ASSESSMENT OF RICE BLAST INTENSITY AND MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF *MAGNAPORTHE ORYZAE*

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DHAKA-1207

DECEMBER, 2021

ASSESSMENT OF RICE BLAST INTENSITY AND MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF *MAGNAPORTHE ORYZAE*

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REGISTRATION NO. 19-10295

A Thesis 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
SEMESTER: JULY – DECEMBER, 2021

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This is to certify that the thesis entitled, **ASSESSMENT OF RICE BLAST INTENSITY AND MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF *MAGNAPORTHE ORYZAE"*** has been *submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE IN PLANT PATHOLOGY** *embodies the results of a piece of bona fide research work carried out by* **Kazi Maliha Fatema, Registration *No.* 19-10295** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma, elsewhere in the country or abroad.*

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

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ACKNOWLEDGEMENTS

All praises to Almighty ALLAH who kindly enabled the author to complete the research work and prepare this thesis successfully.

The author humbly takes this opportunity to place his deep sense of gratitude to his supervisor ***Prof. Dr. F. M. Aminuzzaman****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for her scholastic guidance, valuable suggestions, constant encouragement, affection, immeasurable help and constructive criticism during the entire period of research work and preparation of the thesis.*

The author equally and deeply indebted to his co-supervisor ***Prof. Dr. M. Salahuddin M. Chowdhury****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his kind co-operation, cordial suggestions, constructive criticisms and valuable advice to complete the thesis.*

The author would like to express his gratefulness to the Chairman, Prof. Abu Noman Faruq Ahmmed and other respected teachers, Prof. Dr. Md. Rafiqul Islam, Prof. Dr. Md. Belal Hossain, Prof. Dr. Fatema Begum, Prof. Dr. Nazneen Sultana, and Prof. Dr. Khadija Akhter, Department of Plant Pathology, Faculty of Agriculture, Sher-e–Bangla Agricultural University, for their valuable suggestion, direct and indirect advice, encouragement and co-operation during the whole period of the study.

The author would like to thank the office staff of the Department of Plant Pathology and the Farm Division of SAU for their cooperation and help to complete this research work.

The author deeply expresses his heartfelt respect and gratitude to his beloved father & mother whose everlasting love, unfading faith, continuous inspiration, well wishes and blessings kept him enthusiastic throughout his life and molded him to the current position without which this work could not be completed.

The author

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ABSTRACT

Rice (*Oryza sativa*) is the most important staple food for majority of human population worldwide. Rice blast disease caused by fungus *Magnaporthe oryzae* is one of the most important devastating disease which results complete failure of the crop under severe infection. Survey on rice blast was conducted in 12 farmer´s fields in three village of Bogura districts during Boro season 2020-2021. The incidence of rice blast in different locations varied from 3.01 to 59.92%. The highest incidence (59.92%) of blast disease was recorded from Bortola with a severity score 7 in BRRI dhan 58. The lowest incidence (3.01%) of blast disease was recorded from Bonani with a severity score 1 in BRRI dhan 28. Twenty four isolates were identified from collected neck blast sample for morphological and cultural characterization viz growth character, colony color, surface structure, shape and eleviation. The radial mycelial growth of 24 isolates were ranged from 40 to 60.67 mm at 4 DAI. The highest mycelial growth (60.66 mm) was observed in Mo22 with black color, smooth velvety surface texture, flat and regular shape at 4 DAI. On the other hand, the lowest mycelial growth was observed in Mo13 (40 mm) with brownish white color, rough cottony surface texture and regular shaped colony at 4 DAI. At 7 DAI, the highest mycelial growth (84.33 mm) was observed in Mo16 with brownish color, smooth velvety surface texture, regular, raised and good growth. On the other hand, the lowest mycelial growth (62 mm) was observed in Mo13 with whitish brown color, rough cottony surface texture, irregular shape, raised and good growth. OMA(Oat Meal Agar) found to be suitable for good growth and sporulation of all the isolates of *Magnaporthe oryzae.*

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ABBREVIATION AND ACRONYMS

SAU	Sher-e-Bangla Agricultural University
BBS	Bangladesh Bureau of Statistics
USDA	United States Department of Agriculture
WHO	World Health Organization
Res.	Research
Sci.	Science
FAO	Food and Agricultural Organization
MS	Master of Science
CRD	Completely Randomized Design
et al.,	And others
Viz.,	Namely
e.g.,	Exemple gratia (L), for example
Etc.,	Etcetera
i.e.,	id est (L), that is
%	Percentage
°C	Degree Celsius
mg	Milligram
l	Liter
cm	Centimeter
ml	Milliliter
g	Gram (s)
Kg	Kilogram (s)
LSD	Least Significant Difference
No.	Number
PDA	Potato Dextrose Agar
OMA	Oat Meal Agar
WA	Water Agar

CHAPTER I

INTRODUCTION

Rice is the most important staple food cultivated in 113 countries and principal source of income for about 100 million households in Asia and Africa (FAO, 2004). East and South Asia are the main regions for rice production in the world. More than 90% of the worlds rice is grown and consumed in Asia where 60% of the earths people live (Kole, 2006). China (over 210 million metric tons) is the world leading rice producer followed by India, Indonesia, Bangladesh, Vietnam and rest of the world (FAO, 2017a). Presently the rice production is insufficient to cover the needs and hence several countries have been importing rice. In 2008, Sub-Saharan Africa imported more than US $ 3.6 billion worth of rice, mainly from Asia, to fill the gap between production and consumption (Anonymous, 2011a). Rice is the predominant staple food for 17 countries in Asia, and provides 20% of world´s dietary energy supply which is higher than wheat (19%) and maize (5%) (FAO, 2004). Approximately 50% of consumed calories by the whole population of humans depend on wheat, Rice and maize (Gnanamanickam, 2009). About 23 perecent of calories consumed by people around the world are from rice alone (Nagaraj *et al*., 2019). Although rice has the second place because of planted area but it serves as the most important food source for Asian countries mainly in south-east parts where it is an economic crop for farmers and workers who grow it on millions of hectares throughout the region (Gomez, 2001).

Historically, rice was cultivated l0000 years ago in the river valleys of South and Southeast Asia and China since it served as the most important food for people (Gnanamanickam, 2009). Although Asia is the main place of rice cultivation but it was harvested in other continents like Latin America, Europe, some parts of Africa and even USA (Gomez, 2001). Rice has a high social and economic importance, playing a major role in the world production of cereals, serving approximately three billion people around the world, with a total production of ca. 756.5 million tons of husked grains (Filippi *et* *al*., 2009; SOSBAI, 2018) and global rice demand is estimated to reach 852 million tons by 2035 (Khush, 2013).

Rice (*Oryza sativa*) is the staple food of Bangladeshi people and it constituted about 90% of the total food grain production (Huda, 2001). The rice sector contributes one-half of the agricultural GDP and one-sixth of the national income in Bangladesh. Over 28 million acres of land in Bangladesh are dedicated to rice production (BBS, 2017). The average world yield of rice is 3.84 t/ha, but the average yield of rice in Bangladesh is only 3.02 t/ha (FAO, 2017b). The population of Bangladesh is still growing by two million every year, while the total rice area is gradually shrinking. Rice yield, therefore, needs to be increased (PRB, 2017-2018). Rice is a rich source of protein, carbohydrate, dietary fiber, minerals and vitamins (Gopalan *et al.*, 2007). Rice grain contains on an average 7% protein, 62-65% starch, 1.3% fibre and 0.7% fat. It is also a main source of vitamin B1 (thiamin), B2 (riboflavin), B3 (niacin) and B5 (pantothenic acid). The biological value of rice is 63% whereas biological value of wheat and maize is 49 and 36%, respectively. In Bangladesh, more than 95% of population consumes rice and it alone provides 76% of calorie and 66% of total protein requirement of daily food intake (Bhuiyan *et al*., 2002). Thus, rice plays a vital role in the livelihood of the people of Bangladesh. Both abiotic and biotic factors adversely affect the crop and causes extensive losses to the yield. Drought, cold, acidity, salinity are abiotic factors while pests, weed and diseases are biotic factors (Onyango, 2014). More than 70% diseases have been caused by fungi, viruses, bacteria and nematode (Zhang *et al*., 2009).

Rice crop is subjected to attack by 50 diseases including 6 bacterial, 21 fungal, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders (Hollier *et al*., 1993; Webster and Gunnell, 1992; Jabeen *et al*., 2012). Among the fungal diseases, rice blast is the most important and the most destructive disease of rice, which is caused by the ascomycetous fungus *Pyricularia oryzae* (Teleomorph *Magnaporthe oryzae* Couch) formerly known as *Pyricularia grisea* (Cooke) Sacc. (Couch and Kohn, 2002). The disease was first reported in 1637 from China and was known as rice fever disease (Wang *et. al*., 2014). In Italy, USA and India, the disease was also identified in 1828, 1876 and 1913, respectively (Rahman and Uddin, 2017). Now it is one of the most important diseases occurring in more than 85 rice growing countries causing considerable economic loss (Wang *et al*., 2014). In Bangladesh the blast disease was relatively less important in late sixties and early seventies. The outbreak of blast disease was recorded on 1980 and 1990 in boro season in Bangladesh (Rahman and Uddin, 2017). The incidence and severity of blast disease is very common in Bangladesh as in that period the water scarcity is universal compared to Aman season. In Bangladesh the frequency of blast occurrence has increased with invasion into new areas (north and northwest parts of the country) in recent years. The BRRI dhan29 and BRRI dhan28 are the most popular and mega varieties recognized highly susceptible to blast disease (Anonymous, 2011b).

Moreover, all local and improved aromatic rice varieties grown in wet season are vulnerable to neck blast. The disease is spread worldwide, but the incidence and severity of rice blast disease vary with time, locations and environmental conditions (Bhat *et al*., 2013). This pathogen infects all the developmental stages and all organs of the rice plant (Le *et al.*, 2010; Ou, 1985). Outbreaks of the disease is a recurrent problem and is extremely difficult to control in all rice growing region of the world, including Bangladesh (Dean *et al*., 2005; Khan *et al*., 2014; Talbot, 2003; Valent and Chumley, 1991). Every year blast of rice is responsible for 10- 30% of yield losses worldwide (Talbot and Wilson, 2009). Under favorable condition it can devastate whole rice field within 15 to 20 days and causes upto 100% yield loss (Asibi *et al*., 2019). More than 50% yield losses occur every year in Philippines and approximately 865,000 hectares of rice fields in Japan are being affected by this disease every year (Magar *et al*., 2015). The disease infects wide variety of grasses including rice, wheat and barley, and is particularly more destructive to upland rice crops produced without irrigation, land with frequent and prolonged periods of rain, higher dose of nitrogen in soil, humid region and cool temperature in the daytime (George, 2005). The disease results in yield loss as high as 70-80% (Piotti *et al*., 2005).

The incidence of rice blast was recorded on Boro season (November to May: irrigated ecosystem) and Transplanted Aman (July to December: rainfed ecosystem) in all over Bangladesh (Rahman and Uddin, 2017). At present 267 races of rice blast have already been identified in our environment (Biswas, 2017). Thus, disease pressure is in increasing trend and may be a devastating experience in the near future. This phenomenon is not for Bangladesh but for the other 85 rice growing countries. Rice has tremendous adaptation ability from Hokkaido to Honolulu (Biswas, 2017). Hence, rice blast is considered as a serious and recurrent problem in many of rice growing countries (Rahman and Uddin, 2017). *Pyricularia oryzae* causes damaged to both vegetative as well as reproductive stages (Seebold *et al*., 2004). This fungus can infect all stages of rice development and different parts of rice plants; leaves, stems, nodes and panicles (Talbot and Wilson, 2009). But the greatest loss occurred when the necks and panicles are attacked. The attacked Panicle results in partially filled or unfilled grains (IRRI, 2014). Symptoms are more acute for neck blast, which is characterized by infection at the base of panicle and subsequent rot (Xiao *et al*., 2017). Blast in epidemics form results in complete loss of rice seedlings in bed (Chaudhary, and Sah, 1998). The disease outbreak depend on the weather and climatic conditions of the various regions. The disease occurrence and symptoms vary from country to country (Rameshbabu, 2015). First appearances of lesion as minute of brown speaks on leaf tissue and gradually growing spindle shaped (Hossain *et. al*., 2017). The center is grayish with brown margin. The lesions may extend and thus eventually coalesce the entire leaf into necrotic (Thon *et al*., 2006). Under favorable conditions, the lesions can enlarge rapidly and tend to coalesce, leading to plant death (Wang *et al*., 2014). No grain is formed if infection of the neck occurs before milky stage whereas poor quality grains are formed if the infection occurs later (IRRI). Yield reduction due to neck blast and leaf blast are 70% and 50-70%, respectively (Bastiaans, 1993).

According to Common wealth Mycological Institute (CMI), cultures of rice blast pathogen *Pyricularia grisea* are greyish in color, conidiophores single or in group, simple or rarely branched, show sympodial growth. Single conidia developed at the tip of the conidiophores, arising sympodially and in succession, the shape of conidia either pyriform or obclavate, narrow towards tip, round at the base, three celled, rarely one or two celled, hyaline to pale olive, 19-23 × 7-9 µm, with a distinct protruding basal hilum. The sexual fruiting body of fungus is called perithecia formed within 21 days. Flask-shaped perithecia undergoes meiosis and forms ascospores. Ascospores are in large populations of randomly selected ascospores (Talbot, 2003). *Pyricularia grisea* isolates forms ring like structure having irregular colonies with rough and smooth margins on OMA media (Srivastava *et al*., 2014).

Survey was carried out in different rice field in Nilgiri and Madurai districts of Tamil Nadu for the collection of blast isolates. The blast disease incidence ranged from 8.8 to 42.4 per cent in various rice cultivars. Fifteen isolates of *Magnaporthe oryzae* were isolated from symptomatic tissues and maintained in pure culture. All isolates produced greyish brown to white colour colony with a diameter which ranged from 80-90 mm in PDA medium. Sporulation was carried out by stem bit inoculation method which produced pyriform shaped conidia with the size range of 8-12 × 3.3-3.6 µm. Thus, there exist morphologoical variability among *Magnaporthe oryzae* isolates within the close proximity (Rahila *et al*., 2019). In another study colony color of the rice blast (*Pyricularia grisea*) 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 (Meena, 2005). Keeping this view in mind, the present research work was undertaken with the following objectives:

Objectives

1. To determine the incidence and severity of rice blast in some selected fields of Bogura district
2. To determine the morphological and cultural characteristics of rice blast pathogen *Magnaporthe oryzae*

CHAPTER II

REVIEW OF LITERATURE

2.1. Importance of rice

Akter *et al*. (2019) stated that rice (*Oryza sativa* L.) is a major staple food crop for half of the world's population. It provides 21% of global human per capita energy and 15% per capita protein.

Kirby *et al.* (2017) stated that globally, about 160 million hectares are estimated to be under rice production with an annual production of approximately 500 million metric ton. Liu *et al*. (2013) and Talbot, (2003) reported that every year the harvested rice will face severe yield losses from 10 to 30 per cent and is a major threat to global food security (Liu *et al*., 2013; Talbot, 2003).

Perez-Montano *et al*. (2014) studied that rice is a vital and strategic human food crop for food security worldwide. Salam *et al*. (2014) found that Bangladesh agriculture involves food production for 163.65 million people from merely 8.75 million hectares of agricultural land.

Ameen *et al*. (2014) reported that in Pakistan, rice ranked as the second most consumed cereal crop after wheat and the third most profitable crop after wheat and cotton. Atera *et al*. (2018) also reported that rice (*Oryza sativa* L.) is one of the most important food crops for more than 50% of the world population.

Khandakar *et al*. (2013) reported that in Bangladesh, rice is the most staple cereal crop and central to Bangladesh’s economy, accounting for nearly 20 percent of gross domestic product (GDP) and providing about one-sixth of the national income of Bangladesh.

Molina *et al*. (2012) reported that rice (*Oryza sativa* L.) is consumed as a staple food for half of the world’s population.

Anonymous (2011) reported that India is the largest rice growing country accounting for about one third of the world acreage under the crop. Rice crop occupies about 44.3 mha in the country with a record production of 103.41mt and productivity of 2125 kg/ hectare as estimated during 2011-12. However, in Karnataka rice is cultivated in about 15.39 lakh hectare with an annual production of 42.97 lakh tonnes and productivity of 2938 kg/hectare during the year 2010-11. In another study, Yaduraju and Rao (2013) reported that rice is the main staple food in Asia and the Pacific region, providing almost 39% calories.

FAO (2009) stated in their annual report that rice is grown on 161 million hectares worldwide, with an annual production of about 678.7 million tons of paddy. About 90% of the world´s rice is grown and produced (143 million ha of area with a production of 612 million tons of paddy) in Asia.

FAO (2009) reported that rice is found all over the world and in thousands of varieties; there are more than 8,000 varieties found in India alone where it all originated, and in one of the smaller rice producing countries, the Philippines, there are about 3,500 varieties. Some of these well known varieties include basmati (India), sushi rice (Japan) and jasmine rice (Thailand) each having a different consistency and flavor. Worldwide, rice is grown on 161 million hectares, with an annual production of about 678.7 million tons of paddy and about 90% of the world's rice is grown and produced (143 million ha of area with a production of 612 million tons of paddy) in Asia.

Khush and Jena (2009) reported that the rate of global rice production has not been able to meet the growing demand of the world population every year.

Sharma and Bambawale (2008) reported that In India, rice is grown to an extent of 43.94 Mha with a production of 159.2 Mt and a productivity of 3623.12 kg/ha. In Andhra Pradesh, the crop is grown to an extent of 3.62 M ha with a production of 11.4 Mt and productivity of 3173 kg/ha. In India, the productivity of rice is less than those in agriculturally advanced countries.

Kole (2006) reported that Rice *(Oryza sativa* L.) is the world's most important crop and a primary source of food for more than half of the worlds human population.

According to FAO (1995), rice is the 1st economically important crop in India, China, East Asia South East Asia, Africa and Latin America catering to nutritional needs of 70% of the population in these countries.

2.2. Significance of blast disease of rice

Shirke *et al*. (2016) stated that rice blast is caused by *Magnaporthe oryzae* B. Couch (formerly *Magnaporthe grisea*) which was probably first recorded as rice fever disease in China in 1637.

Gaikwad and Balgude (2016) found that among the several diseases infecting rice, blast disease caused by *Pyricularia grisea (Magnaporthe grisea*) is important in rice in Maharashtra which causes about 10-80 per cent yield loss.

Magar *et al*, (2015) reported that more than 50% yield losses occur every year in Philippines and approximately 865,000 hectares of rice fields in Japan are being affected by rice blast disease every year.

Dar *et al*. (2011) studied that rice blast caused by a filamentous, ascommycete fungus *Magnaporthe oryzae* (syn: *Pyricularia oryzae* Cav.) is one of the most important diseases of rice worldwide and is one of the major hindrances for profitable rice production all over the world. In another study. Dean *et al*. (2012) reported that the tropical humid climates in South East Asia are very conducive to the epidemics of rice blast disease. During the epidemic year, the disease cause yield losses up to 100 per cent.

Sundaram *et al. (*2011) stated that In India, blast epidemics were from sub- Himalayan regions of Jammu and Kashmir, Andhra Pradesh, Tamil Naidu and Coorg regions of

Karnataka and North eastern region comprising the states of Arunachal Pradesh, Manipur, Mizoram, Meghalaya and Assam.

Chandrasekhara *et al*. (2008) reported that rice blast caused by *Magnaporthe oryzae* is one of the devastating disease of rice resulting in yield losses up to 65% in susceptible rice cultivars. And Sukanya *et al*. (2011) reported that rice blast causes at least US$55 million production losses annually in South and Southeast Asia.

Arshad *et al*. (2008) reported that In Pakistan, during the past 20 years, rice blast has occurred frequently in the Punjab province districts of Toba Tek Singh, Faisalabad, Vehari.

Seebold *et al*. (2004) stated that *Pyricularia oryzae* caused damaged to leaf and panicle of rice which indicates that it causes damaged to both vegetative as well as reproductive stages. IRRI (2014) reported that the attacked Panicle results in partially filled or unfilled grains. Moreover, Blast in epidemics form results in complete loss of rice seedlings in bed (Chaudhary and Sah, 1998).

Sesma and Osbourn (2004) reported several rice blast epidemics that occurred in different parts of the world, resulting in 50 to 90% of the grain yield losses. There was a severe epidemic of rice blast in 1978, when incidence of panicle blast was more than 40% in some cultivars.

Sesma and Osbourn (2004) reported that rice blast was first recorded in India during 1913 and a devastating epidemic occurred in 1919 in the Tanjore delta of Tamil Nadu.

Samira *et. al*. (2002) reported that rice blast pathogen can cause damage up to 90% and sometime total crop loss under favorable conditions.

Ahn and Mukelar (1994) reported that blast pathogen may produce new races and break the resistance in introduced variety because of wider adaptability and high degree of variability among the pathogenic races.

Pinnschmidt *et al*. (1994) found that neck blast and the panicle blast are the most damaging phases of the disease and have been shown to significantly reduce yield, grain weight and milling quality.

Bastiaans (1993) reported that yield reduction due to neck blast and leaf blast are 70% and 50%-70%, respectively.

Bonman *et al*. (1989) stated that the rice blast fungus can causes symptoms like leaf blast, nodal blast and neck or panicle blast. The most severe stage is neck blast. Asibi *et al*., (2019) reported that rice blast can devastate whole rice field within 15 to 20 days and causes upto 100% yield loss under favorable condition.

Padmanabhan (1963) stated that rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, blast is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions. Losses due to the blast disease may range up to 90% depending upon the component of the plant infected.

2.3. Survey and disease intensity of rice blast

Mahmud *et al*. (2021) conducted a survey program in eight rice-growing districts of Bangladesh, namely Dinajpur, Rangpur, Bogura, Natore, Meherpur, Rajbari, Mymensingh, and Jashore, to estimate the incidence and severity of leaf and neck blast diseases of rice at vegetative and reproductive stages. The highest leaf and neck blast incidence of 28 and 11% was observed in Dinajpur, whereas it was the lowest in Rajbari and Jashore. The leaf blast severity ranged from 28% in Dinajpur to 2% in Rajbari and Jashore. The highest neck blast severity was recorded in Dinajpur, whereas the lowest was observed at Rajbari and Jashore.

Rahila *et al*. (2019) surveyed in Nilgiri and Madurai districts of Tamil Nadu for the collection of blast isolates. The blast disease incidence ranged from 8.8 to 42.4 per cent in various rice cultivars.

Rayhanul *et al*. (2019) conducted a survey on rice blast in 110 rice field of 12 upazillas under 5 districts of Bangladesh namely Mymensingh, Kishoreganj, Barishal, Naogaon and Cumilla. Among the survey areas Muktagachha, Mymensingh was found as the highest rice blast disease infected area and Bakerganj, Barishal was found as the lowest in Boro season of 2017-2018.

Singh *et al*. (2018) conducted survey in thirteen major rice growing districts viz., Jagdalpur (Bastar), Dantewada, Narayanpur, Bilaspur, Janjgir-Champa, Kanker, Bemetara, Raipur, Dhamtari, Gariyaband, Balrampur, Surajpurand Surguja from August last week to October 2016 and September first week to October 2017. Among the thirteen districts, percent disease index was varied from 20 to 87.78%. The highest percent disease index (PDI) was recorded (87.78%) in Jagdalpur (Bastar) district with Swarna cultivar which is followed by Surguja (85.56%) and Balrampur (84.44%) and lowest PDI was recorded (20%) in Surajpur (Maheshwari) and Bastar (Safari).

Kulmitra *et al*. (2017) conducted survey, collection, isolation and identification of isolates of *Pyricularia oryzae* causing rice blast in southern Karnataka by using host differential lines and concluded that the highest percent disease severity of the rice leaf blast was recorded (38.92%) in Krishnarajpet Taluk and lowest incidence was recorded (27.39%) in Maddur Taluk.

Asfaha *et al*. (2015) reported the assessment of rice blast was carried out in 90 farmers' fields in three districts during the main cropping season from May to October 2013, in the major upland rice growing areas of Kaffa, Benchi-Maji and Sheka zones in South West of Ethiopia. The results of the assessment revealed that the incidence and severity of the disease vary from low to high on the rice fields depending on the agro-ecological zone and cultivars differences. The incidence of rice blast in six different localities varied from

42.01 to 85.69%. The highest mean incidence of rice blast was recorded in Otuwa locality (85.69%) and the lowest incidence recorded in Argoba locality (42.01%). Likewise, blast severity showed similar trend as that of incidence in all six localities. The highest severity was recorded in Otuwa locality with range of 8.88 - 88.8 % and the mean severity values of 55.7% while the lowest severity was recorded in Argoba locality (33.62%).

Shahijahandar *et al*. (2010) recorded prevalence and distribution of blast in Kupwara district of Jammu and Kashmir and reported 25% disease incidence and 15% severity and the incidence was more from transplanting to panicle initiation stage.

Hossain and Kulakarni (2001) conducted survey for blast of rice during Kharif 1999 in different villages of Dharwad, Belgaum and Uttara Kannada districts and reported maximum disease incidence in Haliyal (61.66%) and Mundagod (54.00%) talukas of North Karnataka.

Hossain (2000) studied on fixed plot survey for blast of rice during kharif 1999, in different villages of Dharwad, Belgaum and Uttar Kannada dsistricts , where maximum disease incidence was noticed in Haliyal (61.66%) and Mundagod (54.00%) talukas of Uttar Kannada.

Pawar *et al*. (2000) conducted simple rapid roving disease survey on major field crops during 1999 in Karnataka. The survey was carried out in three districts, viz., Bangalore rural, Tumkur and Hassan. Sixty one per cent of rice blast incidence was recorded in the surveyed villages of Hassan, Alur and Sakleshpur

Verma and Sengupta (1985) reported that, survey for diseases of rice, the principal cereal crop of Tripura, had led to the identification of as many as 17 diseases caused by fungi, bacteria, viruses and nematodes.

2.4. Isolation and identification of causal agent

Nartymov *et al*. (2021) reported that leaf forms of *Pyricularia oryzae* strains isolated from rice plants with leaf form of blast disease have an equally directional growth pattern of a colony with a felt structure, and strains isolated from neck affected plant form often produce a zone of a colony with a clumpy structure.

Hussin *et al*. (2019) studied twelve succeeded isolates which were pre-identified as *Pyricularia. oryzae* by morphological characteristics of spores, followed by verification through (internal transcribed spacer) ITS sequencing. The isolates were evaluated for morphological characteristics, growth rate and sporulation rate, which were grown on two types of media, (filtered oatmeal agar) FOMA and (potato dextrose agar) PDA. Morphological characterization showed that the colony surface of the different isolates varied from smooth and fluffy to rough and flattened mycelia; some were with the present of concentric rings, and some with aerial mycelia.

Rayhanul *et al*. (2019) isolated *Pyricularia oryzae* from infected leaf and panicle and identified based on cultural characteristics and conidia morphology. Mycelial growth of four *Pyricularia oryzae* isolates varied significantly with fair to excellent sporulation ability.

Nagaraj *et al*. (2019) studied on six isolates of rice blast pathogen which were collected from different locations in Coimbatore and Erode districts. The isolates were confirmed phenotypically through morphological characters and also confirmed through molecular methods.

Goretti *et al*. (2017) studied eight different Kenyan isolates of *Pyricularia oryzae* identified from a previous survey using DNA analysis and sequencing of the ITS region from the rice genome. Results revealed variation with respect to colony color, diameter, morphology and conidia shape and size. Sequenced Isolates clustered into four haplo groups (HGs), that is HG1 for isolate 4, HG2 for isolate 6, HG3 for isolate 7a and HG4 for isolate 7b. The new isolates' sequences were submitted to the NCBI GenBank database and the accession numbers assigned as, *Pyricularia oryzae* KY275366, *Pyricularia oryzae* KY275367 and *Pyricularia oryzae* KY275368. The result of the study demonstrated that there is a certain level of genetic diversity among isolates of *Pyricularia oryzae* from various regions of Kenya. The screening for resistance to *Pyricularia oryzae* therefore should take into account the different isolates in order to avoid cryptic error.

Asfaha *et al*. (2015) characterized rice blast *Pyricularia oryzae* isolates and identified based on their growth parameters into six isolates such as Po12, Po28, Po41, Po55, Po72 and Po85. Among the four culture media (oat meal agar, rice flour agar, malt extract agar and potato dextrose agar), the *Pyricularia oryzae* isolates were grown on optimum growth and good sporulation in oat meal agar followed by rice flour agar. The optimum temperature and pH of the growth of the *Pyricularia oryzae* isolates were at 30°C and 6.5, in almost all isolates, respectively. It could be concluded that the rice blast was the most important disease of rice cultivars in South West of Ethiopia.

Santos *et al*. (2010) verified *Magnaporthe oryzae* monosporic isolates of the IA-1 race collected at Projeto Rio Formoso, Formoso do Araguaia Municipality. The period of the cultures stored under laboratory conditions, the maximum quantity of the subcultures to keep the production of the conidia for the colonies satisfactory and the relation of different culture colors in BDA medium with the conidia production. For that, three experiments were carried out in complete randomized design. In the experiment I, the cultures with 10, 14, 18, 22, 26 and 30 days after subculture were used. In the experiment II, the cultures with 0, 1, 2, 3 and 4 subcultures from the original culture were used. In the experiment III, the cultures were classified as black, center ash with wide white edges, ash, center ash with narrow white edges and white. Higher sporulation of the *Magnaporthe oryzae* was obtained on the cultures with 14 days after the subculture, in the second subculture and on the black color.

Akoi (1955) measured the conidia of 16 isolates in potato dextrose agar culture and showed that the everage length of the conidia ranged from 21.2 to 28.4 µm, and the average width from 7.3 to 9.0 µm.

2.5. Media suitable for culturing *Magnaporthe oryzae*

Hussin *et al*. (2020) studied on twelve succeeded isolates which were grown on two types of media, (Filtered Oatmeal Agar) FOMA and (potato dextrose agar) PDA. The growth rate and sporulation rate of each isolate varied based on types of media used. Most of the isolates grew faster on PDA than on FOMA but produced higher number of spores on FOMA as compared to PDA.

Jagadeesh and Devaki (2020) found the morphological and biochemical variations existing among the field isolates of *Magnaporthe oryzae* collected from different agro-climatic zones. The influence of culture media on growth, colony character of *Magnaporthe oryzae* isolates on two different media viz., potato dextrose agar and oat meal agar was studied. Results revealed that there was a significant variation among the isolates of *Magnaporthe oryzae* in different parameters such as growth pattern, colour of media and mycelia, texture and sporulation ($P \leq 0.05$).

Kulkarni and Peshwe (2019) studied with fifteen different natural and synthetic media to understand the nutritional requirements of the blast pathogen naming few Oat meal agar, rice media, rice and soyabean extract media. Mycelial yield, number of spores, radial growth were observed and compared. Based on the requirements of *Magnaporthe oryzae*, founded a new medium named KKSP formulated medium was composed for growth and sporulation studies. It was found that the formulated medium was also suitable medium for the growth of blast pathogen, where high mycelium yield was achieved in shorter time as compared with other standard media used so far. The KKSP formulated medium showed pink pigmentation and newly formed mycelia could be easily identified with morphology and septation pattern with visual observation.

Dutta *et al*. (2019) isolated the blast pathogen from the rice field of Uttar Banga Krishi Viswavidyalaya Farm, purified, and characterized based on morphological and cultural characters. Highest mycelial growth were observed in oat meal agar (OMA) medium of 28.50 mm and 71.33 mm at 2 and 6 days after inoculation (DAI), respectively, whereas in potato dextrose agar (PDA) medium growth of 47.77 mm was observed at 4 DAI. The mycelial growth was highest at 300°C at 2 (27.00 mm) and 6 (72.17 mm) DAI whereas at 4 DAI at 250°C, maximum growth of 43.50 mm was recorded.

Manjunatha and Krishnappa (2019) conducted a study to describe the cultural and morphological characteristics such as color and texture of the leaf blast pathogen *Pyricularia oryzae* on different solid media viz., Oat meal agar, Potato dextrose agar, Host extract agar and Richard's agar medium. Among all the solid media the highest mean mycelial growth (diameter) of 40.80 mm of the fungus *Pyricularia oryzae* was recorded on Host extract agar followed by Oat meal agar (38.33 mm) and least mean mycelial growth of 28.4 mm of the fungius *Pyricularia oryzae* on Rechard's agar. The highest mean dry mycelial weight (mg) Rechard's agar (300.65 mg) followed by Oat meal agar (234.67 mg) and least mean mycelial weight was recorded 96.31 mg in Potato Dextrose agar. In general, for cultural and morphological study of rice blast fungus *Pyricularia oryzae* the host extract agar media is more appropriate among all solid media.

Teli *et al*. (2016) studied on different media such as Potato dextrose Agar (PDA), Oat meal Agar, Ragi flour agar, yeast extract + 2% soluble starch, Host extract + 2% soluble sucrose agar, Potato dextrose agar + Biotin + Thiamine and Rice flour agar. Oat meal agar and potato dextrose agar was found to be best media for radial growth and sporulation of *Magnaporthe grisea*. Maximum conidia length (9.46 µm) and breadth (7.36 µm) was recorded in Oat meal agar followed by Potato dextrose agar and least conidia length (6.15 µm) and breadth (5.11 µm) was recorded in ragi flour media after 20 days of inoculation. Conidial size varied in leaf and neck blast isolates, the maximum mean colony diameter was recorded in Oat meal agar of 88.00 and 89.16 mm in neck and leaf blast, respectively. The maximum sporulation mean index of 3.15 µm in leaf and

3.20 µm in neck blast was recorded on Oat Meal Agar. The best growth of the pathogen was recorded at optimum pH range from 6.0 - 7.0 and temperature of 27°C. Therefore among all the media used for growth, sporulation, conidial size and colony characters of *Magnaporthe grisea* on Oat Meal Agar media was found to be best.

Ravindramalviya (2014) used four culture media for the study of mycelial growth of *Pyricularia grisea* under in vitro. Among them PDA media supported maximum mycelial growth followed by Richard's Agar medium after 168 hours of incubation.

Mahdieh *et al*. (2013) introduced the optimal condition in which *Pyricularia oryzae* grows and sporulates best on common culture media. Three fungal culture media, was considered viz. PDA, PCA and WA, based on which *Pyricularia oryzae* sporulation inducers like rice polish, rice extract or rice leaf segments could be added, and evaluated both for vegetative growth and sporulation. Three light regimens, i.e. continuous light, 16/8 hr light/darkness, and continuous darkness were applied in combination with nine synthetic culture media. Mycelial growth was measured after 11 days, but sporulation was tracked on the 10th, 20th, and 30th day after incubation at 26ºC. The findings indicate that for vegetative growth of *Pyricularia oryzae* PDA culture medium could provide the best medium, regardless of light condition. However, *Pyricularia oryzae* could sporulate when light was provided either continuously or at intervals. A combination of 16/8 hr light/darkness intervals and adding rice materials to culture media could induce *Pyricularia oryzae* for a better sporulation. In order to obtain a high number of conidiab for *Pyricularia oryzae* RPCA can be used as the best culture medium under light alterations. Moreover, aging process increases the total number of conidia.

Hossain (2000) founded that among the non synthetic media, potato dextrose agar supported maxium 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).

Kumar and Singh (1995) obtained the growth pattern of *Pyricularia oryzae* from rice on different culture media and showed maximum linear growth even on malt agar. The varied colour of the colony of same isolates grown on two different media was observed by Vanaraj *et al*. (2013).

Perezsendin *et al*. (1982) reported that the optimum growth and sporulation is favored by high relative humidity and optimum temperature of 26 to 28°C of *Magnaporthe oryzae*. Sun *et al.* (1989) studied the effect of different media on *Pyricularia oryzae* growth and sporulation. They have observed luxurious growth of *Pyricularia oryzae* on PDA medium compared to all other media.

Kulkarni and Govindu (1976) and Awoderu *et al*. (1991) reported that *Pyricularia oryzae* is known to grow vigorously on oat meal agar (OMA), potato dextrose agar PDA) medium at neutral pH and 30°C.

2.6. Morphological Characteristics of Rice Blast

Boyapati *et al*. (2021) studied on the variability in cultural characteristics of fifty isolates of *Pyricularia grisea* which were taken from different regions of Jharkhand state. Out of fifty isolates of *Pyricularia grisea*, colony color of six isolates were found to be as greyish white color, three isolates were blackish grey, six isolates as white color, three isolates as whitish grey color, five isolates were whitish black and twenty seven isolates were recorded as blackish white in color. The growth pattern of 47 isolates of *Pyricularia grisea* showed circular growth pattern and three isolates have irregular growth pattern but elevation of the mycelium differs from flat to raised. Out of fifty isolates of *Pyricularia grisea*, sector formation was observed in seventeen isolates and no sector formation was observed in rest isolates. The radial growth of fifty isolates of *Pyricularia grisea* were ranged from 76.0 mm to 90.0 mm.

Yashaswini *et al*. (2017) recorded the blast pathogenic isolates which were differed and their colony diameter ranged from 71.6 to 87.6 mm. On oat meal agar, colony color of the isolates was usually grey and greyish-white with good growth.

Srivastava *et al.* (2014) isolated *Magnaporthe grisea* and investigated morphological characteristics such as colony color, color of the mycelium, and surface appearance among the isolates. And reported that Blast fungal isolates produced ring like, circular, irregular colonies with rough and smooth margins on oat meal agar media having buff colour, greyish black to black colour.

Vanaraj *et al*. (2013) studied Culture of different isolates of *Pyricularia oryzae* and reported that colonies of *Pyricularia oryzae* appeared as white on oat meal, rice polish and malt extract agar, grey on potato dextrose agar and whitish grey on rice agar.

Pinnschmidt *et al.* (1994) studied that *Magnaporthe grisea* infects above ground parts of the plant. A typical blast lesion on a rice leaf is gray at the centre, has a dark border and it is spindle-shaped. Under favourable conditions, leaf lesions enlarge and coalesce, eventually blighting the entire leaf. Neck blast results in a girdled neck with grayish brown lesions.

Dutta *et al.* (2019) recorded that the conidia in infected leaf sample were three-celled with an average size of 22.42 × 8.59 µm. The conidia on rice grain were predominantly two celled with an average size of 16.45 × 7.46 µm. Conidial production was not observed in any of the media tested including PDA, OMA. However, on rice grain, the fungus produced conidia at 55-60 days.

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

Yaegashi and Udagawa (1978) reported that the fungal hyphae of *Magnaporthe oryzae* are hyaline and septate. When the fungus gets older, the hypha become brown. Generally, growth of the pathogen is relatively more on upper surface making the spot more dark on upper side. Conidiophores are simple, septate, basal portion being relatively darker. Conidia are pyriform in shape and hyaline in colour, produced acrogenously, one after another. Conidium is three celled, the middle cell being much wider and darker, and end cell germinates giving out germ tube. Formation of intercalary or terminal chlamydospores is common, which are globose, thick walled and olive brown.

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental site

The survey was carried out in different rice field of Bogura district. Isolation of *Magnaporthe oryzae* and determination of cultural characteristics were conducted in the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.2. Experimental period

The experiment was conducted during the period from November 2020 to June 2022.

3.3. Survey of rice blast

The survey of rice blast disease was conducted in different farmers field of Bogura district (Figure 1) during Boro season (November to May, irrigated ecosystem) and Transplanted Aman (July to December, rainfed ecosystem). In each season, for the observation of leaf blast and node blast, survey was conducted during post-flowering stage of the rice crop. For the severity of blast disease, a zigzag sampling pattern was followed (Savary *et al*., 1996). At every 50 step interval a single hill (consist of several tillers/plant) was selected and the diseased data was recorded.

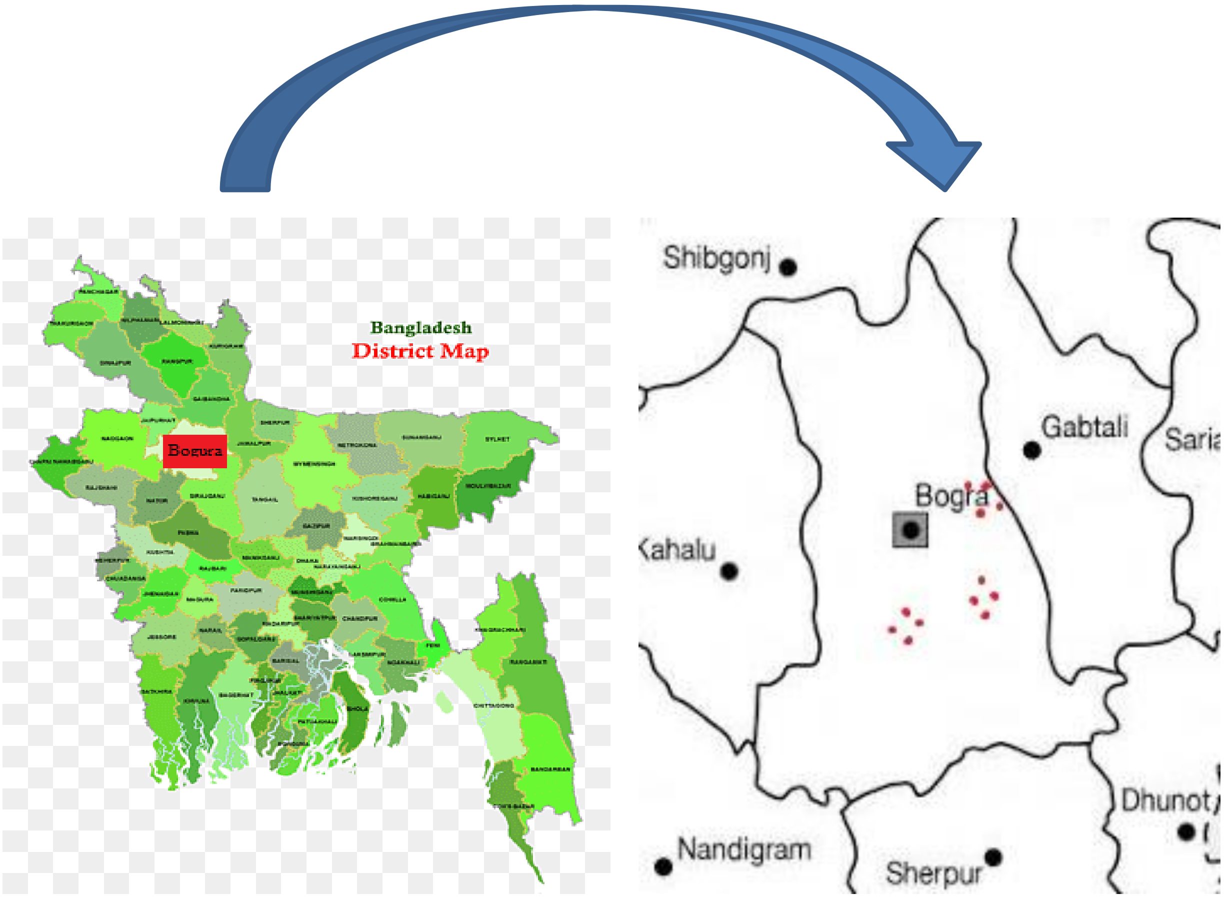

Figure 1. Rice blast survey and sample collection area (●) under Bogura district of Bangladesh

3.4. Incidence and severity of rice blast

3.4.1. Assessment of the disease incidence

Disease incidence was recorded based on percent diseased panicle present in the field. Data were recorded during post flowering stage by observing the symptoms. Three hills were randomly selected from each field. Disease incidence was calculated from the following formula (Rajput and Bartaria, 1995; Greer and Webster, 2001):

$$\text{Disease Incidence (\%DI)} = \frac{\text{Total number of infected plants}}{\text{Total number of inspected plants}} \times 100$$

3.4.2. Assessment of the disease severity of rice blast

Disease severity was recorded during survey of farmer's field. Assessment of disease severity in the field from each unit plot were randomly selected and tagged for granding the severity of disease. Disease severity was recorded as 0 to 9 scale (Table 1) developed by International Rice Research Institute (IRRI, 2014)

Table 1. Scale for panicle blast disease based on symptom

Scale	Symptom on panicle
0	No visible lesion or observed lesions on only a few pedicles
1	Lesions on several pedicles or secondary branches
3	Lesions on a few primary branches or the middle part of panicle axis
5	Lesion partially around the base (node) or uppermost internode or the lower part of panicle axis near the base
7	Lesion completely around panicle base or uppermost internode or panicle axis near base with more than 30% of filled grains
9	Lesion completely around panicle base or uppermost internode or the panicle axis near the base with less than 30 % of filled grains

3.5. Isolation and Identification of pathogen from collected sample

3.5.1. Collection of rice blast sample from selected area

Typical rice blast sample were collected from the rice blast affected areas of Bangladesh. The infecting neck were cut into small pieces and kept in a zip lock plastic with needed information (Like sampling number and sampling sites). Then dry it about 7-8 hours at room temperature. Then the sample were carried to Mycology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka 1207 for isolation and characterization of *Magnaporthe oryzae*. Infected necks were covered with brown paper and kept at 4°C in a refrigerator.

3.5.2. Cleaning and sterilization of glassware

The glass-wares were first cleaned with detergent, then thoroughout cleaning with tap water. Then they were air dried and sterilized in a hot air oven at 160°C for one hour. Working media and water were sterilized at 121°C under 15 psi pressure for 15 minutes in an autoclave machine. Whole Laminar air flow cabinet and work benches were sterilized with 70% ethyl alcohol. Needle, scalpel and inoculation loop were sterilized by spirit lamps flame (Darmady *et al*., 1961).

3.5.3. Isolation and Identification of *Magnaporthe oryzae* from collected sample

The infected neck of diseased samples were cut into small pieces with diseased and healthy part properly and surface sterilized by dipping in a mercuric chloride solution (1: 1000) for 1 minute, rinsed three times with sterile distilled water and placed on a sterile blotter paper to remove excess water. Then the pieces of sample were inoculated immediately in another wet filter paper in a sterile plastic petridish and incubated at 25±1°C for 48 hours (Figure 2). For wet filter paper preparation, at first 9 cm radial blotter papers were sterilized in a hot air oven for 10 minutes at 160°C temperature. Then 2 filter papers were placed in a sterilized plastic petridishes and sterilized distilled water

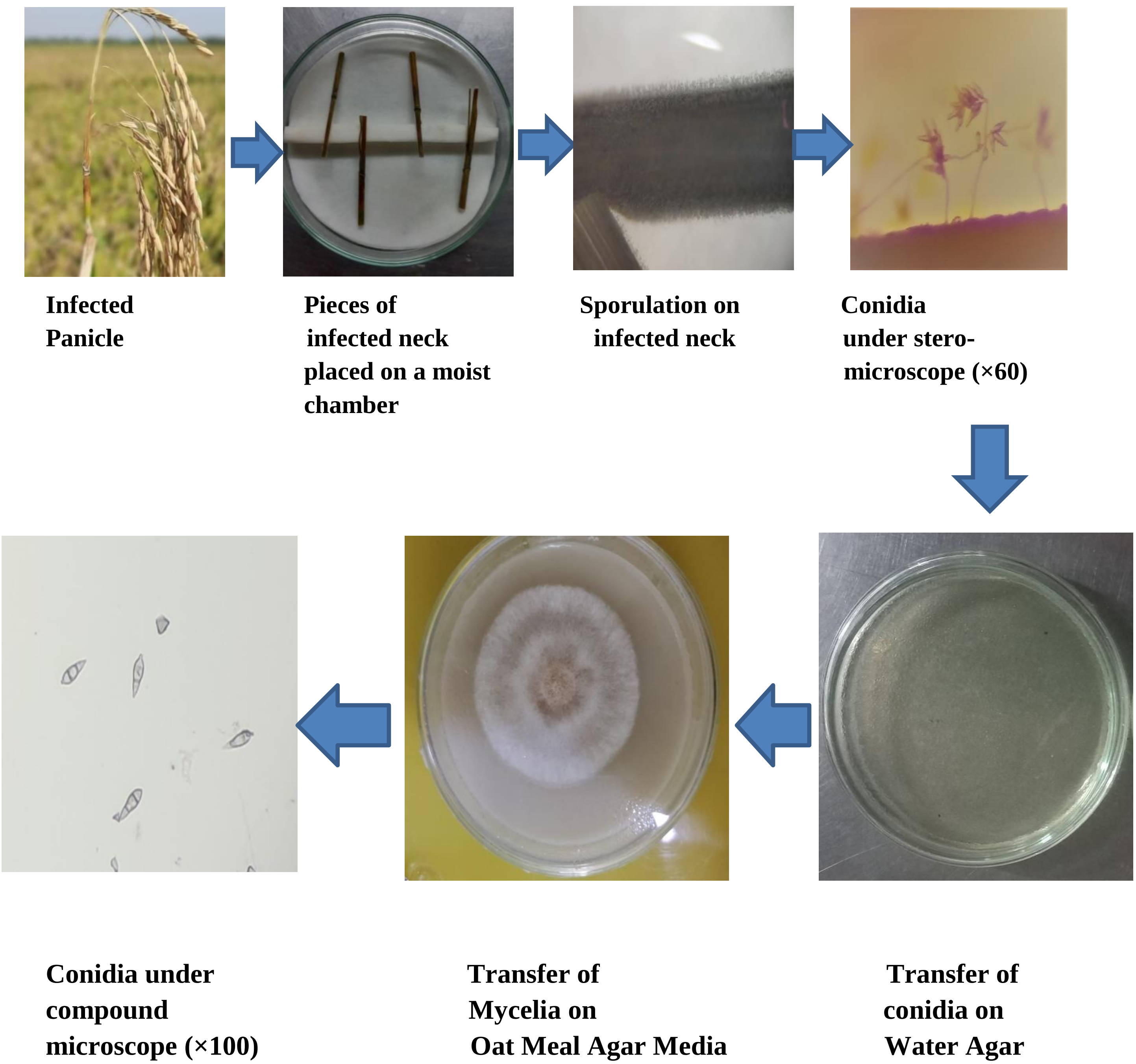

Figure 2. Flow chart of isolation and identification of *Magnaporthe oryzae*

was added with sterilized dropper aseptically. When the whole paper become wet, and no excess water was floated then it was ready for next step the pieces of infected sample were placed on the petri dishes containing wet filter paper. Whole process was done in Laminar air flow cabinet. After incubation, conidia were collected and transferred to water agar with needle by inspecting the plate under steriomicroscope and incubated at 25±1°C for 24 hours. After that mycelial tip was sub cultured from water agar to oat meal agar and incubated at 25±1°C for 10 to 15 days. The isolates were identified based on morphological and cultural characteristics and confirmed under the compound microscope, by observing three celled pyriform conidia.

3.6. Media used for culturing *Magnaporthe oryzae*

The isolates of *Magnaporthe oryzae* was grown on OMA for 7 days at room temperature. From the fungus active growth margin 5 –6 mm discs were blocked out. Sterile petri-dishes with water Agar (WA) and Oat Meal Agar (OMA) were inoculated each with a single 5-mm disc of fungus and incubated for 7 days at room temperature. Three replication were maintained for each medium. The fungal growth was measured at 7 DAI. Further, the colony character of the 32 isolates were grown on OMA and their colony morphology was observed.

3.6.1. Water Agar (WA) medium Preparation

Amount of ingredient for WA media preparation were-

Composition	Quantities (g/litter)
Water	1 L
Agar	20 g

20 grams of agar was suspended in 1000 ml distilled water. Heated for boil to dissolve the medium properly. And mixed properly in the flask. Then the flask was plugged with cotton and sterilized by autoclaving at 15 psi for 15 minutes (Figure 3).

3.6.2. Oat Meal Agar (OMA) medium Preparation

Amount of ingredients for OMA media preparation were-

Composition	Quantities (g/Litter)
Oat Meal	60 g
Agar	17 g
Water	1L

60 gram oat flakes were boiled with 2000 ml water for 15- 30 minutes. And prepared 1000 ml oatmeal containing water. Filtered through muslin cloth and brought filtrate back to volume with water. 20 gram agar was added, mixed properly and melted. Then the flask was plugged with cotton and sterilized by autoclaving at 15 psi for 15 minutes (Figure 3).

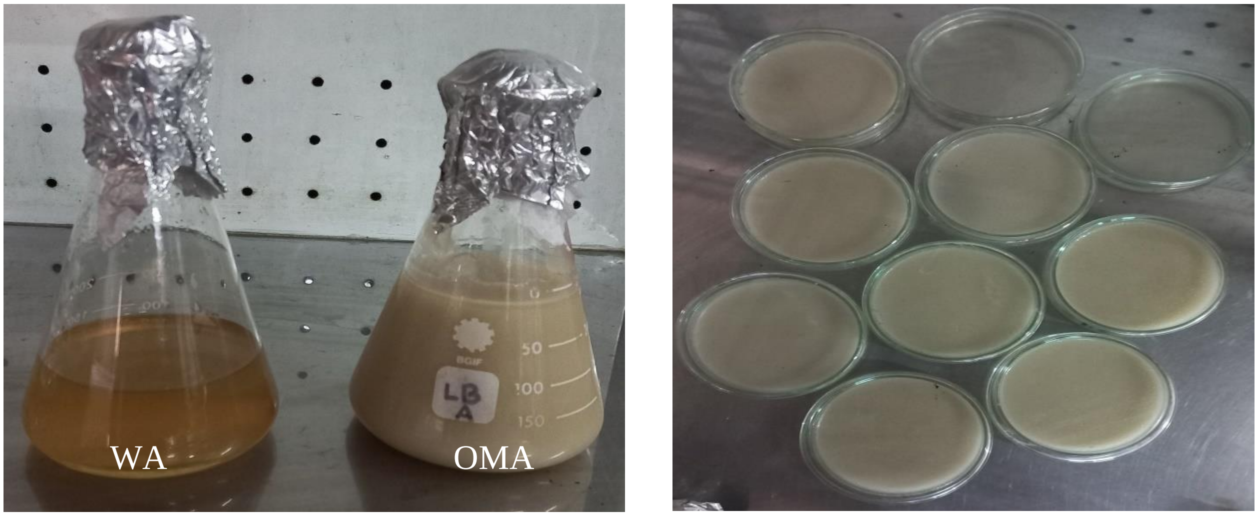

Figure 3. Culture media (WA and OMA) for isolation of *Magnaporthe oryzae*

3.7. Pathogenicity study

Pure culture of each isolate are grown on OMA for 30 days at 25 °C under alternating 14 hours of fluorescent light and 10 hour dark cycle to induce sporulation (Barksdale and Asai 1961). Harvested conidial suspension was filtered and centrifuged at 5000 rpm. By using a heamocytometer, the mass of spore sedimentation was collected, resuspended with sterilized distilled water and spore density was adjusted to a concentration of 1 × 10^5 spore/ml. The conidial spore suspension was sprayed at 3-4 leaf stage on rice leaves in pot and the seedlings were placed under glass house condition at 250°C. The sterile water was used instead of spore suspension served as control in vitro condition. Seedlings were evaluated after 7 days of inoculation.

3.8. Mycelial growth and morphological characterization

Mycelial growth of *Magnaporthe oryzae* on OMA was recorded at 4 days after Inoculation (DAI) and 7 DAI. Data on mycelial growth and growth rate were recorded and morphological character like shape, color, growth character, and surface structure of conidia were also determined. Mycelial growth was characterized by poor, medium or good growth where >6 mm/day growth rate was recorded as poor growth, 6-8 mm/day growth rate was recorded as medium growth and >8.00 mm/day growth rate was recorded as good growth of *Magnaporthe oryzae*.

3.9. Experimental design and statistical analysis of data

The experiment was conducted by using a complete Randomized Design (CRD) with three replications and data was analyzed by using the "Statistix 10" software. Treatment means were compared by Duncans Multiple Range Test (DMRT). Least significant differences (LSD) were calculated by using significant level at P= 0.05.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Survey on rice blast disease

Survey was conducted in Boro season 2020-2021 and Aman 2021 in three villages of Bogura districts. During survey, the rice blast symptoms were observed on leaf and neck. Leaf was recognized as typical blast disease symptom, diamond shape with grayish center and white margin (Wang *et al*., 2014). And in neck brownish black lesion were observed. The incidence and severity of neck blast and leaf blast was recorded from the selected fields listed in (Table 2 and Plate 1).

According to the survey, the disease incidence of rice blast in different location varied from 3.01 to 59.92% (Table 2). From the result it was indicated that the heighest incidence (59.92%) of blast disease was recorded from Bortola (Field 4) with maximum severity score (7) in BRRI dhan 58. And the lowest incidence (3.01%) of blast disease was recorded from Bonani (Field 1) with a severity score 1 in BRRI dhan 28. No disease was recorded on BRRI dhan 29 in Bonani (Field 4) at Bogura district in Bangladesh. These results showed variation in percent disease incidence (PDI) and severity in different location under cultivation.

Mahmud *et al*. (2021) surveyed on eight rice growing districts of Bangladesh namely Rangpur, Dinajpur, Bogura, Natore, Meherpur, Rajbari, Mymensingh, Jashore to estimate the incidence and severity of leaf and neck blast disease of rice. The heighest leaf and neck blast incidence was observed (28 and 11%) in Dinajpur. The lowest leaf blast (2%) was observed in Rajbari and Jeshore. According to Nazifa *et al*. (2021) surveyed three districts of Bangladesh in northern zone. The highest incidence (84.26%) of rice blast was recorded in BRRI dhan 28 from Gobindogonj. The lowest incidence was 13.56%. The highest severity score 9.00 was recorded in Mohimagonj with rice blast incidence 65%.

Table 2. Incidence and severity of rice blast (*Magnaporthe oryzae*) disease in selected location at Bogura district in Bangladesh in Boro season 2020-2021.

Name of village	Field site	Name of variety	Blast disease		
			Incidence (%)	**Severity (%)**	**Degrees of severity**
Sabgram	Field 1	BRRI dhan 28	10.46	4	1
	Field 2	BRRI dhan 28	19.88	6	1
	Field 3	BRRI dhan 29	7.81	4	1
	Field 4	BRRI dhan 29	4.09	5	1
Bortola	Field 1	BRRI dhan 28	3.19	5	1
	Field 2	BRRI dhan 58	16.01	12	3
	Field 3	BRRI dhan 58	55.90	30	5
	Field 4	BRRI dhan 58	59.92	40	7
Bonani	Field 1	BRRI dhan 28	3.01	5	1
	Field 2	BRRI dhan 58	4.13	6	1
	Field 3	BRRI dhan 58	6.09	2	1
	Field 4	BRRI dhan 29	0	0	0

Plate 1. Rice blast infected rice field and sampling sites and of Bogura district of Bangladesh in Boro season 2020-2021

Rayhanul *et al*. (2019) surveyed on 110 rice field of 5 districts of Bangladesh on rice blast. The heighest rice blast incidence (60%) was recorded from Mulktagachha with a severity score 5. But the heighest severity score was 7 in Hossainpur, Kishoreganj. Singh *et al*. (2018) surveyed in thirteen major rice growing districts naming Dantewada, Jagdalpur, narayanpur, Bilaspur, Janjgir-Champa, Kanker, Bemetara, Raipur, Dhamtari, Gariyaband, Balrampur, Surajpurand Surguja from 2016-2017. The disease incidence in different district ranged from 20% on Safari and Maheshwari varieties, Bastar district to 87.78% on Swarna, Jagadalpur district. The heighest disease incidence was 87.78% followed by Surguja (85.56%) and Balrampur (84.44%). Jagadeeshwar *et al*. (2014) reported the incidence of neck blast was 30-35% in flowering stage with North-East monsoon. Shahijahandar *et al*. (2010) surveyed on various rice growing areas during kharif season from (2006-2007). The heighest disease incidence (25%) was observed in node and neck blast and severity (15%) in node blast from district Kupwara. The result of the present study supported by Anwar *et al.* (2009). They surveyed temperate districts of Kashmir for the severity of rice blast and reported the leaf blast severity ranged from 3.7 to 41.3%. Where as height nodal blast was found in Kulgam (7.3%) followed by Khudwani (5.4%).

4.2. Isolation of *Magnaporthe oryzae*

The pathogen was isolated on OMA media from collected sample. There were 24 isolates of *Magnaporthe oryzae* successfully isolated from collected diseased sample . These 24 isolates showed morphological and cultural variation in their character.

4.3. Identification of *Magnaporthe oryzae*

Identification of the *Magnaporthe oryzae* isolates was concluded based on the morphology of the conidia. By using very fine tip needle, conidial masses were picked in glass slide. And the slide was observed under compound microscope with cover slip. Typical pyriform, 2 septate, 3 celled conidia was found with narrowed apex and broad basal. Manjunatha and Krisnappa (2019) aslo found pyriform 2- septate, 3 celled, greyish black conidia (Figure 4).

4.4. Pathogenicity study

Five isolates namely Mo3, Mo4, Mo7, Mo21 and Mo23 produced sufficient conidia and produced typical blast symptom after inoculation. The rest of the isolates did not sporulate sufficiently.

Plate 2. Collected rice blast samples and preparation of samples for incubation after plating

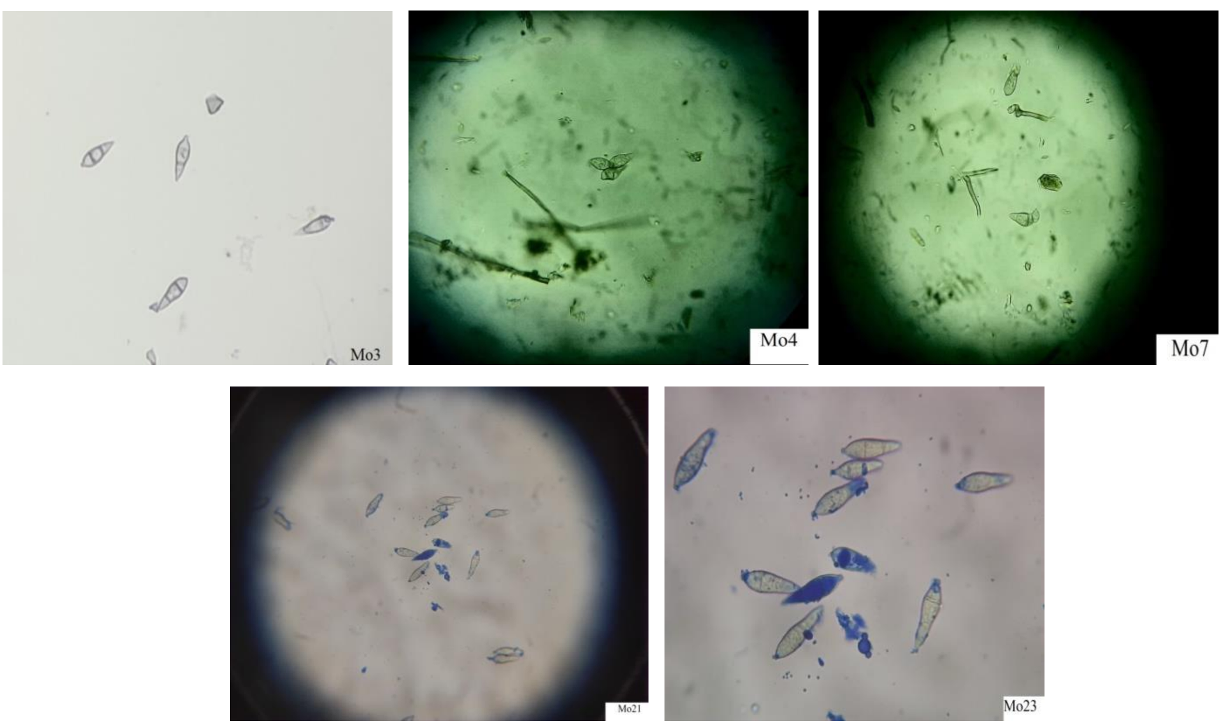

Figure 4. Conidia of five pathogenic isolates of *Magnaporthe oryzae* under compound microscope (×100)

4.5. Morphological characterization of *Magnaporthe oryzae*

4.5.1. Mycelial growth of 24 isolates of *Magnaporthe oryzae* in OMA at 4 DAI

Tweenty four isolates of *Magnaporthe oryzae* were cultured on OMA from the samples, collected from different villages of Bogura district**.** Mycelial growth, growth rate as well as cultural and morphological characteristics like color, surface structure, shape and eleviation were recorded (Table 3 and Plate 3). Cultural and morphological variation among the *Magnaporthe oryzae* isolates on OMA media at 4 DAI was observed. These isolate were differed in color, surface structure, shape, eleviation and mycelial growth. The radial mycelial growth of 24 isolates were ranged from 60.67 mm to 40 mm at 4 DAI. Highest mycelial growth was observed in Mo22 (60.67 mm at 4 DAI and 15.17 mm per day) with black color, smooth velvety surface texture and regular shape, flat and good growth. On the other hand, the lowest mycelial growth was observed in Mo13 (40 mm at 4 DAI and 10 mm per day) with brownish white color, rough cottony surface texture and regular shape, raised and good growth. The colony color of Mo1 and Mo15 isolates were grayish white. However the colony color of Mo2, Mo3 and Mo5 were grayish color. On the other hand Mo4, Mo7 and Mo21 isolates showed black color colony. Mo6, Mo8, Mo9, Mo11, Mo13, Mo14, and Mo22 isolates showed brownish white color colony. Mo16, Mo17, Mo18, Mo19 and Mo20 isolates showed whitish grey color colony. On the other hand Mo10 and Mo23 isolates showed ash color while only Mo24 isolate was light ash color colony. Almost all isolates were raised except three (Mo4, Mo7 and Mo21) isolates were flat type. All 24 isolates were graded as good mycelial growth.

Mycelial growth, morphological and cultural characterization of *Magnaporthe oryzae* were carried out by many scientists in the word. Shaw *et al.* (2019) isolated ten isolates of *Magnaporthe grisea* on OMA media from different rice growing region. They categorized *Magnaporthe grisea* into different group based on colony and morphological character. The colony color was grey, white grey and black. Like our study they found most of the isolates as smooth surface colony.

Table 3. Radial Mycelial growth (mm) and growth rate of 24 isolates of *Magnaporthe oryzae* in OMA media at 4 DAI

Isolates	Radial mycelial growth		Cultural characteristics
	4 DAI (mm)	Average growth rate/day (mm)	
Mo1	49.33 g	12.33	Grayish white color, smooth velvety surface texture, raised, irregular margin, and good growth
Mo2	48 g	12	Grayish color, rough and cottony surface texture, irregular margin, raised and good growth
Mo3	54 cde	13.5	Grayish color in the centre and white color in the margin, cottony and rough surface texture, regular shape, raised and good growth
Mo4	50 fg	12.5	Black color, rough velvety suface texture, irregular shape, flat and good growth
Mo5	55.67 bcde	13.92	Grayish color, rough cottony surface texture, irregular shape, raised and good growth
Mo6	56 bcd	14	Brownisg white color, smooth cottony surface texture, regular shape, raised, good growth
Mo7	49.33 g	12.33	Black color, smooth cottony surface texture, regular shape, flat, good growth

Table 3. Contd.

Mo8	57 bc	14.25	Brownish white color, smooth cottony surface texture, regular shape, raised and good growth
Mo9	55.33 bcde	13.83	Brownish white color, smooth cottony surface texture , regular shape, raised and good growth
Mo10	56 bcd	14	Ash color, smooth velvety surface texure , regular shape, raised and good growth
Mo11	58.33 ab	14.58	Brownish white color, smooth cottony surface texture, regular shape, raised and good growth
Mo12	54.67 cde	13.67	Grayish white color,smooth cottony surface texture, regular shape, raised and good growth
Mo13	40 h	10	Brownish white color, rough cottony surface texture , regular shape, raised and good growth
Mo14	41.67 h	10.42	Brownish white color, rough cottony surface texture, irregular shape, raised and good growth.
Mo15	53.33 de	13.33	Grayish white color, velvety surface texture, irregular shape, raised and good growth
Mo16	54.33 cde	13.58	Whitish Brown color, smooth velvety surface texture, regular in shape, raised and good growth

Table 3. Contd.

Mo17	56.67 bc	14.17	Whitish brown color, smooth velvety surface texture, regular in shape, flat and good growth
Mo18	56 bcd	14	Whitish brown color, smooth cottony surface texture, regular in shape, raised and good growth
Mo19	55 cde	13.75	Whitish brown color, smooth velvety surface texture, irregular in shape, raised and good growth
Mo20	52.67 ef	13.16	Whitish brown color, smooth cottony surface texture, regular in shape, raised and good growth
Mo21	48 g	12	Black color, smooth velvety surface texture, regular in shape, flat and good growth
Mo22	60.67 a	15.17	Brownish white color, smooth velvety surface texture, regular in shape, raised and good growth
Mo23	55.33 bcde	13.83	Ash color , smooth velvety surface texture, regular in shape, raised and good growth
Mo24	53.33 de	13.33	Light ash color, smooth cottony surface texture, regular shape, raised and good growth
LSD value	3.05		

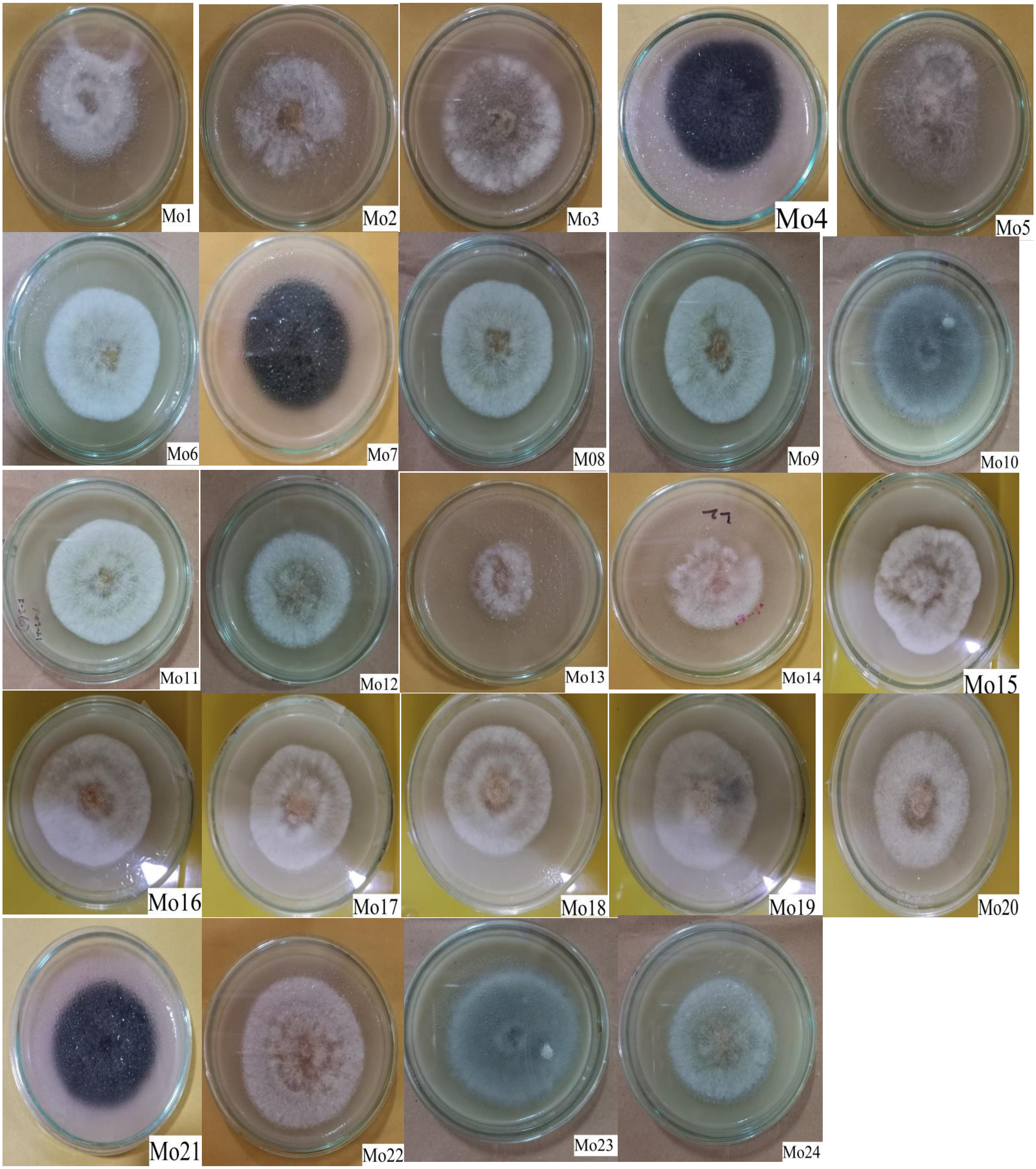

Plate 3. Pure culture of *Magnaporthe oryzae* isolates on OMA at 4 DAI

In another study, Panda *et al*. (2017) collected twenty isolates and categorized into three groups like colony color, texture of the colony and type of growth. The colony color varied from grey, greyish black, dark black, blackish white and greyish black. The maximum colony diameter (48 mm) and the least colony diameter (27 mm) found. Most of the isolates were smooth and few isolates were rough. Most of the isolates were flat mycelial growth except three isolates that supports our study. Khadka *et al*. (2012) used different media and found that OMA media was the best for mycelial growth of *Pyricularia oryzae* isolates from rice and finger millet crop plants. The colony color was grey on OMA media. The maximum mycelial growth was (55 mm) and the least was (2 mm) in OMA from rice blast sample. Bandyopadhyay (2009) also reported that the OMA media produced off- white, good and regular mycelial growth. That corroborate our study. The present study also supported by Kulkarni (1973) reported that OMA was good for the growth of *Pyricularia oryzae* isolates among all the solid and liquid medias. *Pyricularia oryzae* isolates showed comparatively higher mean mycelial growth rate on OMA (15.43 mm per day). Ou (1985) recorded colony color of *Pyricularia oryzae* isolates varied to greyish black to dark jet black color, smooth to irregular margin, medium to good growth on Oat Meal Agar media.

4.5.2. Mycelial growth of 24 solates of *Magnaporte oryzae* in OMA at 7 DAI

Mycelial growth of 24 isolates of *Magnaporthe oryzae* at 7 DAI isolated on OMA from the collected sample from different village of Bogura district and morphological and cultural characteristics were recorded (Table 4 and Plate 4). The results indicate variation among mycelial color, surface structure, shape, eleviation and mycelial growth. The radial mycelial growth of 24 isolates were varied from 84.33 mm to 62 mm at 7 DAI. Highest mycelial growth was observed in Mo16 (84.33 mm at 7 DAI and 12.05 mm per day) brown color, smooth velvety surface texture and regular in shape followed by Mo15 (84 mm at 7 DAI and 12 mm per day). The lowest mycelial growth was observed in Mo13 (62 mm at 7 DAI and 8.86 mm per day) with ash centre and white margin, rough cottony surface texture and irregular shape. Among 24 isolates, 7 isolates (Mo5, Mo6, Mo8, Mo9, Mo11, Mo12 and Mo18) showed gray color, 5 isolates (Mo2, Mo3, Mo13, Mo14 and Mo20) showed whitish brown color, 3 isolates (Mo4, Mo7 and Mo21) showed black color, 3 isolates (Mo16, Mo17 and Mo22) showed brown color, 2 (Mo1 and Mo19) were whitish color, 2 isolates (Mo10 and Mo23) showed whitish ash color, and other 2 isolates (Mo15 and Mo24) were showed grayish white color. All isolates were good growth. Maximum isolates were regular shape, smooth velvety surface texture. Almost isolates were raised except Mo9. MO12 and Mo21.

This type of study was carried out by many scientists in the word. Nazifa *et al*. (2021) used different solid and liquid media for isolation of *Magnaporthe oryzae.* The heighest mycelial growth (20mm) was recorded in OMA having buff color, greyish black to black color, irregular colonies with rough and smooth margin and lowest was recorded in WA (10 mm) at 7 DAI. Similar results also reported by Dutta *et al*. (2019) that the rice blast pathogen *Pyricularia oryzae* was isolated from Uttar Banga Krishi Viswavidyalaya Farm of rice field and characterized based on morphological and cultural characters. Heighest mycelial growth was observed on OMA (71.33 mm) at 6 DAI. Rayhanul *et al*. (2019) isolated four isolates of *Pyricularia oryzae* on OMA. The heighest mycelial growth was 79.50 mm in case of PO4 isolates. The lowest mycelial growth (68.33mm) was recorded

Table 4. Radial mycelial growth(mm) and growth rate of 25 isolates of *Magnaporthe oryzae* on OMA media at 7 DAI

Isolates	Mycelial radius growth (mm)	Average mycelial growth rate/ day (mm)	Cultural characteristics
Mo1	73.33 hi	10.47	White color, smooth velvety surface texture, regular shape, raised, good growth
Mo2	72 ij	10.28	Whitish brown color, rough cottony surface texture, irregular shape, raised, good growth
Mo3	73.33 hi	10.47	Whitish brown color, rough cottony surface texture, irregular shape, raised, good growth
Mo4	76.67 g	10.95	Black color, smooth cottony surface texture, regular shape, flat, good growth
Mo5	70.67 ij	10.09	Light ash color, rough cottony surface texture, regular in shape, raised, good growth
Mo6	82 abcd	11.71	Light ash color, smooth velvety surface texture, regular in shape, raised, good growth
Mo7	77.33 fg	11.05	Black color, smooth cottony surface texture, regular in shape, flat, good growth
Mo8	78.67 efg	11.24	Light ash color, smooth cottony surface texture, regular in shape, raised, good growth

Table 4. Contd.

M09	80.67 cde	11.52	Light ash color, smooth cottony surface texture, regular in shape, raised, good growth.
Mo10	83.67 ab	11.95	Ash color, smooth velvety surface texture, regular in shape, raised, good growth.
Mo11	76 gh	10.86	Light ash color, smooth cottony surface texture, regular in shape, raised, good growth
Mo12	80.67 cde	11.52	Light ash color, smooth cottony surface texture, regular in shape, raised, good growth
Mo13	64.67 k	9.24	Whitish brown, smooth cottony surface texture, regular in shape, raised, good growth
M014	70 j	10	Grayish in color, rough cottony surface texture, irregular shape, raised, good growth
Mo15	84 ab	12	Whitish ash color, smooth velvety surface texture, regular in shape, raised, good growth
Mo16	84.33 a	12.05	Ash color, smooth velvety surface texture, regular in shape, raised, good growth

Table 4. Contd.

Mo17	83.33 abc	11.90	Brownish color, smooth velvety surface texture, regular shape, raised, good growth
Mo18	82 abcd	11.71	Light ash color, smooth velvety surface texture, regular shape, raised, good growth
Mo19	80.67 cde	11.52	Whitish color, smooth velvety surface texture, regular shape, raised, good growth
Mo20	83 abc	11.86	Whitish brown color, smooth cottony surface texture, regular shape, raised, good growth
Mo21	80 def	11.43	Black color ,smooth velvety surface texture, irregular shape,raised, good growth
Mo22	81.33 bcde	11.62	Brownish color, smooth velvety surface texture, regular shape, raised, good growth
Mo23	83.33 abc	11.90	Ash color, smooth velvety surface texture, regular shape, raised, good growth
Mo24	62 k	8.86	Whitish ash in the centre and white in the margin, rough cottony surface texture, irregular shape, raised, good growth
LSD value	2.86		

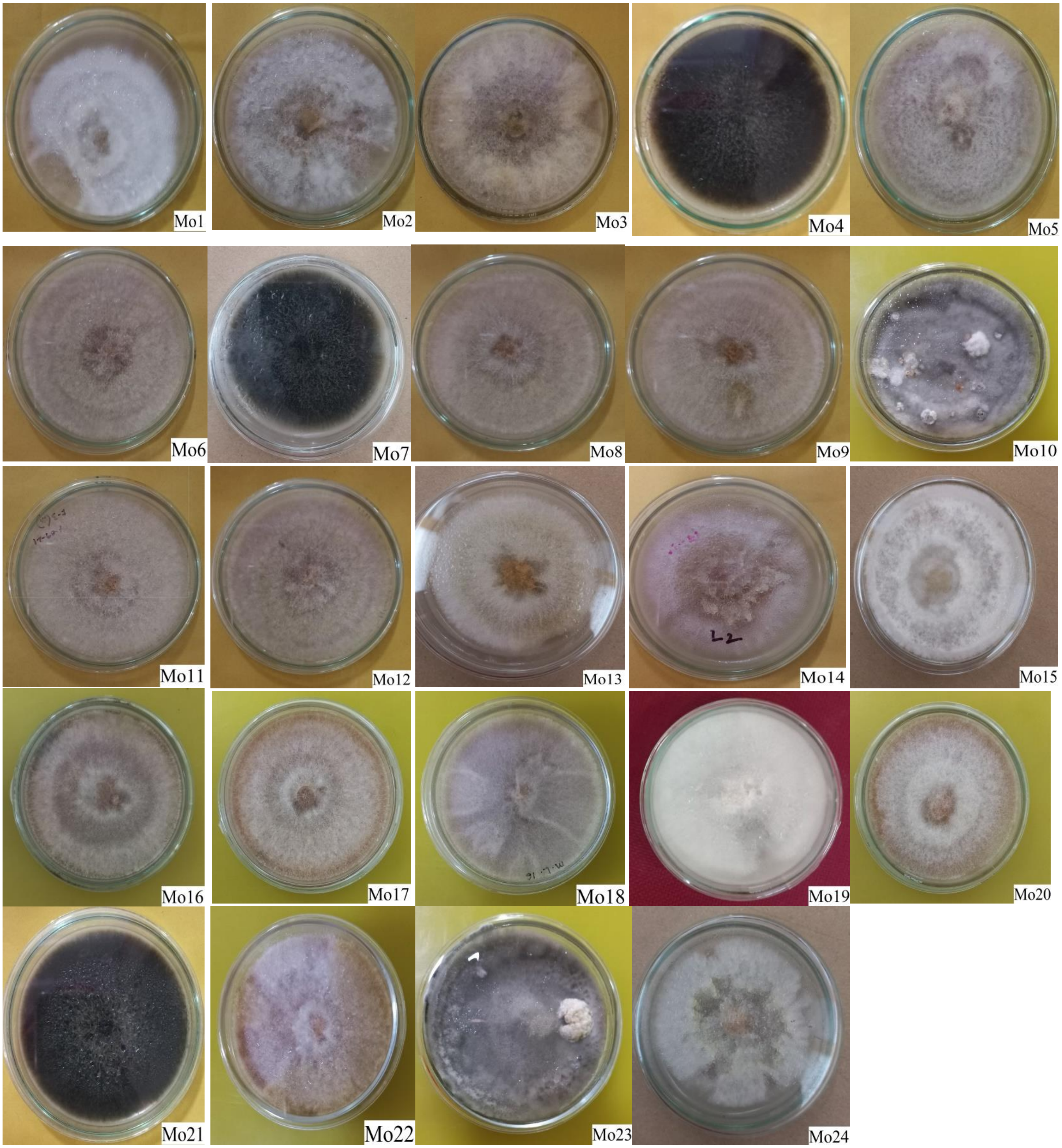

Plate 4. Pure culture of *Magnaporthe oryzae* isolates on OMA at 7 DAI

in Po1 isolates on OMA. Kulmitra *et al*. (2017) used different liquid and solid media for isolated *Pyricularia oryzae* isolates from rice. The heighest mean mycelial growth (77.6 mm) of the fungus was recorded on OMA among all the solid media. The present study supported by Yashaswini *et al*. (2017) isolated twenty *Magnaporthe oryzae* isolates from different rice growing areas. The morphological characteristics like radial mycelial growth, color of the colony, texture, size and shape of the colny were studied. The maximum colony diameter was 87.67 mm and least colony diameter was 71.67mm on OMA media. Most of the isolates showed greyish white colonies with smooth texture followed by greyish white colonies with smooth texture. And other three isolates were grey color with rough texture. Similar study Teli *et al*. (2016) used different media such as Potato dextrose Agar (PDA), Oat Meal Agar (OMA), Ragi flour agar, Yeast extract + 2% soluble starch, Host extract + 2% soluble sucrose agar, Potato dextrose agar + Biotin + Thiamine and Rice flour agar for morphological and cultural characeterization of *Magnaporthe grisea* isolates from rice field. Among these media OMA was found to be best media for radial mycelial growth. The heighest mycelial growth (88 mm) with off white color, smooth, uniform and good growth was recorded on OMA. The present study supported by Asfaha *et al*. (2015) identified six *Pyricularia oryzae* isolates such as Po12, Po28, Po41, Po55, Po72 and Po85. Among different culture media best mycelial growth on OMA. The heighest radial mycelial growth in Po12 (88 mm) with dark gray color, entire, cottony surface texture was observed on OMA. And the lowest mycelial growth was observed in MEA (59 mm) with light gray color, entire, velvety and thick surface texture of Po85 isolates. Gashaw *et al*. (2014) studied with six *Pyricularia grisea* isolates for morphological and physiological variability. Among different media, the maximum mycelial growth showed on OMA (87.3 mm) with grey color, smooth margin, raised and good mycelial growth.

CHAPTER V

SUMMARY AND CONCLUSION

Rice blast caused by *Magnaporthe oryzae* is one of the most destructive disease in any rice growing region of the world. The survey was conducted in the selected field of Bogura district and Laboratory experiment was conducted in the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University following Complete Randomized Design (CRD).

Incidence and severity of rice blast were recorded in some selected fields of Bogura districts and the morphological and cultural characteristics such as growth characters, colony color, surface structure, shape, eleviation of *Magnaporthe oryzae* isolates were recorded.

Among 12 farmer´s fields, the heighest disease incidence (59.92%) was recorded in the field Bortola village with a severity score 7 in BRRI dhan 58 and the lowest incidence (3.01%) of blast disease was recorded from Bonani field with a severity score 1 in BRRI dhan 28. Collected isolates were identified based on 3–celled, pyriform conidia. Pathogenecity tests were confirmed for five (5) isolates among 24 collected isolates.

Twenty four isolates of *Magnaporthe oryzae* were successfully isolated from infected neck of rice. The present study showed that, there were variation on cultural and morphological characteristics among 24 isolates of *Magnaporthe oryzae*. The highest mycelial growth was observed in Mo22 (60.667 mm at 4 DAI and 15.17 mm per day) and Mo16 (84.33 mm at 7 DAI). On the other hand, the lowest mycelial growth was observed in Mo13 (40 mm at 4 DAI and 10 mm per day) and Mo13 (62 mm at 7 DAI) on OMA. All isolates showed good mycelial growth. The present study emphasized a global collection of *Magnaporthe oryzae* to detect and monitor physiologic races of *Magnaporthe oryzae* present in the country.

CHAPTER VI

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