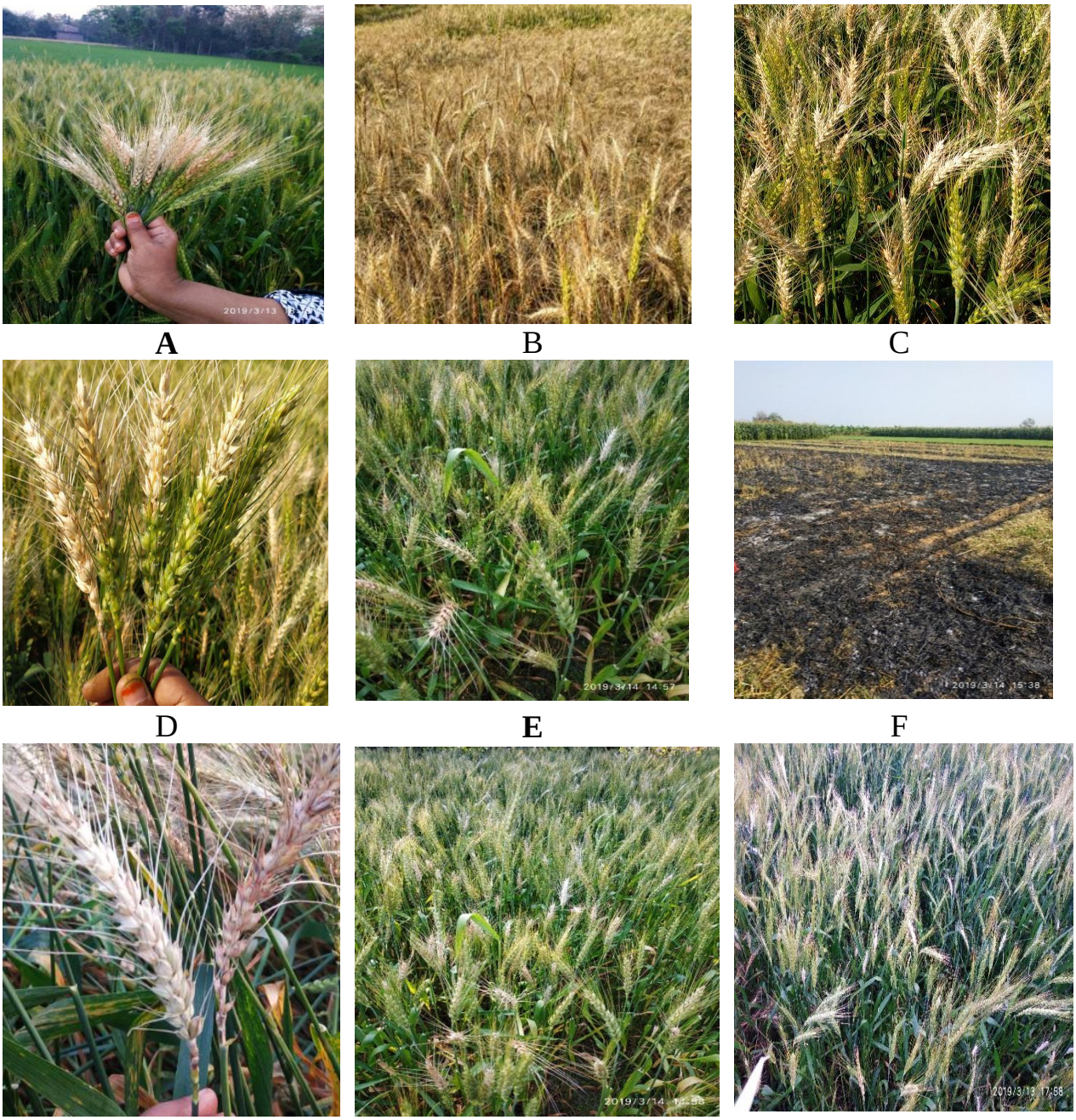


Plate 3. Bleached wheat spikes in a blast-infected field in Meherpur region (A, B, C), in Mujibnagar (D, E, F) and in Chuadanga (G, H, I)

A

B

C

D

E

F

Plate 4. Tagging of healthy panicle and wheat blast infected panicale in the field of Meherpuer (A, B), Mujibnagar (C, D) and Chuadanga (E, F)

4.4. Wheat production of Healthy panicle vs Blast panicle

In Meherpur Sadar for healthy wheat plant, the highest number of seed per panicle was recorded in Pirojpur, it was 47.87 and the lowest number of seed per panicle in Amdoho, it was 28.10g seed per panicle. Seed weight per panicle was maximum in field Pirojpur, it was 2.43g. Lowest weight of seed per panicle was minimum in Kutubpur, it was 1.64g in field. Weight of thousand seed was high in Pirojpur (58.98g) and lowest weight of thousand seed was Amdoho (47.23). For wheat blast disease, maximum seed per panicle was Pirojpur (28.21g) and minimum seed per panicle was Sonapur (16.89). Seed weight per panicle was maximum in field Pirojpur, it was 0.91g. Lowest weight of seed per panicle was minimum in field Pirojpur, it was 0.61g in field. Thousand seeds weight was also lower than healthy plant. The highest thousand seeds weight was Pirojpur (32.26g) and the lowest was Amdoho (28.12g). **(Table 4)**.

In Chuadanga Sadar Upazilla, the highest amount of seed per panicle was recorded in Komurpur and it was 42.03g for healthy plant that was more than diseased plant Charulia and it was 24.02 seed per panicle. Maximum weight of seed per panicle was recorded in Komurpur and it was 3.14g and minimum weight was Monkhali which was also more than blast plant maximum 0.91g and minimum 0.30g respectively. Thousand seeds weight of healthy plant (max 66.83 g and min 35.14g) was also higher than blast affected plant (max 32.75 g and min 21.95g) **(Table 5)**.

In Mujibnagar Sadar, the highest amount of seed per panicle was recorded in Begumpur and it was 40.67 for healthy plant that was more than diseased plant Shibpur and it was 16.09g seed per panicle. Maximum weight of seed per panicle was recorded in also Begumpur and it was 2.31g and minimum weight was recorded in Shibpur and it was 1.61g which was also more than blast plant maximum 0.63g and minimum 0.54g respectively. Thousand seeds weight of healthy plant (max 61.93g and min 55.93g) was also higher than blast affected plant (max 28.95g and min 25.48g) **(Table 6)**.

Table 4. Number of seed/panicles, weight(g) of seed/panicle, 1000-seed weight (g) collected from healthy and bleached panicle as influenced by collection 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)
Kathalpara	BARI Gom-28	42.35 d	2.19 c	51.98 c	18.87 e	0.49 f	31.89 b
Amdoho	BARI Gom-24 (Pradip)	28.10 g	1.76 e	47.53 f	16.89 e	0.61 e	28.12 f
Kathalpota	BARI Gom-24 (Pradip)	29.53 fg	1.78 e	51.01 d	25.02 bc	0.73 c	29.02 f
Amjhupi	BARI Gom-24 (Pradip)	43.09 c	2.20 b	49.87 d	22.03 d	0.59 e	27.02 g
Kutubpur	BARI Gom-24 (Pradip)	28.00 g	1.64 f	47.23 f	22.98 cd	0.75 c	30.67 c
Pirojpur	BARI Gom-26	47.87 a	2.43 a	58.98 a	28.21 a	0.91 a	32.26 a
Buripota	BARI Gom-24 (Pradip)	45.89 b	1.83 d	50.11 e	25.21 b	0.83 b	31.06 c
Rajapur	BARI Gom-28	32.23 ef	1.79 e	58.23 b	24.12 bc	0.67 d	31.10 c
Sonapur	BARI Gom-24 (Pradip)	31.78 e	1.75 e	48.21 f	17.98 e	0.48 f	29.87 c
Chanbil	BARI Gom-26	31.09 e	1.79 d	55.87 b	28.02 a	0..81 b	27. 94 e
CV %		2.76	0.9431	1.41	5.21	2.14	0.3978
LSD (0.05)		1.9134	0.0372	1.3076	1.9879	0.0260	0.2401

Mean with the same letters are not significantly different

Table 5. Number of seed/panicles, weight(g) of seed/panicle, 1000-seed weight (g) collected from healthy and bleached panicle as influenced by collection sites and variety in Chuadanga sadar upazilla.

Villages	Variety	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)
Dariapur	BARI Gom-24 (Pradip)	33.97 c	1.21 c	35.14 f	20.67 c	0.45 d	22.12 c
Monkhali	BARI Gom-24 (Pradip)	34.01 c	1.16 c	33.95 g	21.04 bc	0.42 d	21.95 c
Baguan	BARI Gom-24 (Pradip)	32.77 c	2.39 b	62.12 c	20.98 bc	0.64 c	29.78 b
Charulia	BARI Gom-26	26.76 e	1.30 c	34.21 e	24.02 a	0.89 a	33.10 a
Jotarpur	BARI Gom-24 (Pradip)	37.91 b	2.29 b	60.98 d	21.02 c	0.30 e	21.95 c
Komurpur	BARI Gom 28	42.03 a	3.14 a	66.83 a	21.83 ab	0.69 b	32.75 a
Mohajonpur	BARI Gom-24 (Pradip)	37.96 b	3.53 a	62.75 b	21.47 abc	0.71 b	20.86 c
Hogoldanga	BARI Gom-24 (Pradip)	29.79 d	1.31 c	32.93 h	15.21 d	0.33 e	31.62 a
Ibrahimpur	BARI Gom-28	36.98 b	1.34 c	33.32 e	20.45 ab	0.91 a	21.01 c
Korimpur	BARI Gom-26	43.13 a	3.48 a	65.56 a	21.28 ab	0.69 b	32.18 a
CV %		2.97	11.41	0.2978	6.87	2.65	1.29
LSD (0.05)		1.8163	0.3897	0.2564	2.4336	0.0261	0.519

Mean with the same letters are not significant different.

Table 6. Number of seed/ panicles, weight(g) of seed/panicle, 1000-seed weight (g) 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)
Parkrisnapur	BARI Gom-24 (Pradip)	31.87 d	1.72 e	56.82 f	21.67 a	0.63 a	28.12 c
Hajrahati	BARI Gom-24 (Pradip)	37.90 ab	2.32 a	58.85 c	16.21 c	0.59 ab	29.13 b
Begumpur	BARI Gom-26	40.67 a	2.31 a	61.93 a	18.27 b	0.54 c	29.49 c
Muminpur	BARI Gom-24 (Pradip)	35.23 c	2.09 bc	61.03 b	17.70 b	0.57 bc	28.97 d
Jotarpur	BARI Gom-24 (Pradip)	36.74 bc	2.32 a	58.85 d	21.81 a	0.61 a	31.21 a
Komorpur	BARI Gom-28	31.80 d	1.78 e	57.89 e	21.77 a	0.63 a	27.41 c
Shibpur	BARI Gom-24 (Pradip)	30.17 d	1.61 e	55.93 f	16.09 c	0.54 c	25.48 d
Muminpur	BARI Gom-28	33.21 d	1.96 d	58.71 d	21.90 a	0.61 a	28.14 c
Bollolpur	BARI Gom-26	36.98 bc	2.15 b	57.23 e	22.02 a	0.62	28.95 b
Maniknagar	BARI Gom-24 (Pradip)	35.86 c	2. 12 b	59.16 c	17.43 b	0.54 c	31.10 a
CV %		3.41	1.75	0.0498	6.79	4.21	0.3926
LSD (0.05)		2.0591	0.0653	0.0587	2.4063	0.0418	0.1934

Mean with the same letters are not significantly different.

4.5. Isolation, identification and purification of *M. oryzae triticum*

Samples were placed in moist chamber and examined under sterio microscope and found conidia then pure culture was made and from this pure culture conidia of the pathogen were confirmed under compound microscope (**Fig 3**).

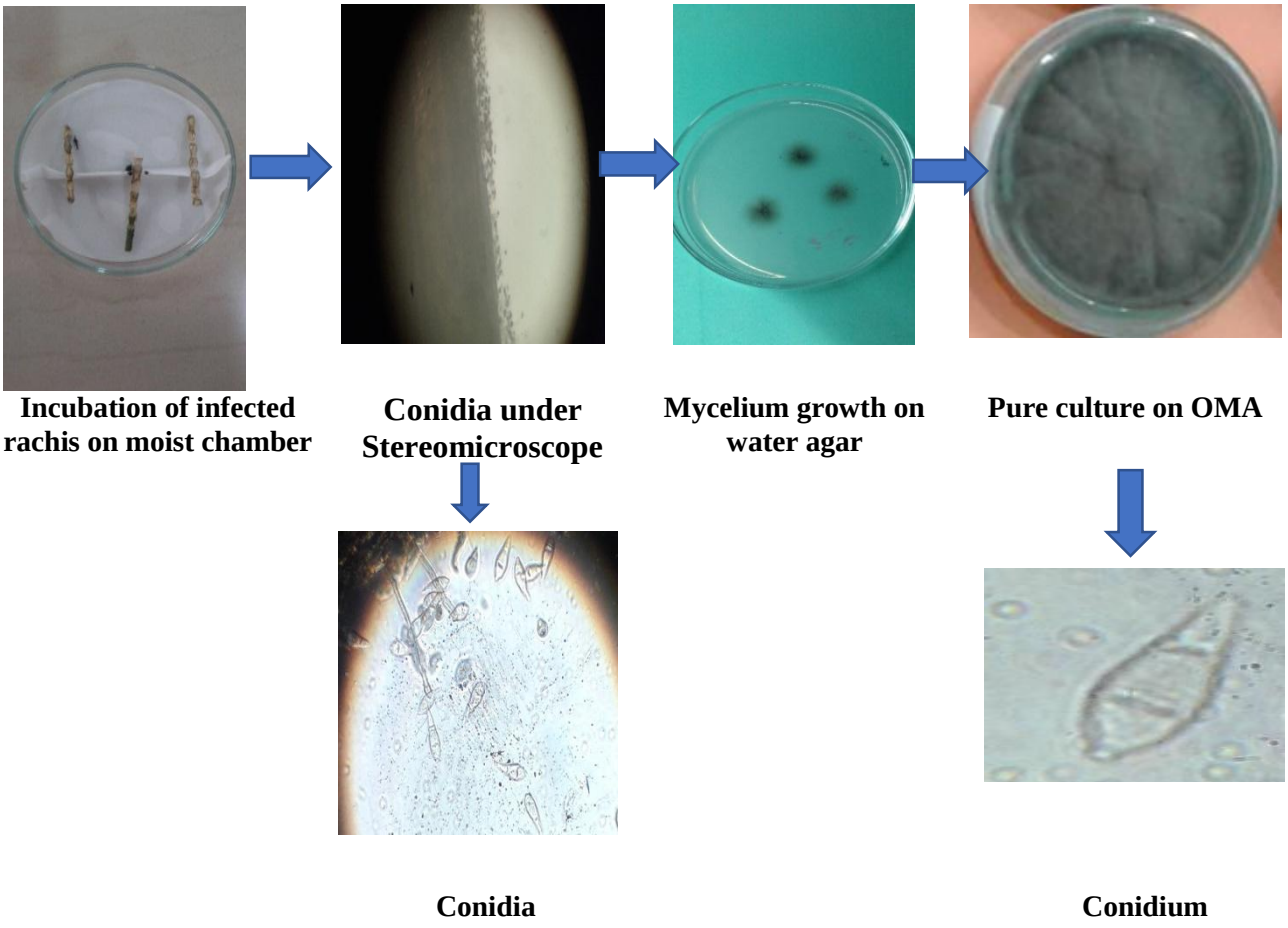


Fig 3. Flow chart of isolation, identification, and culture of *Magnaporthe oryzae triticum* on OMA media

4.6. Cultural and morphological characterization of MoT isolates

Significant variation among the colony characteristics of the different isolates were observed in culture plates which were collected from different localities. All the 12 MEMoT, 12 MUMoT and 11 CHMoT isolates grown on PDA media were observed the mycelial color, surface texture and shape and growth (**Plate 5**). On the PDA medium, the isolate MEMoTo1, MEMoT04 and MUMoT06 colony color was dark gray and the light gray color in the isolate, MEMoT03, MEMoT08, MUMoT02, MUMoT08, MUMoT12, CHMoT04, CHMoT05, CHMoT08 and CHMoT09 and off-white color in isolates MUMoT11, grayish black color in the other five isolates like MUMoT03, MUMoT07, CHMoT01 and CHMoT06, grayish white color in the other five isolates like MEMoT02, MEMoT06, MEMoT10, MEMoT12 and MUMoT01, dark black color colony MEMoT07 and MUMoT09 were observed. **(Table 7)**

On the PDA media, the colony of the isolates CHMoT08 were light black in color whereas the isolate MEMoT05 and MUMoT09 were light gray. The surface texture of the isolates MEMoT01, MEMoT2, MEMoT06, MEMoT07, MUMoT01, MUMoT03, MUMoT06, MUMoT08, CHMoT01, CHMoT06 and CHMoT10 rough velvety and colony medium in growth, MUMoT05 and CHMoT11 rough cottony and other surface texture of isolates smooth cottony and poor in growth were observed. Colony of the isolates MEMoT01, MEMoT06, MEMoT07, MEMoT09, MEMoT12, MUMoT04, MUMoT07, MUMoT12, CHMoT03 and CHMoT05 margins were irregular and other colony of the isolates margin were regular. Other all colonies were good growth. **(Table 7)**

Mew and Gonzales, (2002) stated that the colony color of Mo isolates was appeared gray on PDA medium. Mew and Misra, (1994) reported that the colonies of Mo isolates on PDA medium showed blackish. Mew and Gonzales, (2002) also indicated that the *M. oryzae triticum* pathogen colonies on PDA medium grow very slowly and colony on the reverse side of the agar plates were black.

For spore characteristics of different isolates of Mo were observed on OMA media. The results showed that all of the conidia in each isolate was pyriform in shape, base rounded, apex narrowed, two-septate, with three celled observed in isolates of Mo2, Mo11, Mo16 and Mo19 and one-septate with two celled was observed in other isolates. The conidium in each isolate was observed hyaline to pale olive colors. Ono and Nakazato, (1958) observed that the size of conidia of *M. oryzae triticum* varied with the culture media. The sizes as well as shape of the spores and colonies of filamentous fungi are the most important factors in fungal identification. The present study results were also supported by the other workers (Mew and Gonzales, 2002; Meena, 2005 and Afshana *et al.*, 2011)

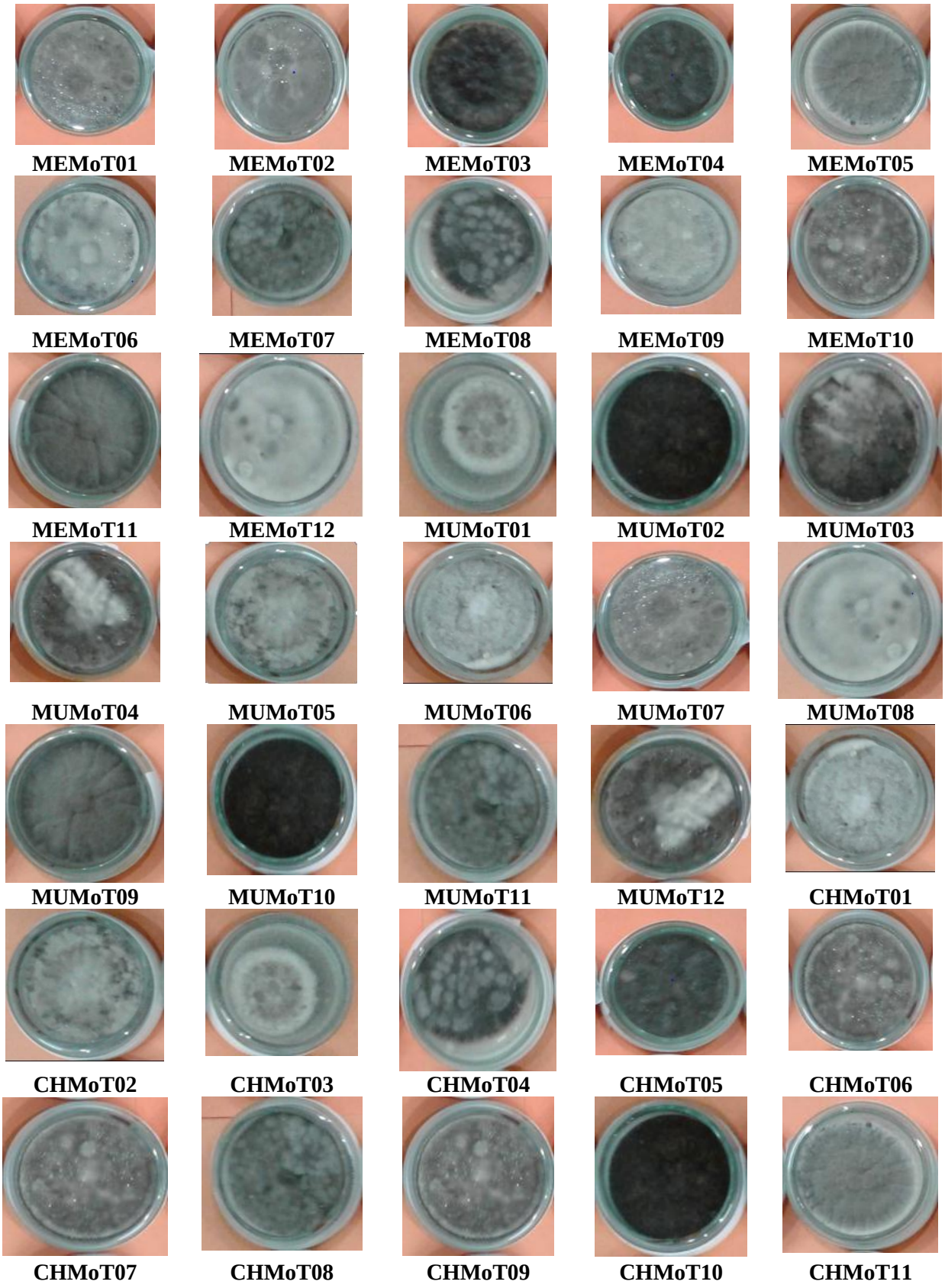


Plate 5. Mycelial growth of 35 isolates of *M. oryzae triticum* on PDA media at 30 days after inoculation

Table 7. Cultural characteristics of 35 isolates of *Magnaporthe oryzae triticum* on PDA

Sl. No.	Isolates	Colony			Mycelial growth
		Color	Surface texture	Shape	
01	MEMoT01	Dark gray	Rough, velvety	Irregular	Medium growth
02	MEMoT02	Grayish white	Rough, velvety	Regular	Medium growth
03	MEMoT03	Light gray	Smooth, cottony	Regular	Poor growth
04	MEMoT04	Dark gray	Smooth, cottony	Regular	Medium growth
05	MEMoT05	Whitish black	Smooth, cottony	Regular	Medium growth
06	MEMoT06	Grayish white	Rough, velvety	Irregular	Medium growth
07	MEMoT07	Dark black	Rough, velvety	Irregular	Good growth
08	MEMoT08	Light gray	Smooth, cottony	Regular	Good growth
09	MEMoT09	Whitish	Smooth, cottony	Irregular	Good growth
10	MEMoT10	Light grayish	Smooth, cottony	Regular	Medium growth
11	MEMoT11	Dark black	Smooth, cottony	Regular	Good growth
12	MEMoT12	Grayish white	Smooth, cottony	Irregular	Poor growth
13	MUMoT01	Grayish white	Rough, velvety	Regular	Medium growth
14	MUMoT02	Light gray	Smooth, cottony	Regular	Medium growth
15	MUMoT03	Grayish black	Rough, velvety	Regular	Medium growth
16	MUMoT04	Grayish white	Smooth, cottony	Irregular	Poor growth
17	MUMoT05	Light gray	Rough, cottony	Regular	Medium growth
18	MUMoT06	Dark gray	Rough, velvety	Regular	Medium growth
19	MUMoT07	Grayish black	Smooth, cottony	Irregular	Medium growth
20	MUMoT08	Light gray	Rough, velvety	Regular	Medium growth
21	MUMoT09	Whitish	Smooth, cottony	Regular	Medium growth
22	MUMoT10	Grayish black	Smooth, cottony	Regular	Good growth
23	MUMoT11	Off-white	Smooth, cottony	Regular	Poor growth
24	MUMoT12	Light gray	Smooth, cottony	Irregular	Good growth
25	CHMoT01	Grayish black	Rough, velvety	Regular	Medium growth
26	CHMoT02	Grayish black	Smooth, cottony	Regular	Medium growth

Sl. No.	Isolates	Colony			Mycelial growth
		Color	Surface texture	Shape	
27	CHMoT03	Grayish black	Smooth, cottony	Irregular	Good growth
28	CHMoT04	Light gray	Smooth, cottony	Regular	Medium growth
29	CHMoT05	Light gray	Smooth, cottony	Irregular	Good growth
30	CHMoT06	Grayish black	Rough, velvety	Regular	Good growth
31	CHMoT07	Light black	Smooth, cottony	Regular	Good growth
32	CHMoT08	Light gray	Smooth, cottony	Regular	Good growth
33	CHMoT09	Light gray	Smooth, cottony	Regular	Good growth
34	CHMoT10	Yellowish	Rough, velvety	Regular	Poor growth
35	CHMoT11	Yellowish	Rough, cottony	Irregular	Medium growth

4.7. Mycelial growth of wheat blast isolates on PDA media

The results revealed that there is a considerable variation among the colony diameter of the *M. oryzae triticum* isolates on PDA media. The mean of radial mycelial growth of different isolates on PDA were optimum for all the cultures of MoT isolates. The radial mycelial growth of MoT isolates CHMoT09 (25.67 mm) on the 7thdays, CHMoT05 (53.33 mm), CHMoT06 (56.33 mm), CHMoT07 (55.83 mm) CHMoT08 (56.33 mm) and CHMoT09 (57.00 mm) on the 14th days and CHMoT08 (75.50 mm) on the 30th days showed significantly highest on PDA media. On the other hand the radial mycelial growth of MoT isolates MEMoT10 (14.17 mm) and MUMoT05 (14.00cm) on the 7th days, MEMoT01 (25.50cm), MEMoT02 (25.17cm), MEMoT03 (26.67cm) and MEMoT11 (28.67cm) on the 14th days and MEMoT09 (40.50cm) and MEMoT01 (40.50) on the 30thdays showed significantly lowest on PDA media (**Table 8)**.

Mijan Hossain, (2000) observed that among the non-synthetic media, potato dextrose agar supported maximum radial growth (85.00 mm), next was host extract + 2 per cent sucrose agar medium (80.33 mm) followed by oat meal agar (75.00 mm).

Cruz *et al.*, (2009) observed the higher sporulation on wheat meal culture medium in alternate light, dark regime.

Table 8. Mycelial growth of 35 isolates of *M. oryzae triticum* at different days of incubation on PDA media

Sl. No.	Isolates	Mycelial Growth (mm)		
		7 DAI	14 DAI	30 DAI
01	MEMoT01	14.67 fg	25.50 g	40.83 i
02	MEMoT02	16.67 e-g	25.17 g	41.67 g-i
03	MEMoT03	16.00 e-g	26.67 g	41.50 hi
04	MEMoT04	16.83 e-g	32.67 d-g	49.00 f
05	MEMoT05	15.00 fg	30.33 e-g	48.00 f
06	MEMoT06	16.33 e-g	30.33 e-g	47.50 fg
07	MEMoT07	14.17 g	31.33 d-g	49.17 f
08	MEMoT08	16.17 e-g	31.33 d-g	50.00 f
09	MEMoT09	16.00 e-g	29.67 fg	40.50 i
10	MEMoT10	14.33 g	31.33 d-g	48.00 f
11	MEMoT11	15.67 e-g	28.67 g	45.50 f-i
12	MEMoT12	14.83 fg	30.33 e-g	47.00 f-h
13	MUMoT01	16.83 e-g	30.33 e-g	48.00 f
14	MUMoT02	16.17 e-g	30.33 e-g	50.00 f
15	MUMoT03	15.50 e-g	31.33 d-g	49.00 f
16	MUMoT04	17.00 e-g	31.33 d-g	44.17 f-i
17	MUMoT05	14.00 g	30.33 e-g	47.83 f
18	MUMoT06	15.67 e-g	33.00 d-g	50.00 f
19	MUMoT07	15.50 e-g	31.33 d-g	48.50 f
20	MUMoT08	15.67 e-g	29.67 fg	47.00 f-h
21	MUMoT09	16.17 e-g	31.00 d-g	45.50 f-i
22	MUMoT10	16.33 e-g	31.33 d-g	48.50 f
23	MUMoT11	16.50 e-g	31.33 d-g	50.00 f

Sl. No.	Isolates	Mycelial Growth (mm)		
		7 DAI	14 DAI	30 DAI
24	MUMoT12	16.67 e-g	34.00 d-g	66.50 de
25	CHMoT01	18.00 ef	39.00 c-f	72.00 a-d
26	CHMoT02	21.50 cd	40.00 c-e	64.00 e
27	CHMoT03	23.50 a-c	43.67 bc	75.00 ab
28	CHMoT03	22.83 a-c	44.67 bc	72.00 a-d
29	CHMoT04	24.50 a-c	53.33 a	70.00 a-d
30	CHMoT05	24.50 a-c	56.33 a	69.00 c-e
31	CHMoT06	25.10 ab	55.83 a	69.50 b-d
32	CHMoT07	25.17 ab	56.33 a	75.50 a
33	CHMoT08	25.67 a	57.00 a	74.50 a-c
34	CHMoT09	21.83 b-d	49.50 ab	50.00 f
35	CHMoT10	18.83 de	40.17 cd	45.67 f-i
	CV (%)	9.96	13.74	5.78

Means with the same letter are not significantly different.

DAI= Days after inoculation

4.8. Percent mycelial growth rate per day on PDA media

Different mycelial growth rate was observed when 35 isolate of *M. oryzae triticum* was cultured on PDA media. On the basis of percent growth rate of mycelia per day, all isolates were distributed into five groups **(Fig 4)**. Lowest growth rate was between 7.0 to 7.9 and five isolates (MEMoT02, MEMoT09, MUMoT01, CHMoT02, MUMoT09) were situated in this group. Among 35 isolates, percent growth rate per day of one third isolates (12) were 7.9 to 8.8, where isolates were MEMoT01, MEMoT04, MEMoT05, MEMoT10, MEMoT11, MEMoT12, MUMoT04, MUMoT08, MUMoT10, CHMoT06, CHMoT07 and CHMoT10. Highest growth rate was found in Isolate MUMoT12 (10.79). Mycelial growth between 8.8% to 9.7% as recorded from eleven isolates (MEMoT03, MEMoT06, MEMoT07, MUMoT02, MUMoT06, MUMoT07, CHMoT01, CHMoT03,

CHMoT04, CHMoT09, CHMoT11) and six isolates (MEMoT08, MUMoT03, MUMoT05, MUMoT11, CHMoT05 and CHMoT08) growth rate were between 9.7% - 10.6%.

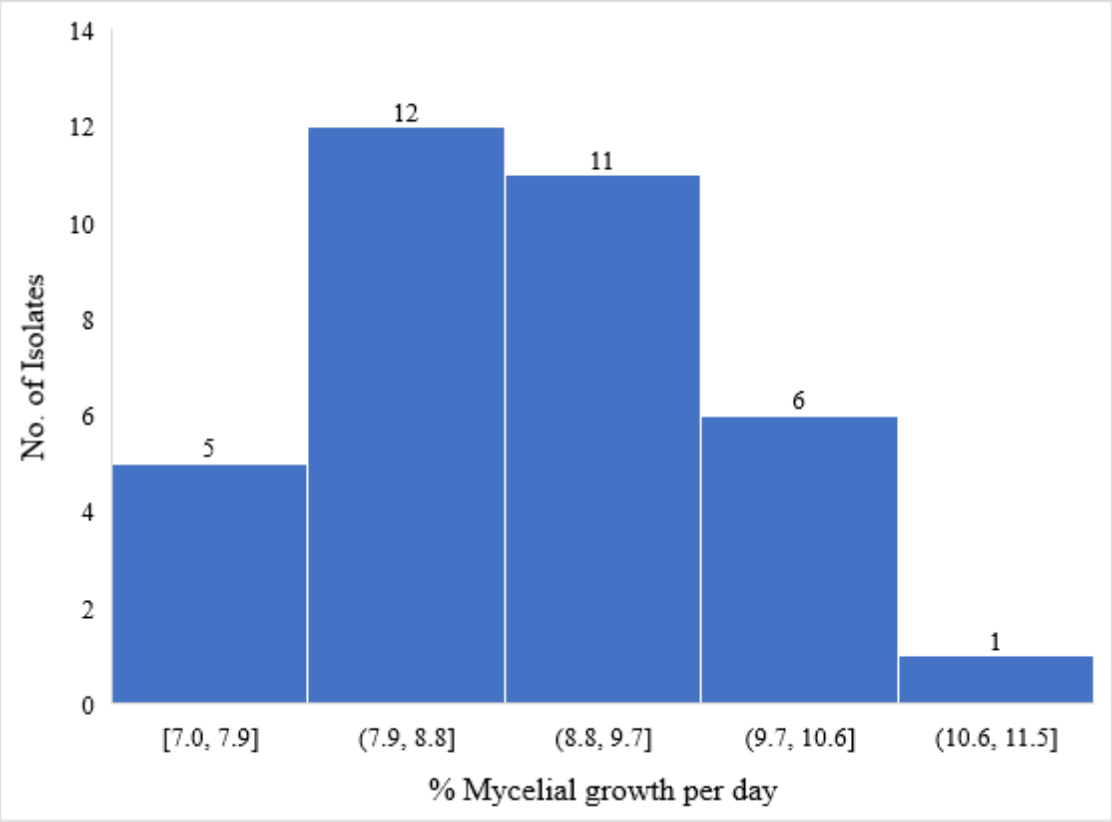


Fig 4. Histogram of mycelial growth rate per day of 35 isolates of *Magnaporthe oryzae triticum* on PDA media

4.9. Diversity of *M. oryzae triticum* isolates

Isolates of *M. oryzae triticum* showed different growth rate on PDA media *in-vitro.* The isolates were classified based on the percent growth rate at different age of isolates on PDA media into three cluster groups, cluster I, cluster II and cluster III **(Fig 5).** Twenty-five isolates were grouped in cluster I; 6 isolates were cluster II and 4 isolates were in cluster III. Among the 25 isolates of cluster I no distance was found between isolate Mo8 and isolate Mo23, which indicate those two may be same isolate. Most of the isolates were grouped into cluster I and the distance among the isolates of cluster I was very low which indicates low variation among the isolates. High variation was observed between clusters I and cluster II & cluster III. These findings indicate that different types of wheat blast isolates are distributed among the wheat blast affected area in Bangladesh.

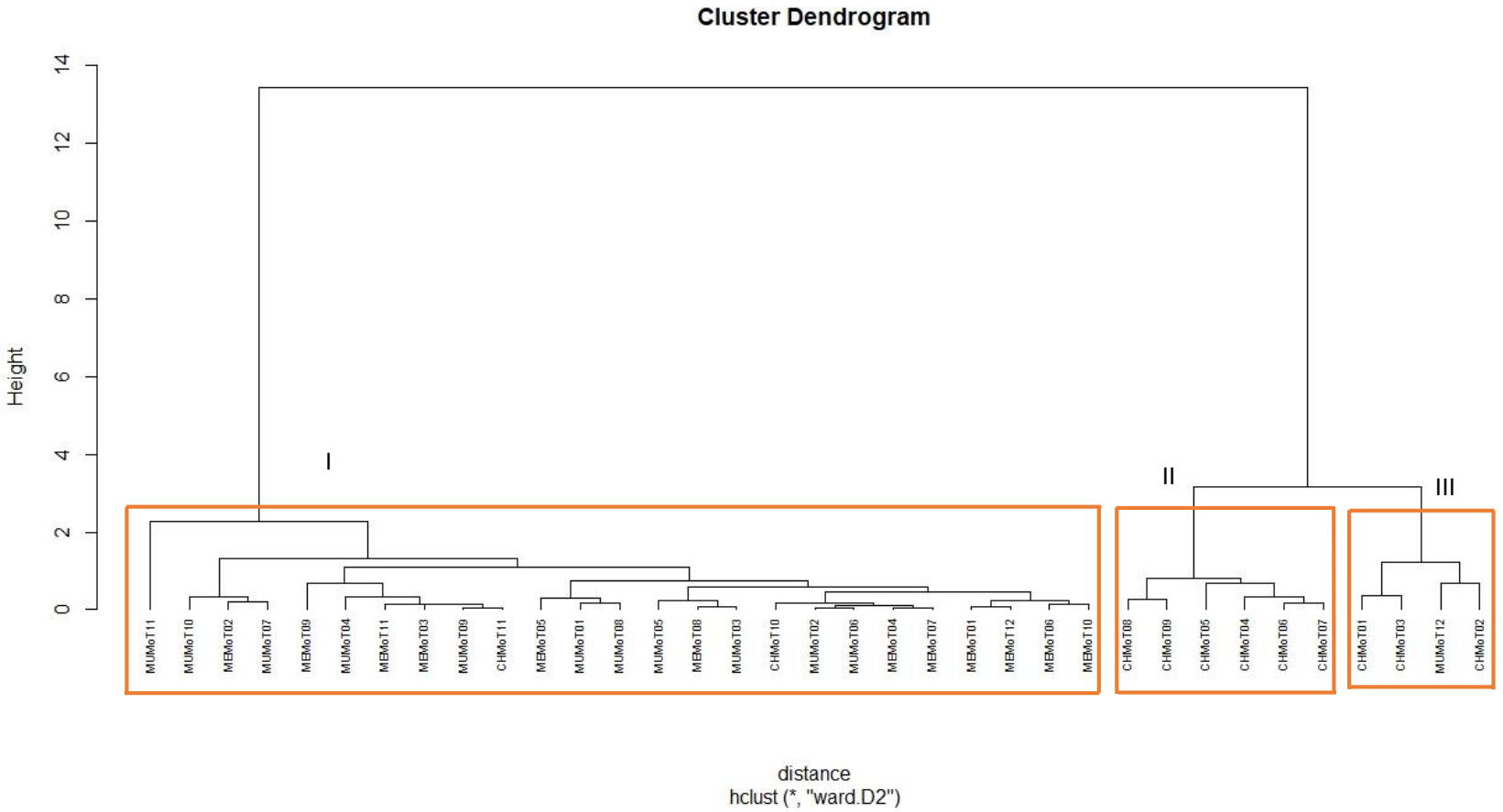


Fig 5. Classification of wheat blast isolates on the basis of the percent growth rate at different age of isolates *Magnaporthe oryzae triticum* grown on PDA media. Cophenetic Correlation Coefficient is 0.939

4.10. Pathogenicity test for *Magnaporthe oryzae triticum* isolates

Pathogenicity test results revealed that the disease symptoms and development of 12 MEMoT, 12 MEMoT and 11 CHMoT isolates on susceptible local cultivar after inoculation with the inoculum of the test *M. oryzae triticum* isolates. The diamond and spindle shaped with gray center and dark brown to necrotic margins were observed on all of the wheat seedlings after the 7th day of inoculation. After 7 day of inoculation, 86.66% disease incidence with 63.2 % of average disease severity was recorded in *M. oryzae triticum* inoculated wheat plants; whereas the disease was not developed in un inoculated wheat plants. The *M. oryzae triticum* isolates were re-isolated from the infected lesions and compared with the original culture and thus Koch's postulates were proved. The re-isolation revealed that the isolated fungi from diseased wheat seedlings were found to be identical with those used for artificial inoculation. Although the reaction types showed by local susceptible wheat cultivar to *M. oryzae triticum* isolates were similar in pot but the disease severity was more intense in the field of the selected localities. All the isolates were the causative agents for blast disease of wheat. Pathogenicity test revealed that all *M. oryzae triticum* isolates were able to infect local susceptible wheat (**Fig 6**).

A **B**

Fig 6. *Magnaporthe oryzae triticum* seedling (A). Eye shaped water-soaked lesion with on inoculated leaf (B)

4.11. *In-vitro* evaluation of *Magnaporthe oryzae triticum* against botanical

4.11.1. Colony character of *M. oryzae triticum* on PDA media supplemented with different plant extracts

The growth characteristics like color of substrate/media, color of colony and margin of colony of *M. oryzae triticum* PDA media supplemented with different plant extracts were observed in this study. The color of the colony of *M. oryzae triticum* was grey ash centre and black margin in case of PDA media. Whereas, PDA media supplemented with *Azadirachta indica* (Neem leaf) extraxt was showed white with grey, *Allamanda cathartica* (Allamanda leaf) extract, *Aloe vera* (Allovera) leaf extract, and *Nigella sativa* (Black cumin) seed extracts showed white colony color. *Allium cepa* (Onion bulb) extracts, *Allium sativa* (Garlic clove) extracts and *Curcuma longa (*Turmeric rhizome) extracts and *Zingiber officinale* (Ginger) showed rhizome extract showed grey with white color colony This result indicated that PDA media supplemented with different plant extracts have an impact on the colony color of *M. oryzae triticum* **(Plate 7 and Table 9).**

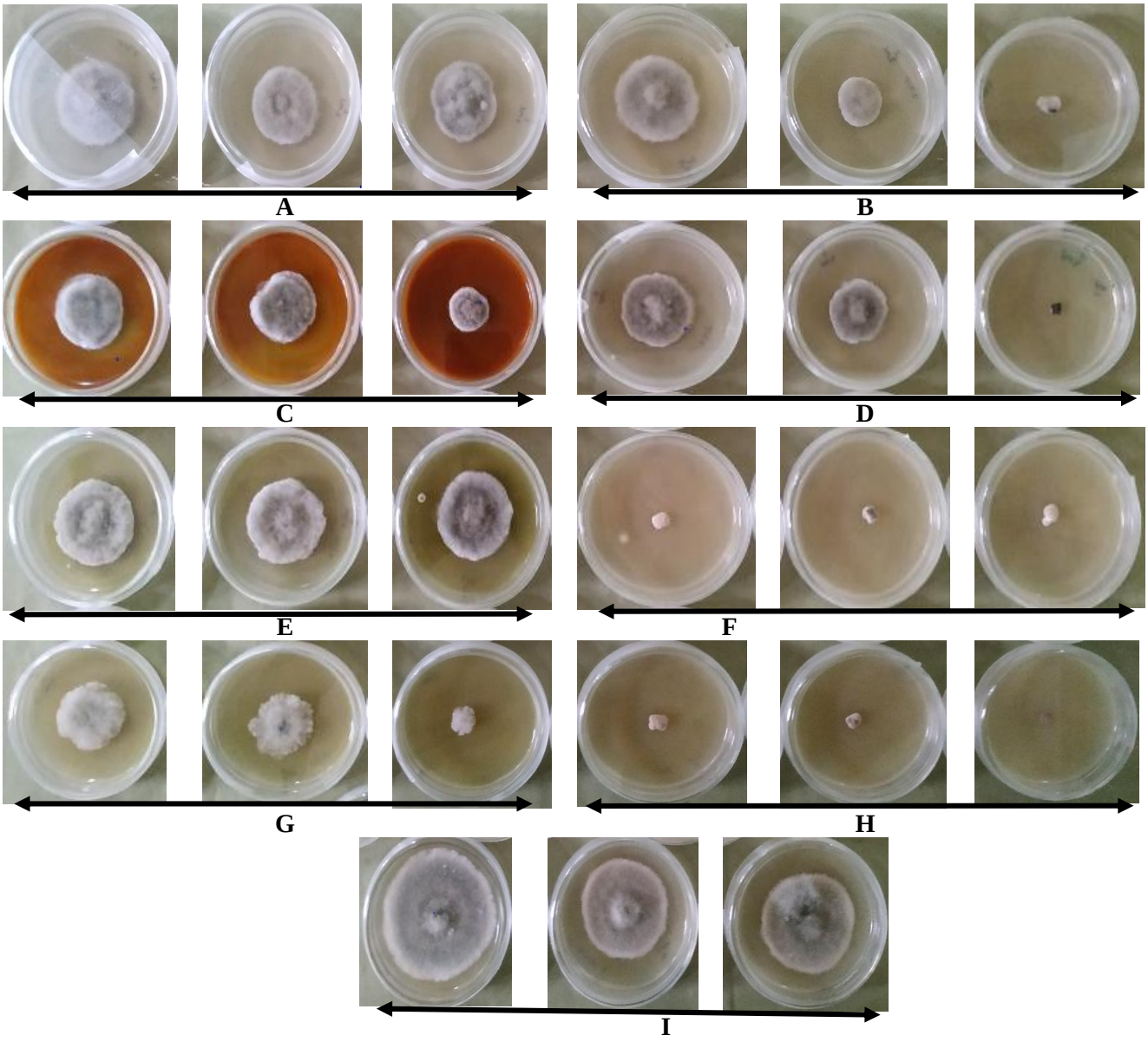


Plate 7. Mycelial growth, color and appearance of *M. oryzae triticum* on PDA media supplemented with different plant extracts with ethanol extracts of botanicals (7 DAI). A. *Allium cepa,* B. *Allium sativum,* C. *Curcuma longa,* D. *Zingiber officinale,* E. *Azadirachta indica,* F. *Nigella sativa,* G. *Allamanda cathartica,* H. *Aloe vera,* I. Control

4.11.2. Efficacy of different botanical extracts on mycelial growth of *M. oryzae triticum*

All botanicals were found significantly effective in reducing mycelial growth of the pathogens compare to control **(Table 9**). The result revealed that *Aloe vera* (Allovera leaf) extracts was found most effective in minimizing the mycelial growth at 7 days but in 14 days *Nigella sativa* (Black cumin seed) extracts and *Aloe vera* (Allovera leaf) extracts both botanicals can minimize the mycelial growth of pathogens. (**Table 9**). The observations support the findings of (Sireesha and Venkateswarlu, 2013) who found the efficacy of plant parts extract of Neem seed kernel, Neem oil, Pongamia spp. The present findings also gain support from the work of Sandeep (2015) who observed the similar pattern of growth suppression in blast and brown spot pathogens using leaf extract of Neem (*Azadirachta indica*), Emblica (*Emblica officinalis*), Karanj (Pongamia glabra) and Babool (*Acacia nilotica*) against rice blast fungus.

Table 9. Efficacy of botanicals on mycelial growth of *M. oryzae triticum*

Sl. No.	Treatments	Radial mycelial growth (mm)	
		7 DAI	14 DAI
01	*Allium cepa* extracts	35.22 b	42.00 d
02	*Allium sativum* extracts	21.67 e	52.56 b
03	*Curcuma longa* extracts	26.78 d	47.11 c
04	*Zingiber officinale* extracts	26.78 d	36.78 f
05	*Azadirachta indica* extracts	31.56 c	52.00 b
06	*Nigella sativa* extracts	4.11 g	10.00 g
07	*Allamanda cathartica* extracts	26.22 d	40.33 e
08	Aloe vera extracts	5.22 f	8.89 g
09	Control	49.56 a	68.78 a
	CV (%)	3.68	3.02

Means with the same letter are not significantly different.

DAI= Days after inoculation

4.11.3. Effect of concentration level on mycelial growth of *M. oryzae triticum*

Three concentration level of botanicals (1:4 w/v @0.1%, 1:2 w/v @0.2%, 1:1 w/v @0.4%) was tested against the pathogen and (1:1) found the best concentration in both 7 days and 14 days **(Table 10)**. The mycelial growth of *M. oryzae triticum* decreases with the increasing concentration of all plant extracts tested. The results are similar with the findings of (Al-Hazmi, 2013) who reported that Neem leaf extract were mostly affective in growth retardation of the *Helminthosporium* sp. fungi when applied at the highest concentration (1:1 w/v).

Table 10. Effect of different concentration level of botanicals on mycelial growth of *M. oryzae triticum*

Concentration	Radial Mycelial growth (mm)	
	7 DAI	14 DAI
1:0.25 w/v @0.1%	30.37 a	47.59 a
1:0.50 w/v @0.2%	25.89 b	40.74 b
1:1 w/v @0.4%	19.44 c	31.15 c
CV (%)	3.68	3.02

Means with the same letter are not significantly different.

DAI=Days after inoculation

4.11.4. Effects of ethanol extracts of botanicals on mycelia growth and colony characters of *Magnaporthe oryzae triticum*

Antimicrobial activities of eight botanicals with specific concentration were assayed and results on presented in **Table 11**. The data revealed that botanicals were found significant in suppression of mycelia growth at higher concentration over untreated check in the fungal pathogens.

The result reveals that the minimum mycelial growth was recorded from *Nigella sativa*: 1:1 w/v @ 0.4% (3.00 mm) combination at 7 days culture age which is statistically similar with *Aloe vera*: 1:1 w/v @ 0.4% (3.33mm) and *Nigella sativa*:1:0.50 w/v @ 0.2% (4.33 mm) combination. Control plates (only PDA: 1:0.50 w/v @ 0.2% and only PDA: 1:1 w/v @ 0.4%) were always observed highest growth of mycelium. At 14 days culture age, lowest mycelial growth was observed in *Aloe vera*: 1:1 w/v @ 0.4% combination (6.00 mm). In 2nd lower growth recorder from *Aloe vera*: 1:0.50 w/v @ 0.2% (9.00 mm) and *Nigella sativa*: 1:1 w/v @ 0.4% (9.00) combination, which were statistically identical each other. Control plates (only PDA: 1:0.25 w/v @ 0.1% (50.00) and only PDA: 1:0.50 w/v @ 0.2% (49.00) and only PDA: 1:1 w/v @ 0.4% (49.67)) were observed highest growth of mycelium both at 7 days culture age and 14 days culture age.

The highest concentration of botanicals extracts was more pronounced compare to low concentration in reducing the radial growth of the fungus as observed in case of blast pathogen. The mycelial growth of *M. oryzae triticum* decreases with the increasing concentration of all botanical's extracts tested compare to control. The findings are similar to Amadioha (2000) who reported that the cold-water extract of neem compared favorably with Cardendazim at 0.1% a.i. in controlling the rice blast *in-vivo*. In an experiment, (Sireesha and Venkateswarlu, 2013) found plant parts extract of Neem seed kernel, Neem oil, *Pongamia* spp are efficient to control fungal growth. Research of Sandeep, (2015) is also support the present findings in which they observed the similar pattern of growth suppression in blast and brown spot pathogens using leaf extract of

Neem (*Azadirachta indica*), Emblica (*Emblica officinalis*), Karanj (*Pongamia glabra*) and Babool (*Acacia nilotica*). Extracts Panchagavya and *Asafoetida* spp. extract in descending order against rice blast fungus. Hajano *et al.,* (2012) observed higher dose of garlic completely inhibited the mycelial growth of *M. oryzae triticum*. Gohel and Chauhan, (2015) reported that Neem leaf extract were found effective but comparably less significant than standard fungicides and bio-agent in minimizing leaf blast intensity in rice. Hubert *et al.*, (2015) observed that extracts from *C. arabica, N. tabacum, A. vera, A. indica,* were found significant to manage rice blast disease *in-vitro* and *in-vivo*. The research is also in accordance with (Khoa *et al.,* 2011) who observed that foliar spray of aqueous extracts of herbal plants have been found effective in reducing rice blast severity. Results on suppression of *M. oryzae triticum* under various concentration of plant extracts were significant. Al-Hazmi, (2013) reported that Neem leaf extract were mostly affective in growth retardation of the *Helminthosporium* sp. fungi when applied at the highest concentration (1:1, v: v).

Table 11. Effects of ethanol extracts of botanicals on mycelia growth and colony characters of *Magnaporthe oryzae triticum*

Treatments	Ethanol botanicals ratio (w/v)	Radial mycelia growth (mm)		Colony character	
		7 DAI	14 DAI	Colony color	Shape
Allium cepa (0nion)	1:0.25	38.33 b	58.33 c	Gray ash	Regular
	1:0.50	36.00 c	41.67 hi		
	1:1	31.33 f	26.00 l		
Allium sativum (Garlic)	1:0.25	31.00 f	60.33 bc	Gray ash	Regular
	1:0.50	23.00 i	56.33 d		
	1:1	11.00 m	41.00 i		
Curcuma longa (Turmeric)	1:0.25	33.33 e	59.00 bc	Gray with white margin	Regular
	1:0.50	29.00 g	49.00 f		
	1:1	18.00 k	33.33 j		
Zingiber officinale (Ginger)	1:0.25	34.00 de	46.33 g	Gray with white margin	Regular
	1:0.50	26.67 h	40.33 i		
	1:1	19.67 j	23.67 m		
Azadirachta indica (Neem)	1:0.25	39.00 b	61.00 b	Grey with white	Regular
	1:0.50	30.33 fg	51.67 e		
	1:1	25.33 h	43.33 h		
Nigella sativa (Black cumin)	1:0.25	5.00 o	11.33 n	White	Regular
	1:0.50	4.33 o-q	9.67 no		
	1:1	3.00 q	9.00 o		
Allamanda cathartica (Allamanda)	1:0.25	35.00 cd	51.00 e	White	Regular
	1:0.50	30.00 fg	40.33 i		
	1:1	13.67 l	29.67 k		
Aloe vera (Allover)	1:0.25	7.67 n	11.67 n	White	Regular
	1:0.50	4.67 op	9.00 o		
	1:1	3.33 pq	6.00 p		
Control	Only PDA	50.00 a	69.33 a	Grey with white	Regular
	Only PDA	49.00 a	68.67 a		
	Only PDA	49.67 a	68.33 a		
CV%		3.68		3.02	

Means with the same letter are not significantly different

DAI= Days after inoculation

CHAPTER V

SUMMARY AND CONCLUSION

About 80 % of people in Bangladesh depend directly on agriculture for their food and livelihood, with wheat being the third most important crop next to rice. Wheat blast is caused by *Magnaporthe oryzae triticum* is a new disease in Bangladesh and caused up to 100% yield loss.

The primary aim of this study was to assess the wheat blast disease prevalence, incidence and severity on upland wheat cultivars in the Meherpur Sadar, Mujibnagar and Chuadanga Sadar upazilla and to characterize the wheat blast pathogenic isolates collected from various Meherpur Sadar, Mujibnagar and Chuadanga Sadar upazilla. It was observed in all assessed localities at variable levels. The results of the assessment revealed that the intensity (incidence and severity) of the blast disease varied from slight to high intensity from field to field and localities to localities depending on the agro-ecological zone and environmental conditions prevailing in each locality. During this assessment, cultivar such as BARI Gom-24 (Pradip) was scored the highest incidence and severity level of blast disease whereas the cultivars such as BARI Gom-26, BARI Gom-28 were scored were the lowest incidence and severity.

Thirty-five MoT isolates were identified from infected field. The *M. oryzae triticum* pathogen colonies on PDA medium grow very slowly and colony on the reverse side of the agar plates were black. Based on the findings of the present study the PDA media showed significantly the highest radial mycelial growth of Mo isolates CHMoT09 (25.67 mm) on the 7^{th}days, CHMoT06 (56.33 mm), CHMoT07 (55.83 mm), CHMoT08 (56.33 mm) and CHMoT09 (57.00 mm) on the 14^{th} days and CHMoT08 (75.50 mm) on the 30^{th} days.

On the basis of growth rate on PDA media, 35 isolates of *M. oryzae triticum* were classified into three cluster groups, cluster I, cluster II, cluster III. Twenty-five isolates were grouped in cluster I. Most of the isolates were grouped into cluster I. Four isolates were grouped into cluster II and 6 isolates were into cluster III. High variation was observed between clusters I and cluster II & cluster III. The result indicates the diversity of wheat blast isolates in wheat blast affected areas of Bangladesh.

Aloe vera (Allovera leaf) extracts and *Nigella sativa* (Black cumin seeds) extracts @ 0.4% (*Aloe vera*: 1:1 w/v @ 0.4% and *Nigella sativa*: 1:1 w/v @ 0.4%) were the most effective under *in-vitro* both in 7 day and 14 days culture age. However, this experiment with botanicals needs to be coined out to assess the field with different concentrations and frequencies in controlling blast of wheat.

CHAPTER VI

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CHAPTER VII

APPENDICES

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

Appendix-II: ANOVA for radial mycelial growth on PDA 7 DAI (Days After Inoculation)

Source	Degree of freedom	Sum of Squares	Mean Square	F value	P value (>F)
Isolates	34	1387.73	40.816	12.706	0.00 ***
Error	70	224.86	3.212		
Total	104	1612.59			

Appendix-III: ANOVA for radial mycelial growth on PDA 14 DAI (Days After Inoculation)

Source	Degree of freedom	Sum of Squares	Mean Square	F value	P value (>F)
Isolates	34	9553	280.97	11.398	0.00 ***
Error	70	1725.5	24.65		
Total	104	11278.5			

Appendix-IV: ANOVA for radial mycelial growth on PDA 30 DAI (Days After Inoculation)

Source	Degree of freedom	Sum of Square	Mean Square	F value	P value (>F)
Isolates	34	13264	390.12	40.367	0.00 ***
Error	70	676.5	9.66		
Total	104	13940.5			

Appendix-V: ANOVA for healthy seed per panicle in Meherpur

Source	DF	Sum of Square	Mean Square	F Value	Pr (> F)
Replication	2	9.8000	4.9000	0.57	0.5770
Treatment	9	85.3667	9.4852	1.10	0.4114
Error	18	155.5333	8.6407		
Total	29	250.7000			

Appendix-VI: ANOVA for wheat blast infected seed per panicle in Meherpur

Source	DF	Sum of Square	Mean Square	F Value	Pr (> F)
Replication	2	14.0667	7.0333	1.64	0.2220
Treatment	9	53.3333	5.9259	1.38	0.2672
Error	18	77.2667	4.2926		
Total	29	144.6667			

Appendix-VII: ANOVA for Effects of ethanol extract of botanicals on mycelia growth at 7 DAI (Days After Inoculation)

Source	Df	Sum Sq	Mean Sq	F value	P-value
Treatment	8	14367.4	1795.93	2078.146	0.00 ***
Concentration	2	1628.9	814.46	942.443	0.00 ***
Treatment: Concentration	16	809.5	50.6	58.546	0.00 ***
Error	54	46.7	0.86		

Appendix-VIII: ANOVA for Effects of ethanol extract of botanicals on mycelia growth at 14 DAI (Days After Inoculation)

Source	Df	Sum Sq	Mean Sq	F value	P-value
Treatment	8	27562.5	3445.3	2385.214	0.00 ***
Concentration	2	3684.5	1842.2	1275.393	0.00 ***
Treatment: Concentration	16	1550.6	96.9	67.095	0.00 ***
Error	54	78	1.4		

Appendix-IX: Preparation of culture media

The composition of the media used in this thesis work are given below:

Potato Dextrose Agar (PDA)

Composition	Quantities (g / litter)
Potato (peeled and sliced)	200
Dextrose	20
Agar	20
Chloramphenicol	0.05
Water	1000ml