SURVEY ON RICE BLAST AND MORPHOLOGICAL CHARACTERIZATION OF *MAGNAPORTHE ORYZAE* ON DIFFERENT CULTURE MEDIA

MD. SHARIFUL ISLAM

DEPARTMENT OF PLANT PATHOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

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BY

MD. SHARIFUL ISLAM

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Approved by:

----------	**----------**
Dr. F. M. Aminuzzaman	**Dr. M. Salahuddin M. Chowdhury**
Professor	**Professor**
Department of Plant Pathology	Department of Plant Pathology
Sher-e-Bangla Agricultural University	Sher-e-Bangla Agricultural University
Supervisor	**Co-Supervisor**

Prof. Dr. Fatema Begum
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University, Dhaka-1207

CERTIFICATE

This is to certify that the thesis entitled, **"SURVEY ON RICE BLAST AND MORPHOLOGICAL CHARACTERIZATION OF *MAGNAPORTHE ORYZAE* ON DIFFERENT CULTURE MEDIA.***" 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 in PLANT PATHOLOGY,*** *embodies the result of a piece of bona fide research work carried out by* ***MD. SHARIFUL ISLAM****, Registration No.* ***14-06187*** *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.

Dated: 15th January 2022
Place: Dhaka, Bangladesh

Prof Dr. F.M. Aminuzzaman
Supervisor
Department Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207, Bangladesh
Mobile: 01733717936
*E-mail:*aminsaupp@yahoo.com

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ABSTRACT

Rice appears to be the most important food crop, accounting for a quarter of all calories consumed Rice blast is one of the most common significant obstacles to rice cultivation in any rice growing region of the world. Field survey on rice blast (*Magnaporthe oryzae*) was conducted in 30 field sites of Sonatola upazila belong to Bogura District during boro season 2019-2020. Blast incidence and severity ranged from 0-81.8% and 0-65% respectively. The highest blast severity was recorded in Basmoti rice field of Pakulla village under Pakulla Union. Twenty (20) isolates of *Magnaporthe oryzae* was identified and morphologically characterized on Water Agar (WA), Potato Dextrose Agar (PDA), Potato Sucrose agar (PSA) and Oat Meal Agar (OMA). Radial mycelia growth rate of all isolates ranged from 5.5 to 22.5, 7.5 to 77.2, 5.5 to 61.5 and 7.5 to 72.5 in Water Agar (WA), Potato Dextrose Agar (PDA), Potato Sucrose agar (PSA) and Oat Meal Agar (OMA) respectively. Most of the isolates were blackish white in color, rough cottony surface structured and irregular shaped. Mycelial growth of *Magnaporthe oryzae* isolates significantly varied on different culture media.

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SOME COMMONLY USED ABREVIATIONS

FULLWORD	ABBREVIATION
Agro-Ecological Zone	AEZ
Agricultural	Agril.
And others	et al.
Accessions	ACC
Bangladesh Rice Research Institute	BRRI
Bangladesh Bureau of Statistics	BBS
Biological	Biol
Centimeter	cm
Disease Incidence	DI
Ecology etc.	eco.
Figure	Fig.
Food and Agricultural Organization	FAO
Gram	gm
Journal	J.
Kilogram	Kg
MiliMeter	mm
Number	No.
Oat Meal Agar	OMA
Page	pp.
Percent	%
PotatoDetrose Agar	PDA
Potato Sucrose Agar	PSA
Completely Randomized Design	CRD
Replication	R
Research	Res.
Science	Sci.
Sher-e-Bangla Agricultural University	SAU
Water Agar	WA

APPENDICES

CHAPTER I
INTRODUCTION

Rice (*Oryza sativa* L.*)* is the most widely produced cereal crop on the planet. It belongs to the Poaceae family and is one of the oldest cultivated crops (Islam and Catling, 2012; Qiu *et al*., 2019). (USDA-NRCS, 2019). Rice is a staple food for more than half of the world's population (BER 2015). (Muthayya *et al*., 2014) 90% of the world's production is produced and consumed primarily in Asia (GRiSP, 2013). Bangladesh has surpassed India as the world's fourth-largest rice producer (CGIAR, 2019; FAO, 2018). In recent years, Bangladesh has not only achieved rice self-sufficiency, but has also begun to enter the export market (BER 2015). Furthermore, Bangladesh is the world's most densely populated country, with 160 million people eating rice as a primary cuisine (UN, 2019; UNFPA, 2019). Rice production is the country's main source of food security (Kashem and Faroque, 2013). Rice production takes up 75% of Bangladesh's cultivable area, yielding 36.3 million tons of rice each year (BBS, 2018). Rice production in Bangladesh is improving every day, although there is still a 20.7% rice yield disparity (Kabir et al., 2016). Furthermore, due to climate change, there are various biotic and abiotic stressors that have formed a new challenge for rice cultivation. Due to numerous biotic stresses, rice output is reduced by about 37% per year (IRRI, 2019; Mondal *et al*., 2017). Bangladesh's food security is jeopardized as a result of these circumstances. There are various illnesses in Bangladesh's rice fields that affect rice yield throughout the year. Rice blast is the most important disease among them, and it has become a major danger to our productivity.

Magnaporthe oryzae causes rice blast, which is the most common fungal disease seen in most rice-growing locations across the world (Ou, 1985). The pathogen is known to attack rice crops at various stages, including seedling, tillering panicle emergence, and pathogen infecting various parts, including leaf, sheath, stem, neck, panicles, and grain discolorations, and due to severe pathogen infection, the leaves become dried and appear to be brunt in both the nursery and main field.

During the maturity stage of the crops, the pathogen infects neck panicles, causing chaffyness of the panicles and browning of the grain, resulting in a reduction in rice output (Gato, 1965). The disease can harm all of the plant's aerial parts. The majority of infections occur on the leaves, resulting in spindle-shaped lesions with a gray or white center caused by blast disease, or on the panicles, which turn white and die before being filled with grain (Scardaci *et al*., 1997). Although certain strains of *M. oryzae* that do not attack rice can hurt weeds in the rice field, *M. oryzae* is very specific to rice. *Magnaporthe oryzae* quickly generates hundreds of spores on a rice plant, which are easily dispersed via the air, by wind or rain, and onto nearby plants (Rick and Lee, 2000).

Blast was originally described more than three centuries ago in Asia, and it is today found in over 85 nations. It can be found in irrigated lowland, rain-fed upland, or deep water rice fields and is very adaptive to environmental circumstances (Rao, 1992). When predisposition factors (high mean temperature values, relative humidity more than 85-89 percent, presence of dew, drought stress, and excessive nitrogen fertilizer) favor epidemic growth, yield loss can reach 70-80% (Piotti *et al*., 2005).

Understanding evolution in the plant pathosystem requires a thorough examination of genetic variation in plant pathogen populations (MC Donald *et al*., 1989). Earlier research (Ou, 1985) focused on pathotypic variability, while more recent investigations have used molecular markers to characterize population variation. The use of molecular markers in population genetic studies has revealed epidemiologic data at previously unattainable levels of precision.

The main driver of resistance breakdown in rice against rice blast disease is pathogenic variation (Urashima, 2002). Different sources of pathogenic variation in *M. oryzae* have been documented in several investigations. Single-spore isolates arising from single lesions and mono conidial sub-cultures have shown significant pathogenic variation in *M. oryzae* (Correa-Victoria *et al*., 1993). Pathogenicity of isolates from the same lesion can vary, as can pathogenicity of single-spore subcultures compared to single-spore cultures (Gad *et al*., 2013).

Para-sexual recombination is one of the ways of variation in *M. oryzae*, according to Zeigler *et al*., 1997. Further research into pathogenic changes during sexual hybridization could lead to proof of pathogenic variation in the fungus's asexual stage.

Magnaporthe oryzae strains are not pathogenically homogeneous, and many major rice-growing countries have documented the presence of pathogenic races (Anwar *et al*., 2009). Although a culture of *M. oryzae* can vary its pathogenicity, it cannot be utilized to assign pathogenic race (Veeraraghaban, 1986). Veeraraghaban, in a 1986 study, classified pathogenic isolates by evaluating the response of rice varieties to blast disease, and came up with three categories: resistance, moderate resistance, and susceptible rice varieties. The technique of differentiating pathogenic races of pathogens using differential rice genotypes of blast disease has been widely adopted.

In a survey Nazifa *et.al*. (2021) found a variation in blast incidence and severity of rice under different field sites and different varieties. Rayhan *et.al*. (2019) also found varying incidence on severity of rice blast in different location of Bangladesh. In both of these studies they identified several isolates with different growth and cultural characteristics on different media. The pathogen was very destructive and highly changeable in genetic makeup and able to arrive as a new race or variants. So, continuous monitoring on different isolates in different rice growing region is utmost important. The present research work was therefore conducted with the following objectives.

I. To determine incidence and severity of rice blast is some selected field site of Sonatola upazila under Bogura district.

II. To determine cultural and morphological variation among isolates of *Magnaporthe oryzae*.

CHAPTER II

REVIEW OF LITERATURE

The available literature of work done on blast disease of rice concerning to this dissertation is presented in the following heading and sub headings.

2.1. Significance of *Oryzae sativa* (Rice)

FAO (2015) reported that rice accounts for 20% of global diatery supply, while wheat and maize account for 19% and 5%, respectively. During 2012-13 and 2013-14, global production increased by 1%, from 472 million tonnes to 41 million tonnes. (January 2015 rice commodity profile)

FAO's (2009) stated that annual report, rice is grown on 161 million hectares worldwide, with Asia producing and growing 90 percent of the world's rice.

Faure and Mazaud (1996) Reported that rice is the most economically important main food crop in India, China, East Asia, South East Asia, Africa, and Latin America, meeting the nutritional needs of 70% of the population in these countries.

Khush and Toenniessen (1991) reported that it is produced primarily in Asia (90 percent) on subsistence farms, with the grain destined for local consumption and only 4 percent exported to international markets. China and India account for half of the production area.

Reversat (1995) Silue and Notteghem (1991), Alam (1988), John *et al*. (1985), and Attire and Fatokun (1983) discovered that *Oryza sativa* cultivars are frequently under-adapted to various abiotic and biotic conditions in Africa. *Oryza glaberrimabas* have been found to have a moderate to high level of resistance to blast, rice yellow mottle virus, rice gall midges, insects, and nematodes.

Seebolf *et al*. (2004) stated that *Oryza glaberrima* is traditionally found in a variety of West African agroecosystems, but it has been largely abandoned in

favor of high yielding *Oryza sativa* cultivars with superior agronomic performance.

Von Barun (2007) reported that rice is one of the world's three most important food crops and the primary source of nutrition for nearly half of the world's population. Its production is concentrated in Asia (90%) in subsistence agriculture farms, with the grain destined for domestic consumption and only 4% exported to international markets.

Yaduraju (2013) stated that rice is the main food in Asia and the Pacific region, accounting for nearly 39 percent of calories.

2.2 Importance of rice blast Disease (*Magnaporthe oryzae*)

Agrios (2005) stated that multiple rice blast epidemics have occurred in various parts of the world, with production losses ranging from 50% to 90% of the predicted crop.

Arshad *et al*. (2008) reported that rice blast has been found in the districts of Faisalabad, Toba Tek Singh, Vehari, and places like Gaggo Mandi in Pakistan for the previous two decades.

Baker et al. (1997) and Scardaci *et al*. (1997) explained that rice blasts caused 50% losses in the Philippines, according to Awoderu and Esuruoso (1975). Rice blast disease killed 30-50 percent of rice plants in South America and Southeast Asia.

Blast disease induced by *Magnaporthe oryzae* cavara [Synnym *Magnaporthe grisea* sacc., anamorph of *Magnaporthe grisea* (Hebert) Yaegaish and Udagawa] increases rice output statistics in Pakistan, according to Jia *et al.* (2000).

Blast disease, caused by the fungus *Magnaporthe oryzae*, is one of the key constraints in rice production, according to Oerke and Dehne (2004).

Bonman (1989) stated that Tsai (1982), Padmanabhan (1965), and Goto (1965), the illness causes a large loss of yield, which has been reported to be as high as 70-80% during an epidemic season.

Hasioka (1950) discovered that vulnerable cultivates cultivated in disease-prone locations can suffer significant losses. Rice is grown in a variety of environments, however upland cultivations are more prone to blast. It has been hypothesized that blast outbreaks are related to plant age and resistance.

Kato (2001) reported that rice blast disease causes yield losses ranging from 1-100 percent in Japan and 70 percent in China.

Leong (2004) stated that the rice blast disease is also a severe concern in the penna river nelts and Godavari in Andhra Pradesh, more than fifty more grass species are susceptible to the blast fungus. It causes illness on the leaves, nodes, and panicles of seedlings and older plants. It can be found in both submerged and deep water rice, as well as irrigated lowland and rain-fed upland rice. Rice blast is the most deadly disease seen in large areas of rice cultivation in Latin America, Africa, and Southeast Asia, and it is a global issue in rice production. Rice blast disease is a global issue in the rice industry. Rice blast disease poses a major threat to world food security and agricultural trade.

Magnaporthe grisea (Anamorph *Magnaporthe grisea* Sacc. Synonym *Magnaporthe oryzae* Cav.) Causes rice blast disease in rice-growing areas around the world, according to IRRI (1976).

Magnaporthe oryzae is one of the most important fungal pathogens of rice, according to Manandhar *et al*. (1998) due to its widespread distribution and destructive character. The fungus can infect any portion of the rice plant's aerial structure, including the seeds. They also suggested that the fungus could be transmitted from seeds to seedlings in a systemic manner.

Manadhar *et al*. (1992) the disease produced a 10-20% yield drop in susceptible types in Nepal, with production reductions reaching 80 percent in severe cases.

Nutsugah *et al*. (2008) stated that this disease is the greatest limitation to production in the largest area of African agriculture, with yield losses ranging from 3-77 percent. In both upland and lowland rice production systems, the fungus can infect plants at any stage of growth and growth. Lowland rice grown in Asia's temperate and subtropical temperatures is extremely susceptible to the disease, whereas tropical upland rice is only susceptible when irrigated.

Prabhu and Morais (1986) reported that the disease causes a large yield loss, which can be as high as 70-80% during an outbreak. Lesions on sensitive cultivars may seem grayish green and water soaked at first, with a darker green border and they can quickly grow to several centimeters in length. The general area necrosis at the junction of the two tissues is a symptom of collar node infection. After the plant is broken at the collar region, collar infections can kill the entire leaf and extend a few millimeters into and around the sheath.

Puri *et al*. (2006) investigated the effect of blast disease on rice plants in 45 Indian regions and discovered that in low land rice growing areas, the highest percent Disease Index (PDI) was found at the dough stage (30.45 percent), followed by booting stage (29.77 percent), and tillering stage (15.4 percent).

Rice blast disease is harmful in around 85 nations where the rice plant is cultivated, in both paddy and upland settings, according to Chiba *et al*. (1996). Rice blast is found anywhere rice is grown, however the severity of the disease varies greatly depending on the environment and cropping strategy. Environments with frequent and persistent dew spells, as well as low daytime temperatures, are more conducive to blast.

Rice blast disease, caused by the fungus *Magnaporthe oryzae*, is one of the principal constraints on rice production, according to Oerke and Dehne (2004). During the 1990s, annual rice losses due to this fungus were projected to be 35 percent of global production.

Rice blast, caused by *Magnaporthe oryzae*, is one of the most damaging diseases of rice, according to Chandrasekhara *et al*. (2008) resulting in yield losses of up to 65 percent in sensitive rice cultivars.

Rice blast, caused by the fungus *Magnaporthe oryzae*, is one of the most serious diseases of rice. (Couch and Kohn 2002).

Rice blasts in India have resulted in losses of up to 75% of grains, according to Padmanabhan (1965).

Shahriar *et al*. (2020) explained that the illness, rice blast has emerged into a pioneering model system for exploring host-pathogen interactions, With 60% of the world's population relying on rice as their primary source of colonies, the disease may have devastating implications. The virulent pathos-types cause a high frequency of disease. Pathogenicity study is the only way to determine pathos-types using a collection of diverse rice varieties that are usually different and carry varied resistance genes. In the late 1960s and early 1970s, the blast in Bangladesh was relatively unimportant. Blast disease outbreaks were reported in Bangladesh during the Boro season in 1980 and 1990.

The culture of *Magnaporthe oryzae* was described by Hawksworth (1990) of the Commonwealth Mycology Institute (CMI) as grayish, conidiophores single or in fascicles, simple, rarely branched, and demonstrating sympodial growth. Conidia developed singly at the tip of the narrower toward tip, rounded at the base, three septate, hyaline to pale olive, 19-23 7-9 m, with a prominent protruding basal hilum Chlamydospore are thick-walled spores with a diameter of 5-12 m that are frequently formed in culture.

Zhu *et al*. (2005) reported that the neck blast is the most devastating phase of the disease and can occur without the presence of severe leaf blast. Infected nodes appear black-brown and dry, and they are frequently found in a banded pattern. This type of infection frequently causes the Culm to break, resulting in the rice plant's death Zhu *et al*. (2005).

2.3 Survey and disease intensity of rice blast

Ali *et al*. (2009) measured leaf blast severity that ranged from 3.7 to 41.3 percent in Kashmir's temperate areas, according to a survey. Anantang district's klugam (7.3 percent), khudwani (5.4 percent), and lamoo (3.8 percent) zones all had highest node blasts. Every district had the most devastating phase of neck blast severity, with an average range of 0.3-4.9 percent. They also tasted for the ID-1, ID 2, IC-25, and IB-4 races, which were founded by Kashmirband. In the Anantang district, the ID-1, ID-2, and IC-17 races were the most common.

Blast symptoms appear at all phases of plant growth, according to Rameshbabu (2015). On leaves, lesions are often spindle-shaped, with a wide core and pointed ends. Large lesions usually have the shape of a diamond, with a grayish center and a brown rim. Lesions on the leaves spread quickly and tend to consolidate under favorable conditions, resulting in full necrosis of infected leaves and a burnt look from a distance. Castilla *et al*. (2009) studied rice blast for a long period and discovered that the pathogen infects all ground components at various growth phases, including the leaf, collar, nodes, internodes, base or neck, and other parts such as the panicle and leaf sheath. A typical blast lesion on a rice leaf is grey in the middle with a black border and is spindle shaped, according to the researchers. The disease thrives in an environment with frequent and protracted dew periods, as well as cool temperatures during the day.

Dar *et al*. (2010) documented the frequency and distribution of rice blast in Jammu and Kashmir's Kupwa district, reporting a 25% illness incidence and 15% severity, with the incidence increasing from transplanting to panicle commencement stage.

During the Boro (irrigated ecosystem) and Transplanted Aman (Julrain fed ecosystem) seasons, Hossain *et al*. (2017) recorded the incidence and severity of rice blast disease in eleven agro-ecological zones (AEZs) in Bangladesh. In terms of disease incidence and severity, the irrigated ecosystem (Boro season) (21.19 percent) outperformed the rain-fed ecosystem (Transplanted Aman season) (11.98 percent) (AEZs).

Hossain and Kulakarmi (2001) conducted a rice blast survey in different villages of Dharwad, Belgaum, and Uttar Kannada districts during Kharif 1999, and found that the disease was most prevalent in the Haliyal (61.66 percent) and Mundagod (54.00 percent) talukas of North Karnataka.

Magnaporthe oryzae was finally proposed by Yaegashi and Udagawa (1978) as a perfect stage of *Magnaporthe oryzae* (cooke). Instead of *Ceratospheria oryzae*, use Sac. In their research, the scientists discovered that fungal hyphae are hyaline and septate. However, as the fungus ages, the hypha turn brown. In general, pathogen growth is more concentrated on the upper surface, making the spot darker on that side. Conidiophores are simple, septate structures with a relatively farker basal portion. Conidia are pyriform and hyaline in color, and they are produced agrogeneously, one after the other. Conidium is a three-celled organism, with the middle cell being much wider and darker, and the end cell germinating and producing a germ tube. Intercalary or terminal clamydospores, which are globoses with thick walls and an olive brown color, are common.

Manandhar (1996) reported that the blast pathogen targets all of the plant's above-ground elements, including leaves, nodes, panicles, and seed. It can also be found on the leaf sheath, Culm rachis joints, and even the glume. The pathogen can infect all above ground sections of a rice plant at different growth stages, according to Pinnschmidt *et al*. (1994), including the leaf, collar, node, internodes, base, or neck, and other regions of the panicle, as well as the leaf sheath.

Manandhar *et al*. (1998) *Magnaporthe oryzae* is one of the most important fungal pathogens of rice, according to due to its widespread distribution and destructive character. The fungus can infect any portion of the rice plant's aerial structure, including the seeds. They also suggested that the fungus could be transmitted from seeds to seedlings in a systemic manner.

Manibhushan (1994) reported that it causes minute dark specks on leaves that evolve into spindle-shaped lesions with pointed ends on both sides. The spots have a brownish grey center with a greenish grey rim. The size, color, and shape

of the lesions, on the other hand, vary depending on environmental conditions and varietal response; in warm weather, the lesions increase and combine, causing the entire leaf to wither.

Munoz (2008) looked explored how temperature and relative humidity affected the concentration of *Magnaporthe oryzae* spores in the air.

Ramappa *et al*. (2002) conducted a study on severely blast-affected paddy fields in Tamil Nadu, India, and discovered a 76 percent reduction in grain yield when rice blast infection occurred immediately after flowering of the rice plants.

Tebeest *et al*. (2007) reported that the symptoms on leaves can vary according on the environment, the age of the plant, and the level of resistance of the host cultivars. Lesions on sensitive cultivars may begin as gray-green and water-soaked lesions with a dark green border that quickly develop to several centimeters in length, typically becoming light tan in color with necrotic margins. Lesions on resistant cultivars are often tiny (1-2 mm) and brown to dark brown in hue.

The symptoms on the leaves, according to Chevalier *et al*. (1991) stated that blast symptoms can vary according on the environmental conditions, the age of the plant, and the level of resistance of the host cultivars. Lesions on sensitive cultivars may begin as grey-green and water-soaked lesions with a dark green border that quickly develop to several centimeters in length, typically becoming light tan in color with necrotic margins. Lesions on resistant cultivars are often tiny (1-2 mm) and brown dark in hue.

2.4. Identification and isolation of causal agent

Dutta (2017) stated that serious yield losses due to epiphytotic blast disease have been recorded in various regions of India, including Tanjore delta, Nellore, Hyderabad, Bombay, parts of Orissa, Kashmir, and Kerala. Magnaporthe pathogen isolated in PDA first appears white, then blackish fungal growth is observed in the media. The leaves began to produce symptoms on the fourth day;

initially, the spots were small, yellow, round to oval. Later, the spots enlarge and spindle in shape, with an ash-colored center. The spot resembled the rice blast spots found in the field in appearance.

Leaf pieces with leisons were surface sterilized with 0.5 percent sodium hypochlorite solution, washed with sterile distilled water, and incubated at 25ºC for 2-3 days. For 15-25 days, petri dishes were incubated at 25ºC in the dark or with artificial fluorescent light on a 12 h light/dark photoperiod. As stock cultures, mono conoidal isolates of the recovered fungi were grown on half-strength potato dextrose agar slants in test tubes.

2.5. Suitable Culturing media for *Magnaporthe oryzae*

Leaf blast fungus can attack rice plants at any stage of growth, causing severe leaf necrosis and obstructing grain filling, resulting in decreased grain number and weight, according to Ram *et al*. discovered (2007). When the last node is attacked, sterility ranges from partial to complete. They also discovered that isolates of the fungus from different hosts had variable responses in mycelia growth and sporulation media. *Magnaporthe oryzae* radial mycelia growth and days of sporulation were investigated using three fungal isolates from rice, finger millet, and panicum sm. Prune agar (PA), oat agar meal (OMA), potato dextrose agar (PDA), finger millet leaf decoction agar, finger millet polish agar (FPA), and finger millet meal agar were tested on six different media. The isolates from finger and millet had the highest RMG, whereas the isolates from rice had the lowest. The isolates from rice had the least days of sporulation (1 week). Whereas the isolates from finger millet had the longest (>2 weeks). PA and OMA were shown to be the optimum media for mycelia growth and sporulation of the isolates, both in terms of the media and isolates used.

Magnaporthe grisea from rice was examined on several solid culture media by Kumar and Singh (1996). On melt extract agar and leonine agar, they discovered that the maximum colony diameter of rice isolate happened.

Magnaporthe isolates from rice and common weeds in paddy fields sporulated abundantly on sterilized barley and sorghum grains, according to (Du *et al*. 1995).

Mahdeih (2013) reported that PDA culture medium could be the best medium for *M.oryzae* vegetative growth regardless of light condition. *M.oryzae*, on the other hand, could sporulate when exposed to light, either continuously or intermittently. *M.oryzae* abettor sporulation is induced by a combination of 16/8 hr light/dark intervals and the addition of rice materials to culture media.

Malviya (2014) investigated *M.grisea* mycelia growth in vitro using four different culturing media. After 168 hours of incubation, PDA media supported the most mycelia growth, followed by Richard's Agar medium. After 168 hours of incubation, sporulation of *M.grisea* was observed in traces in PDA medium RAM. The czapek-Dox medium, on the other hand, was found to be ineffective for both vegetative growth and sporulation of the test pathogen.

Metz and Chandra (2011) investigated the effect of five media on vegetative mycelia growth, pigmented mycelia production, and conidial production in *M.oryzae*: potato dextrose agar (PDA), oat meal agar (OMA), V8 agar (V8A), prune juice agar (PJA), and st. Augustine grass agar (STAA). Following inoculation, various media were kept at 26o C for 11 days under diurnal (12 hr) fluorescent light. He discovered that vegetative mycelial growth was higher on V8A, PDA, and OMA, whereas pigment mycelia grew higher on OMA, STAA, and V8A. Conidial highest production was observed on STAA.

Potato dextrose agar (PDA) was reported to enable linear growth of *M. grisea* among non-synthetic media by Mijan (2000). On the 14th day of incubation, this fungus reached its maximal growth. Richard's medium was the best synthetic media for maximum fungal growth.

Priya *et al*. (2013) reported that colonies of *M. oryzae* appeared white on oat meal, rice polish, and malt extract agar, grey on potato dextrose agar, and whitish grey on rice agar after cultivation of several isolates of *Magnaporthe oryzae*. On maize stem pieces, spore induction was faster than on rice and panicum repens. Conidia

of Pennisetum purpureum isolates were substantially larger than those of other isolates when spores of 11 M. oryzae isolates were analyzed. Rice isolates from Erode and geopichettipalayam have considerably shorter and narrower spores.

2.6. Morphological characterization of *Magnaporthe oryzae*

Blast fungal isolates generated ring-like, circular, irregular colonies with rough and smooth borders on oat meal agar media with buff, grayish black, and black hue (Getachew *et al*. 2014). The colony diameters of distinct groups ranged from 67.4 to 82.50 mm, and the colonial shape was pyriform, with a rounded base and narrowing towards a pointy or blunt time. On OMA, all of the isolates' colony color was usually grey and growing well. All of the isolates had a smooth colony border and enhanced mycelia growth.

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Castilla *et al*. (2009) studied rice blast for a long period and discovered that the pathogen infects all ground components at various growth phases, including the leaf, collar, nodes, internodes, base or neck, and other parts such as the panicle and leaf sheath. A typical blast lesion on a rice leaf is grey in the middle with a black border and is spindle shaped, according to the researchers. The disease thrives in an environment with frequent and protracted dew periods, as well as cool temperatures during the day.

Tebeest *et al*. (2007) reported that the symptoms on leaves can vary according on the environment, the age of the plant, and the level of resistance of the host cultivars. Lesions on sensitive cultivars may begin as gray-green and water-

soaked lesions with a dark green border that quickly develop to several centimeters in length, typically becoming light tan in color with necrotic margins. Lesions on resistant cultivars are often tiny (1-2 mm) and brown to dark brown in hue.

The culture of *Magnaporthe oryzae* was described by Hawksworth (1990) of the Commonwealth Mycological Institute (CMI) as grayish, conidiophores single or in fascicles, simple, rarely branching, and demonstrating sympodial growth. Conidia pyriform to obclavate, constricted toward the base, three septate occasionally one or two septate, hyaline to pale olive, 19-23 7-9 m, with a conspicuous protruding basal hilum, developed singly at the tip of the conidiophores at points arising sympodial and in succession. Chlamydospores are thick-walled chlamydospores with a diameter of 5-12 m that are frequently formed in culture.

The symptoms on the leaves, according to Chevalier *et al*. (1991) can vary according on the environmental conditions, the age of the plant, and the level of resistance of the host cultivars. Lesions on sensitive cultivars may begin as grey-green and water-soaked lesions with a dark green border that quickly develop to several centimeters in length, typically becoming light tan in color with necrotic margins. Lesions on resistant cultivars are often tiny (1-2 mm) and brown dark in hue.

CHAPTER III

MATERIAL AND METHODS

3.1. Experimental Site

The experiment was 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 June 2019 to December 2020.

3.3. Survey area and collection of blast disease sample

Rice blast disease survey was conducted at farmers' field sites of 30 selected fields of Sonatola upazila under Bogura district.

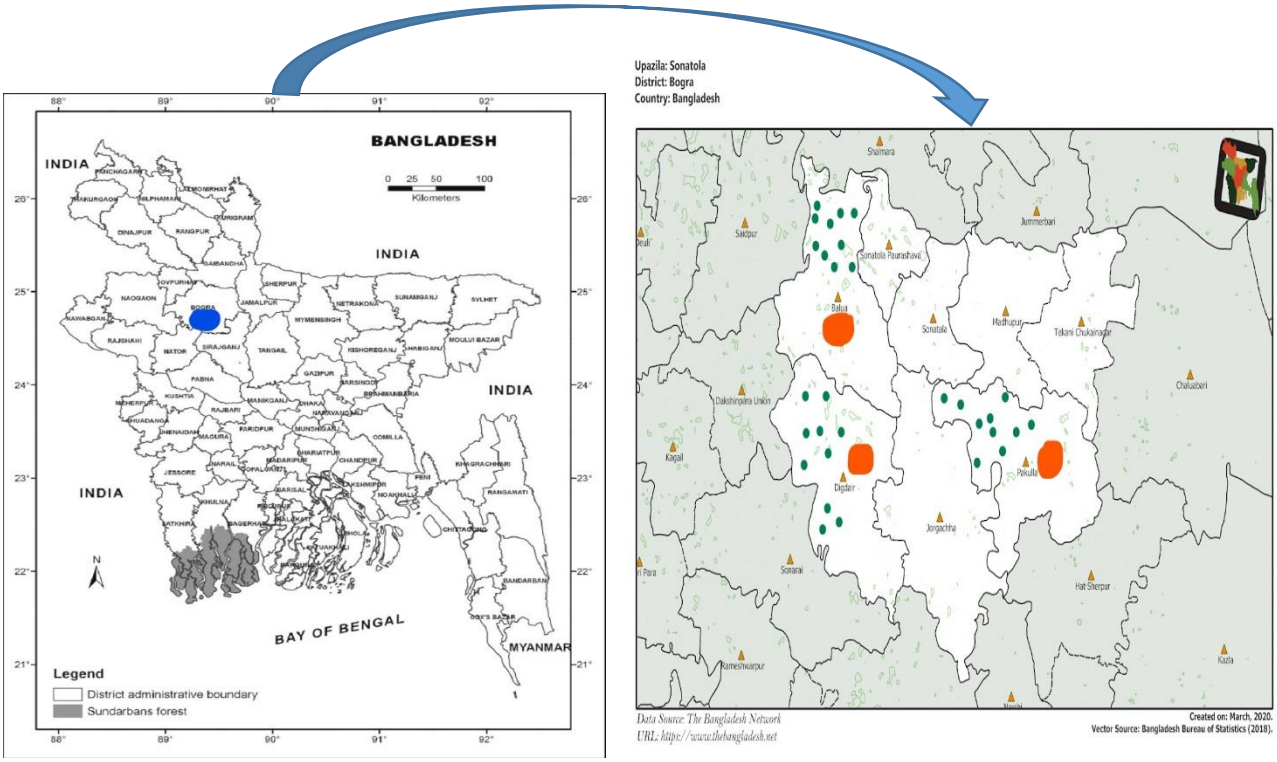


Figure 1. Rice blast sampling site (●) under Sonatola upazila of Bogura district.

3.4. Incidence and severity of rice blast

Assessment of disease severity in the field from each unit plot were randomly selected and disease severity of leaf blast (*Magnaporthe orygae*) of rice was estimated following Singh's (2000) 0-9 scale, where 0= no lesion observed; 1= 1% leaf area covered; 3= 10% area covered; 5= 25% leaf area covered; 7=50% leaf area covered; 9= more than 50% leaf area covered. Disease incidence was assessed using the following formula

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

3.5. Isolation and identification of *Magnaporthe oryzae*

After collecting the pathogen infected neck, that necks' were cut into small pieces and dry it about 7-8 hour at room temperature. Then that neck were covered with "brown paper" and kept them into a cool temperature. Infected neck portion of panicle used for isolation of *Magnaporthe oryzae* (Plate 1). The infected area was cut into small pieces and surface sterilized by soaking it in 0.1% HgCl2 or 10% Clorox for 1 minute and then rinsed three times with distilled water. After placing the tissues in sterile water soaked filter paper in a plastic petridish and incubating at 25°±1° C for 48 hours, conidia were collected and transfered to water agar by inspecting the plate under a stereo microscope. After that, a mycelia tip was sub cultured and incubated at 25°±1°C for 7 to 10 days after being transferred from water agar to potato dextrose agar. The isolates were identified based on morphological and cultural characteristics. After confirmation under the microscope, a single conidium was transferred to create a mono conidial isolate on potato dextrose agar (PDA) media.

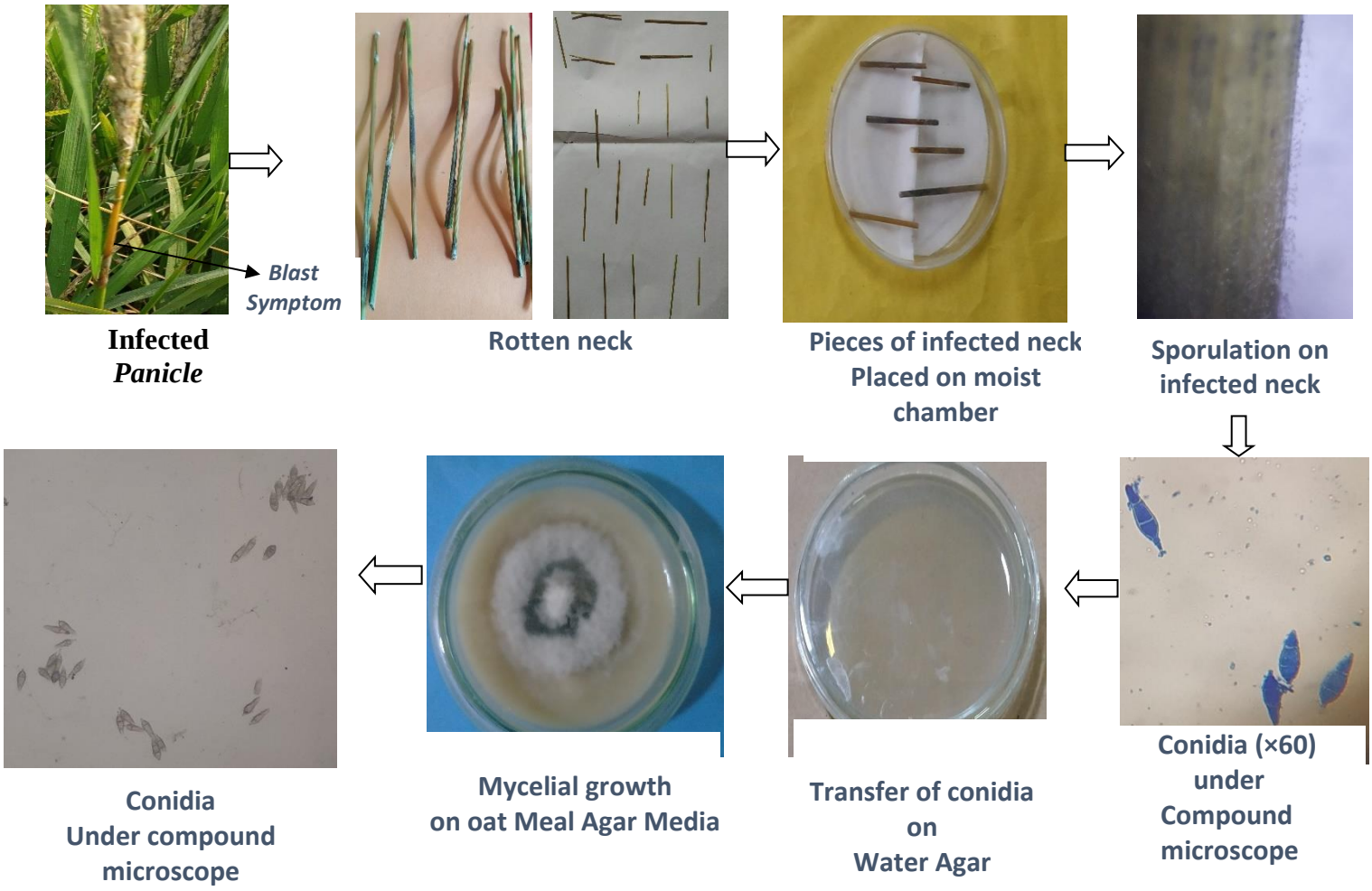
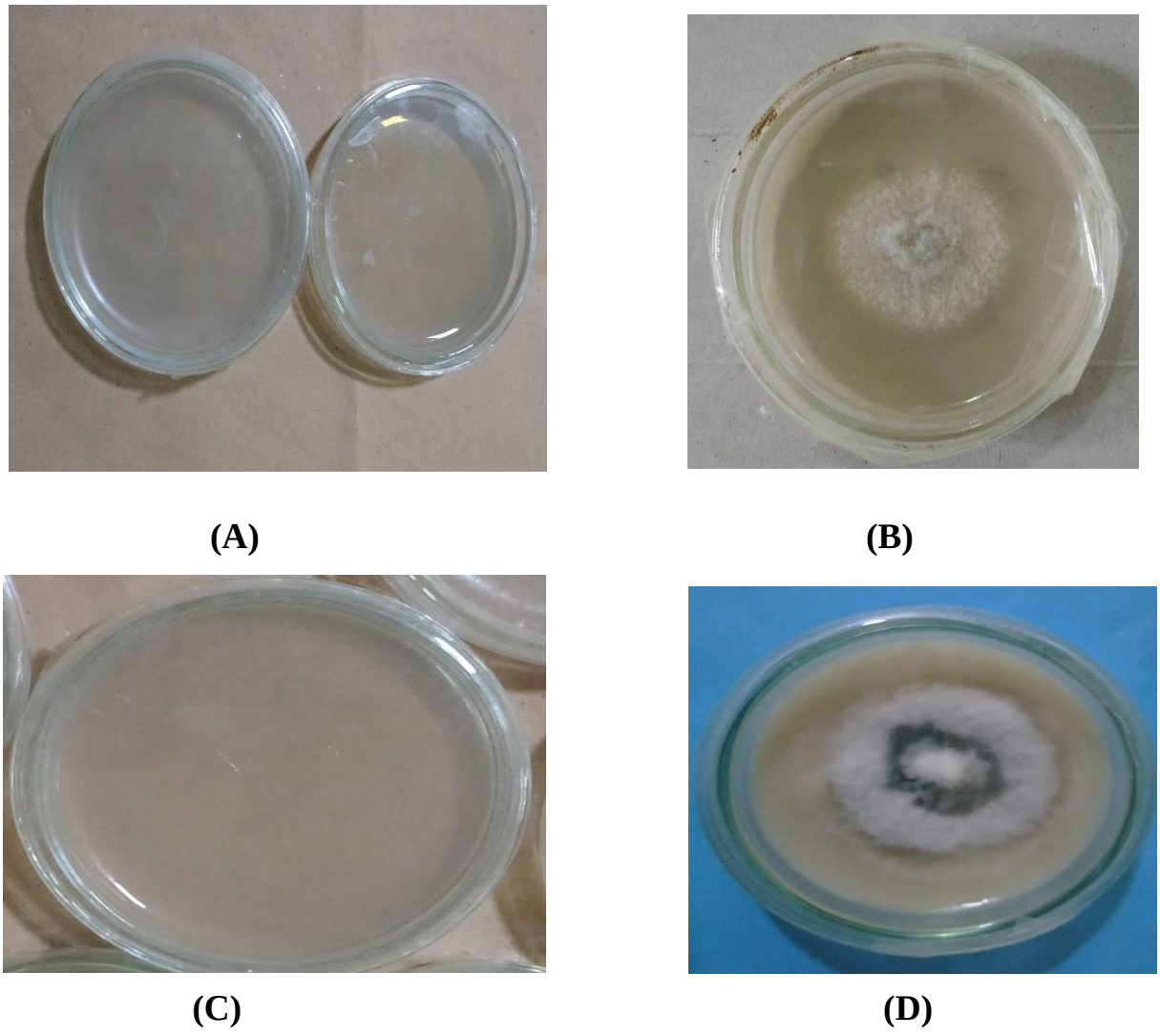


Plate 1. Flow chart of isolation and identification of *Magnaporthe oryzae*.

3.6. Media used for culturing *Magnaporthe oryzae*

M. oryzae isolates were cultivated on PDA for 10 days at room temperature. 5-mm discs were blocked out from the fungus's active growth margin. The fungus was inoculated into sterile petri dishes with water agar (WA), Potato dextrose Agar (PDA), Potato Sucrose Agar (PSA) media and Oat Meal Agar (OMA), incubated at room temperature for 15 days. For each medium, three replications were kept. At 7 DAI, the fungal growth was assessed. In addition, the isolates' colony characters were cultivated on PDA and their colony shape was studied.



(A) **(B)**

(C) **(D)**

Plate 2. Pure culture of *Magnaporthe oryzae* on (A) Water Agar (WA) media (B) Potato Dextrose Agar (PDA) media (C) Potato Sucrose Agar (PSA) Media (D) Oat Meal Agar (OMA) Media.

3.6.1. Water Agar (WA)

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

3.6.2. Potato Dextrose Agar (PDA)

Composition	Quantity (g/litre)
Potato (peeled and sliced)	200g
Dextrose	20g
Agar-agar	20g
Distilled water	1L

3.6.3. Potato Sucrose Agar (PSA)

Composition	Quantity (g/litre)
Potato (peeled and sliced)	200g
Sucrose	20g
Agar-agar	20g
Distilled water	1L

3.6.4. Oat Meal Agar (OMA)

Composition	Quantity (g/litre)
Oat Meal	60g
Agar	12.5g
Water	1L

3.7. Pathogenicity study

To induce sporulation, pure cultures of each isolate are cultivated on OMA for 15 days at 25℃ under an alternate 14-hour fluorescent light and 10-hour dark cycle (Barksdale and Asai, 1961). Harvested conidial suspension was filtered and centrifuged at 5000 rpm. Using a heamocytometer, the spore mass sedimentation was collected, re suspended in sterilized distilled water, and the spore density was adjusted to 1×10^{6} spore/ml. The spore suspension was sprayed on susceptible rice cultivars used in pots at the 3-4 leaf stage, and the seedlings were kept in a glasshouse at 25°C. Under in-vitro conditions, sterile water was used instead of spore suspension as a control. After 10 days of inoculation, seedlings were assessed on the growth of leaf blast.

3.8. Experimental design and statistical analysis

The experiment was carried out using a complete randomized design (CRD) with three replications and statistical analysis performed using Statistix 10 software. Using significance level of P=0.05, the least significant differences (LSD) were determined.

CHAPTER IV
RESULTS AND DISCUSSION

Magnaporthe oryzae causes rice blast disease, which is the most devastating and a severe threat to rice production. Various evolving patterns of blast disease as a result of climate change, as well as new vulnerable varieties chosen for cultivation by farmers.

4.1 Rice Blast Disease Survey

In Boro season (2019-2020), a survey was conducted in three villages of Bogura district's at Sonatola upazila. The incidence and severity of "neck blast" and "leaf blast" in selected fields listed in Table 4 that was recorded in survey areas.

(A)

(B)

(C)

(D)

Figure 2. Survey on rice blast disease; (A) field view of blast infected rice field In Charalkandi village. (B) Data recording on rice blast. (C) Sample collection of rice blast. (D) Blast infected rice field in Pakulla village.

Table 1. Incidence and severity of rice blast (*Magnaporthe oryzae*) disease in selected location at Sonatola Upazila of Bogura district in Bangladesh in Boro season 2019-2020.

Name of Union	Name of Village	Field Site	Name of variety	Blast Disease		
				Incidence %	Severity %	Degree of severity
Pakulla	**Pakulla**	Field 1	BRRI DHAN 28	62.60	15	3
		Field 2	BASMOTI RICE	18.20	6	1
		Field 3	BASMOTI RICE	23.25	8	3
		Field 4	BASMOTI RICE	11.20	2	1
		Field 5	BRRI DHAN 29	5.40	2	1
		Field 6	BRRI DHAN 28	30.20	10	3
		Field 7	BRRI DHAN 28	12.20	4	1
		Field 8	BRRI DHAN 28	81.84	10	3
		Field 9	BRRI DHAN 29	22.62	5	1
		Field 10	BRRI DHAN 29	0	0	0
Digdair	**Charalkandi**	Field 1	BASMOTI RICE	55.20	28	5
		Field 2	BASMOTI RICE	30.10	65	9
		Field 3	BASMOTI RICE	0	0	0
		Field 4	BASMOTI RICE	79.36	50	7
		Field 5	BASMOTI RICE	20.50	5	1
		Field 6	BRRI DHAN 28	30.60	12	3
		Field 7	BRRI DHAN 28	62.20	32	5
		Field 8	BRRI DHAN 28	70.00	50	7
		Field 9	BRRI DHAN 29	0	0	0
		Field 10	BRRI DHAN 29	44.20	27	5
Balua Hat	**Balua**	Field 1	BRRI DHAN 28	32.40	40	7
		Field 2	BRRI DHAN 28	37.30	10	3
		Field 3	BASMOTI RICE	20.20	12	3
		Field 4	BASMOTI RICE	40.20	4	1
		Field 5	BRRI DHAN 28	35.16	3	1
		Field 6	BRRI DHAN 28	25.17	13	3
		Field 7	BRRI DHAN 28	0	0	0
		Field 8	BRRI DHAN 28	18.90	45	5
		Field 9	BRRI DHAN 29	35.10	8	1
		Field 10	BRRI DHAN 29	70.20	52	9

According to the survey, Pakulla has the highest rate of blast disease (81.84), with a severity score of 3 in BRRI dhan 28 and the lowest disease incidence was 5.40 in BRRI dhan 29. The opposing perspective in Charalkandi, the highest blast disease incidence was 79.36, with a severity level of 7 in field 4 cultivated with basmoti rice, but the disease incidence was found to be 30.10, with a severity score of 9 in field sites 2 cultivated with. In Balua Hat the highest blast disease

incidence was 70.20 in field site 10 cultivated with BRRI dhan 29, with a severity level 9.

From the field survey data it has been found that blast incidence and severity varied among union, village and field sites. The result of the present study was supported that by Nazifa *et.al*. (2021).They surveyed in Gobindogonj upazila under Gaibandha districts where the highest incidence was recorded in BRRI dhan 28 (84.26%) followed by BRRI dhan 81 with 70.61% blast incidence. Beside this field cultivated BRRI dhan 28 where recorded the lowest incidence (13.56%) was recorded in BRRI dhan 28 in Mohimagonj upazila under Gabindogonj district. Highest incidence was recorded 79.36% disease incidence cultivated with BRRI dhan 28 and the lowest disease incidence was 13.56% cultivated with BRRI dhan 29.

The present study also corroborate with the study of Rayhan *et. al*. (2019). They reported from their survey that, the highest disease incidence was recorded from Muktagacha (60%) with a severity of 5. The highest severity of blast disease was observed in Hossainpur, Kishoreganj (7) but the disease incidence was only 20%.

The result of the present study was also supported by Ali *et al*. (2009). They determined that the severity of leaf blast ranged from 3.7 to 41.3 percent in Kashmir's temperate areas. Highest node blasts were found in the Anantang district's klugam (7.3 percent), khudwani (5.4 percent), and lamoo (3.8 percent) zones. With an average range of 0.3-4.9 percent, each district had the most devastating phase of neck blast severity. Dar *et al*. (2010) investigated the frequency and distribution of rice blast in the Kupwa district of Jammu and Kashmir, finding a 25% sickness incidence and 15% severity, with the incidence increasing from transplanting to panicle initial stage. *Magnaporthe oryzae* is one of the most important fungal pathogens of rice, Manandhar *et al*. (1998) stated that due to its widespread distribution and destructive character. The fungus can infect any portion of the rice plant's aerial structure, including the seeds. They also suggested that the fungus could be transmitted from seeds to seedlings in a systemic manner.

Manadhar *et al*. (1992) the disease produced a 10-20% yield drop in susceptible types in Nepal, with production reductions reaching 80 percent in severe cases. Motlagh and Javadzadeh (2010) observed blast on affected leaves of rice cultivars collected from rice fields in Iran's Guilan province.

4.2 Confirmation of *Magnaporthe oryzae*

The conidial masses were picked up with a fine pointed needle and placed in a glass slide. The slide was examined using a compound microscope and a cover slip. All the isolates were found pathogenic and produce typical spindle shaped lesion where inoculation on susceptible rice seedling BRRI dhan 28. The pathogen was re-isolated and identified based on three celled pyriform conidia.

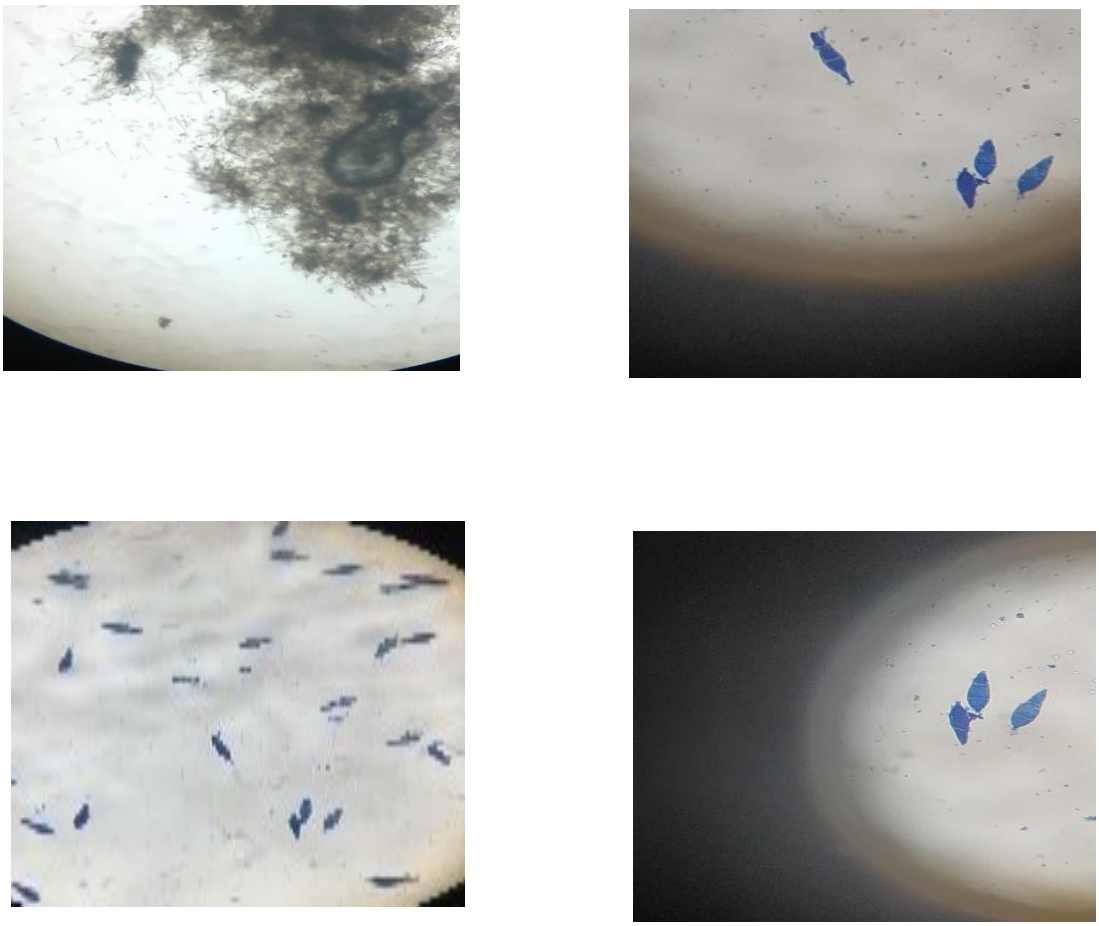


Plate 3. Microscope view of *Magnaporthe oryzae* A. (× 40°) B. (× 100°) C. (× 60°) D. (× 100°)

4.3. Morphological characterization of *Magnaporthe oryzae*.

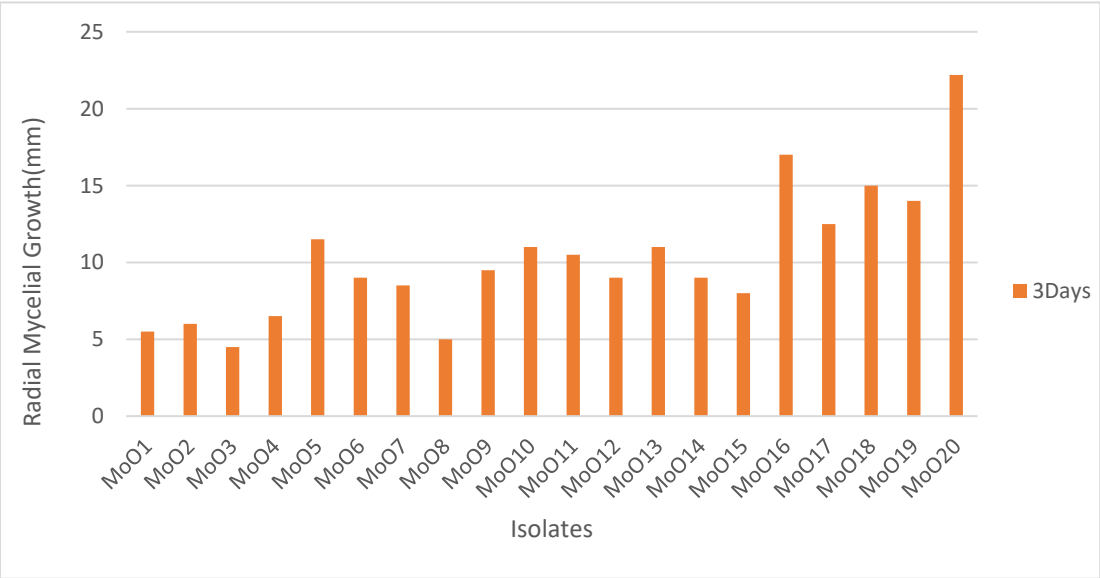


Figure 3. Radial mycelial growth of different isolates of *Magnaporthe oryzae* in Water Agar (WA) at 3 DAI.

On Water Agar, 20 isolates of *Magnaporthe oryzae* were cultured, and their mycelial growth and growth rate were measured (Appendix II and Figure 3) as well as morphological characteristics such as growth character, color, surface structure, and form. MoO20 had the fastest growth, measuring 22.2 mm at 3 DAI and 7.4 mm per day, with White colony color and a rough surface texture with an irregular shape of colony. MoO8 had the slowest growth, measuring5 mm at 3 DAI and 1.67 mm growth per day, with White colony hue and a rough, surface structure.

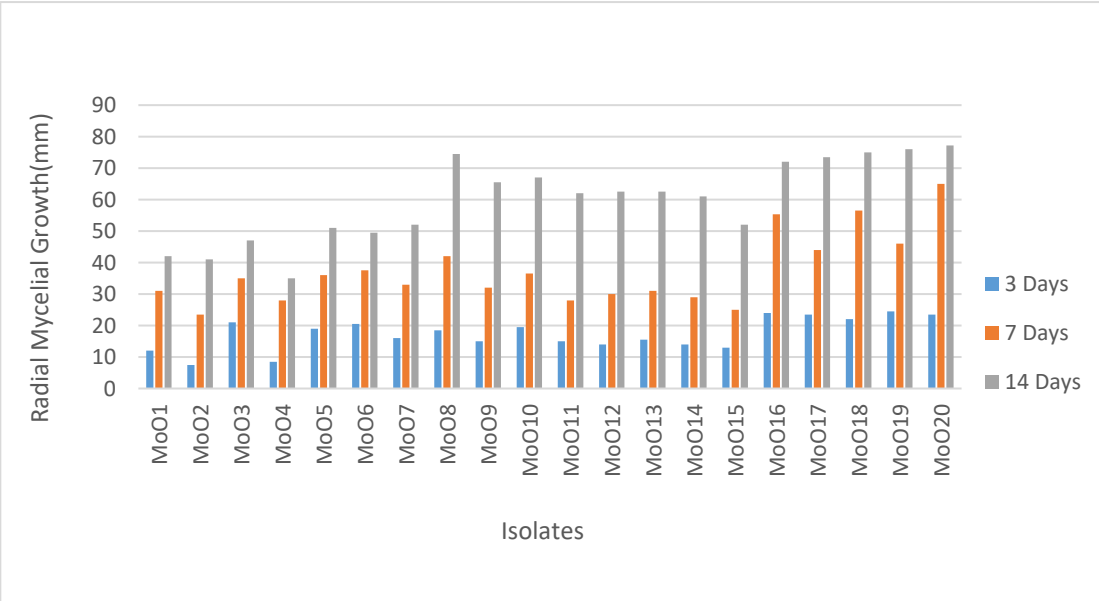
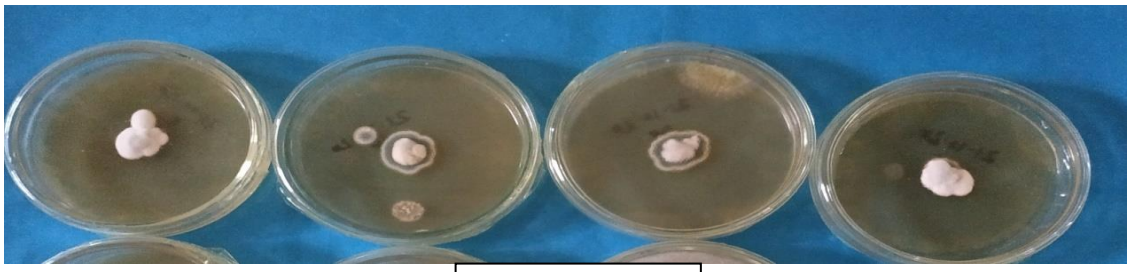
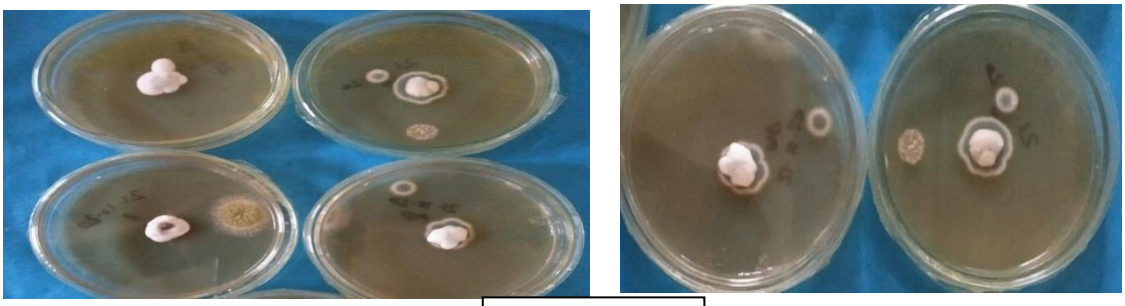


Figure 4. Radial mycelial growth of different isolatesof *Magnaporthe oryzae* in Potato Dextrose Agar (PDA) at 3 DAI, 7 DAI and 14 DAI.

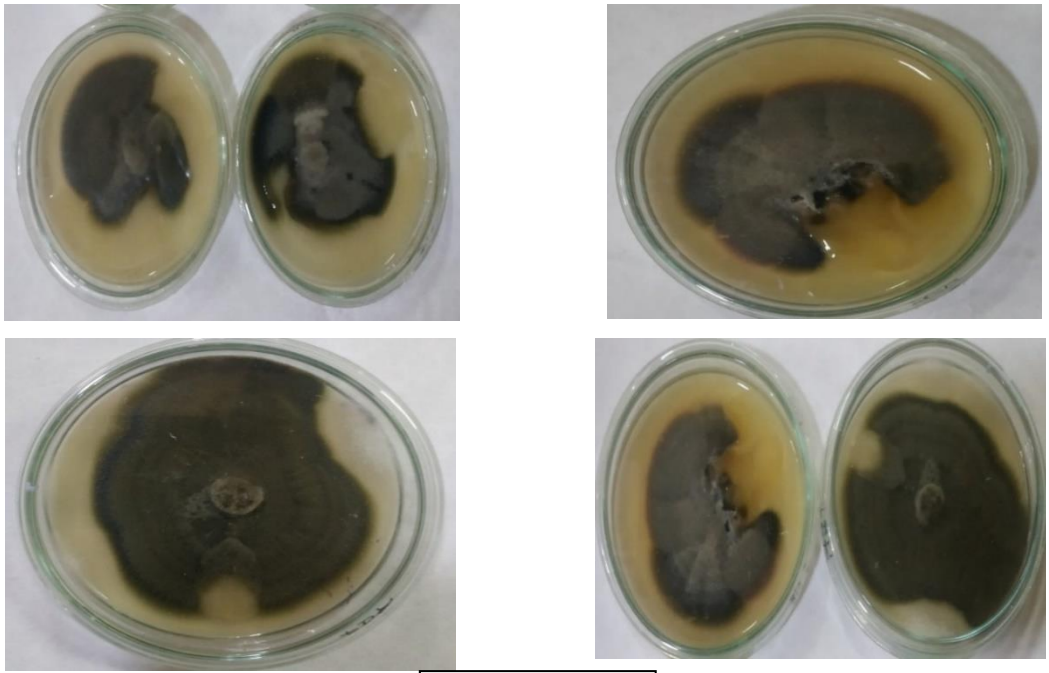
On Potato Dextrose Agar (PDA), 20 isolates of *Magnaporthe oryzae* were cultured, and their mycelial growth and growth rate were measured (Appendix III and Figure 4) as well as morphological characteristics such as growth character, color, surface structure, and form. MoO19 had the fastest growth, measuring 24.5 mm at 3 Days at 8.17mm per day, with blackish white colony color and a rough surface texture with an irregular shape. MoO5 had the slowest growth, measuring 8.5mm at 3 Days at 2.83 mm per day, with blackish white colony hue and a rough cottony surface structure with an irregular shape. On the other hand on 7 and14 days MoO20 fastest growth measuring 65 and 77.2 mm and 9.2 mm and 5.51 mm per day, with blackish colony color and a rough surface texture with an irregular shape. MoO2 had the slowest growth, measuring 23.5and 41 mm at 7 and 14 Days and 3.36 and 2.93 mm per day, with blackish white colony hue and a rough cottony surface structure with an irregular shape.



A. 3 Days



B. 7 Days



C. 14 Days

Plate 4. Radial mycelial growth of the isolates of *Magnaporthe oryzae* on Potato Dextrose Agar (PDA) media of A. (3 DAI), B. (7 DAI), C. (14 DAI)

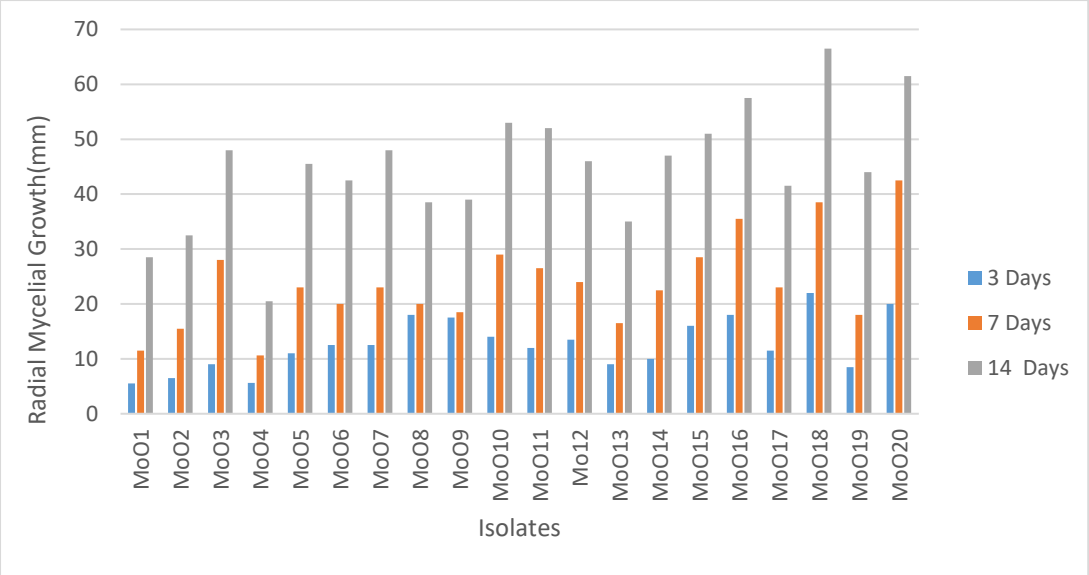
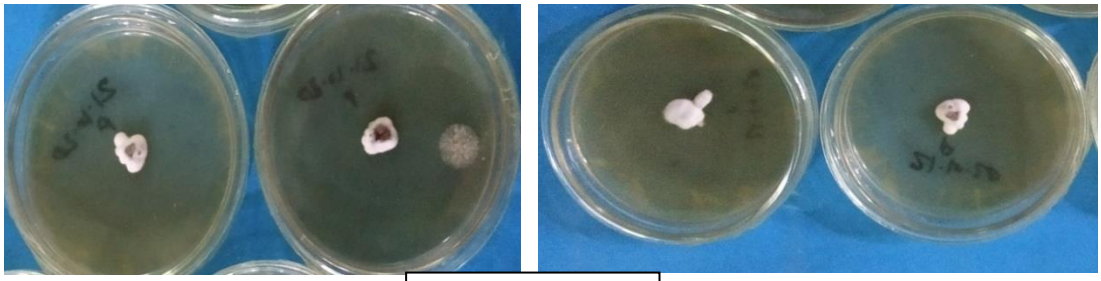
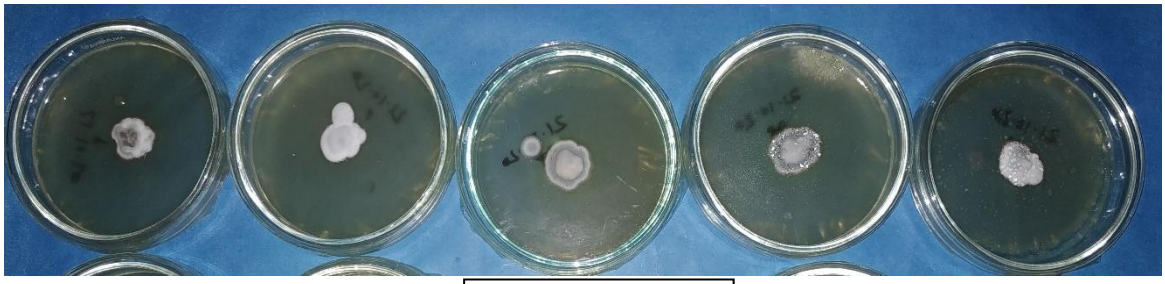


Figure 5. Radial mycelial growth of different isolatesof *Magnaporthe oryzae* in Potato Sucrose Agar (PSA) at 3 DAI, 7 DAI and 14 DAI.

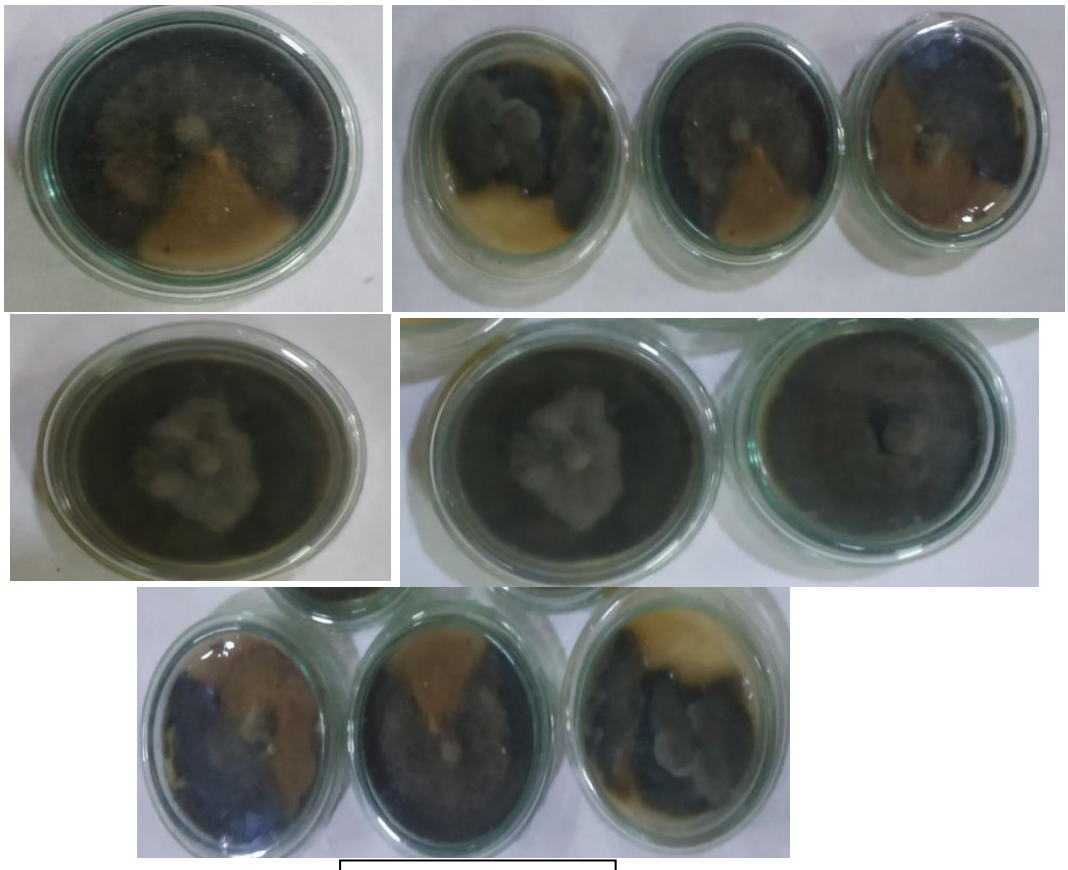
On Potato Sucrose Agar (PSA), 20 isolates of *Magnaporthe oryza*e were cultivated, and their mycelial growth and growth rate were documented (Appendix IV and Figure 5) as well as morphological characteristics such as growth character, color, surface structure, and form. MoO20 had the fastest growth, measuring 20 mm and slowest growth 5.5 mm in MoO1 at 3 Days and 6.67 and1.83 mm per day, with blackish and blackish white colony color and a rough surface texture with an irregular shape. MoO20 had the fastest growth, measuring 42.5 mm and slowest growth 11.5 in MoO1at day 7 and 6.07 and 1.64 mm per day, with blackish and blackish white colony color and a rough surface texture with an irregular shape. MoO20 had the fastest growth measuring 61.5 mm and slowest growth 20.5 mm in MoO4 at 14 Days and 8.782.93 mm per day, with blackish and blackish white colony color and a rough surface texture with an irregular shape.



A.3 Days



B. 7 Days



C. 14 Days

Plate 5. Radial mycelial growth of the isolates of *Magnaporthe oryzae* on Potato Sucrose Agar (PSA) media of A. (3 DAI), B. (7 DAI), C. (14 DAI)

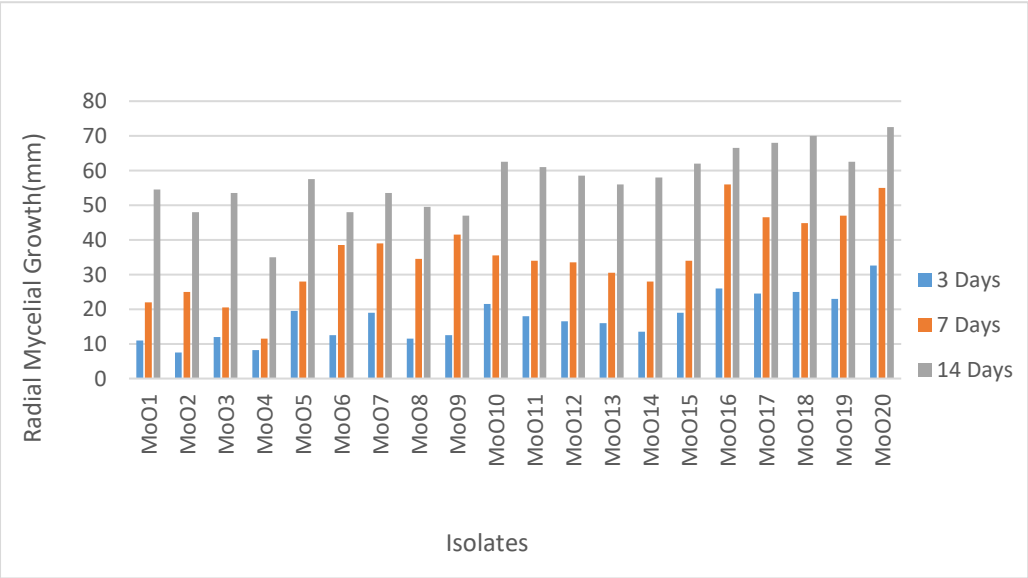
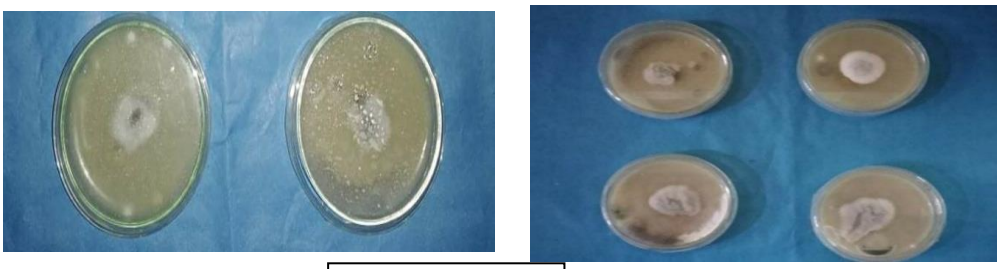
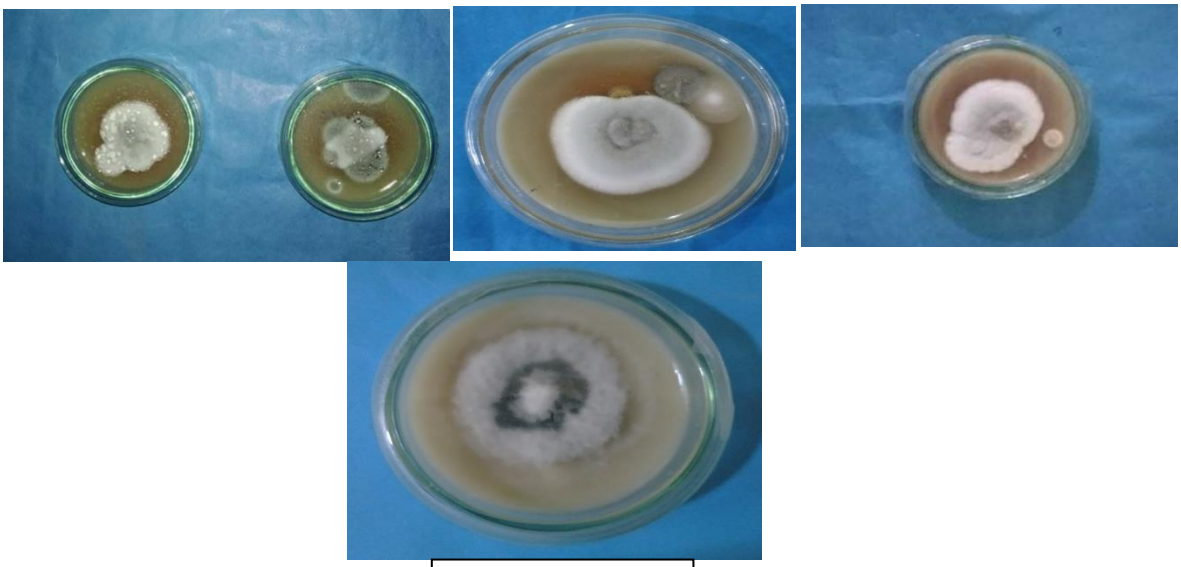


Figure 6. Radial mycelial growth of different isolates of *Magnaporthe oryzae* in Oat Meal Agar (OMA) at 3 DAI, 7 DAI and 14 DAI.

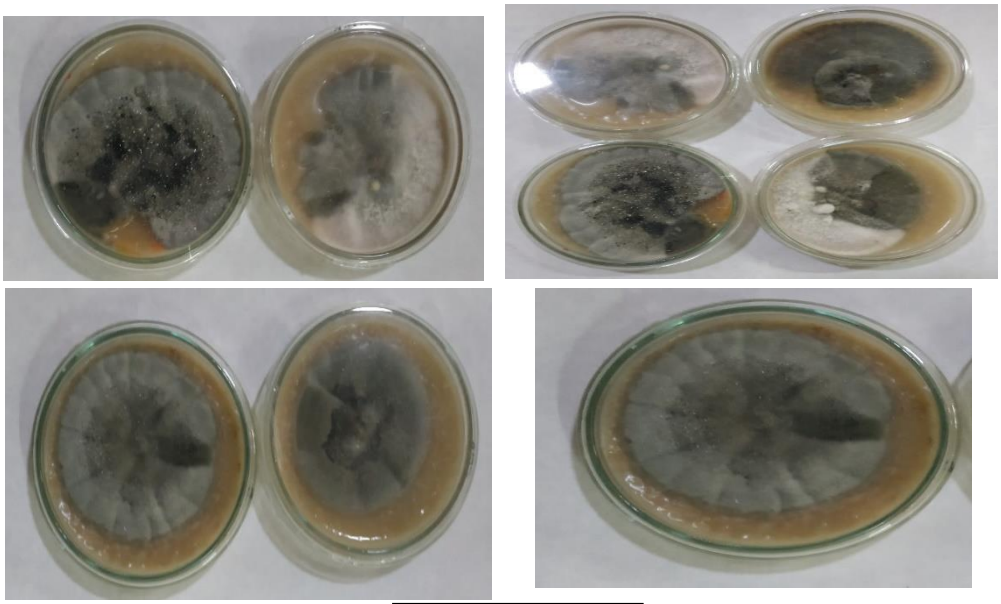
On Oat Meal Agar (OMA), 20 isolates of *Magnaporthe oryza*e were cultivated, and their mycelial growth and growth rate were documented (Appendix V and Figure 6) as well as morphological characteristics such as growth character, color, surface structure, and form. MoO20 had the fastest growth, measuring 32.5, 55 and 72.5 mm at Day 3, 7 and 14 and 10.83, 7.86 and 5.18 mm measuring per day with blackish white colony color and a rough surface texture with an irregular shape. . MoO2 had the slowest growth, measuring 7.5 at day 3 and 2.5mm measuring per day and 11.5 in MoO4 at 7 Days and 1.64 mm per day, with blackish white colony color and a rough surface texture with an irregular shape and MoO9 had the slowest 47 mm growth at day 14 and 3.36 mm growth per day with blackish white colony color and a rough surface texture with an irregular shape.



A. 3 Days



B. 7 Days



C. 14 Days

Plate 6. Radial mycelial growth of the isolates of *Magnaporthe oryzae* on Oat Meal Agar (OMA) media of A. (3 DAI), B. (7 DAI), C. (14 DAI).

4.4. Mycelial growth of *Magnaporthe oryzae* on different cultural media:

Table 2. Mycelial growth of *Magnaporthe oryzae* at day 3 in different growth media.

Culturl Media	**Radial mycelial growth(mm)**
Potato Dextrose Agar (PDA)	38.15 a
Oat Meal Agar (OMA)	36.64 a
Potato Sucrose Agar (PSA)	27.08 b
Water Agar (WA)	10.26 b
LSD (P= 0.05)	**1.95**

In-vitro condition the mycelial growth of the isolates under different culture media significantly varied from one another (Table 2). From the graph revealed that, growth of isolates at day 3 in different media become different (Figure 7). The highest growth become observed in Potato Dextrose Agar (PDA) Media and the lowest growth become observed in Water Agar (WA) media. Mycelial growth of *Magnaporthe oryzae* isolates significantly varied on different culture media. Highest mycelial growth was obtained on PDA that was statistically similar to the growth on OMA. The lowest mycelial growth was obtained on WA.

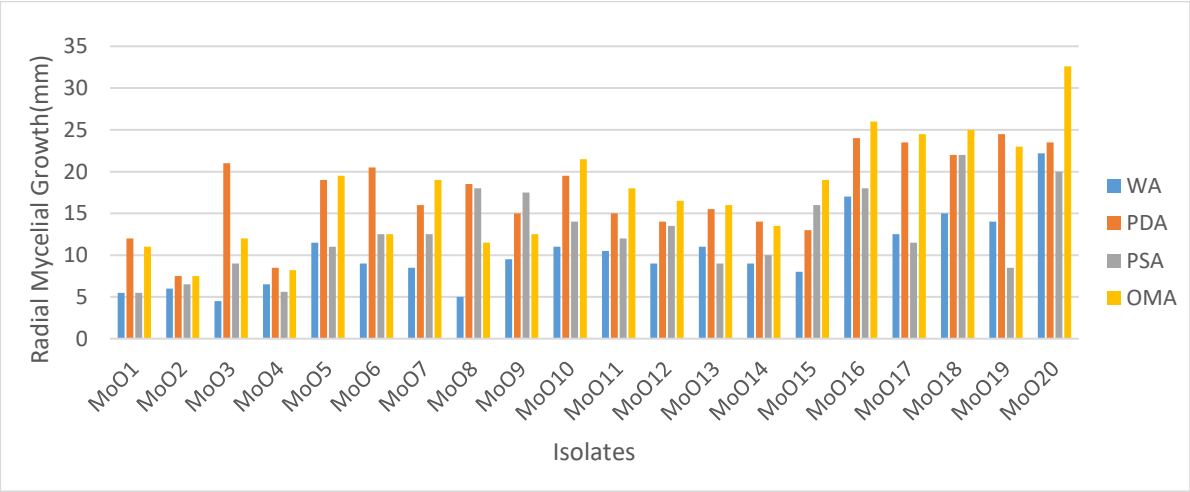


Figure 7. Radial mycelial growth of *Magnaporthe oryzae* at 3 DAI in different growth media.

Table 3. Average radial mycelial growth of isolates of *Magnaporthe oryzae* on WA, PDA, PSA and OMA.

Isolates	Radial mycelial growth (mm)
MoO20	48.78 a
MoO18	45.11 a
MoO16	44.25 a
MoO17	38.02 b
MoO19	37.52 bc
MoO10	36.07 bcd
MoO11	32.98 cde
MoO8	32.27 de
MoO15	31.79 de
MoO12	31.78 de
MoO7	31.35 de
MoO5	30.94 e
MoO9	30.67 e
MoO14	30.23 e
MoO6	29.84 e
MoO13	29.09 e
MoO3	28.783 e
MoO1	22.96 f
MoO2	21.98 f
MoO4	17.09 g
LSD (P=0.05)	**4.81**

There are 7 groups (a, b, c etc.) in which the means are not significantly different from one another. From the table we can find out the average mycelial growth parameter of isolates of *Magnaporthe oryzae* on WA. PDA, PSA and OMA. Among the mean value the highest value observed in MoO20 (48.78) and the lowest value in MoO4 (17.09) and the LSD value about 4.81.

Table 4. Group of *Magnaporthe oryzae* based on cultural characteristics on different cultural media.

Culture Media	Cultural group	Isolates	No of Iolates in Per sample	% of the sample
Water Agar	White, Rough and Irregular	MoO1, MoO2, MoO3, MoO4, MoO5, MoO6, MoO7, MoO8, MoO9, MoO10, MoO11, MoO12, MoO13, MoO14, MoO15, MoO16, MoO17, MoO18, MoO19, MoO20.	20	100
Potato Dextrose Agar	Blackish White, Rough cottony and Irregular	MoO1, MoO2, MoO3, MoO4, MoO5, MoO6, MoO7, MoO8, MoO9, MoO10, MoO11, MoO12, MoO13, MoO14, MoO15, MoO19,	16	80
	Blackish, Rough cottony and Irregular	MoO16, MoO17, MoO18, MoO20	4	20
Potato Sucrose Agar	Blackish White, Rough cottony and Irregular	MoO1, MoO2, MoO3, MoO4, MoO5, MoO6, MoO7, MoO8, MoO9, MoO10	10	50
	Blackish, Rough cottony and Irregular	MoO11, MoO12, MoO13, MoO14, MoO15, MoO16, MoO17, MoO18,MoO19, MoO20	10	50
Oat Meal Agar	Blackish White, Rough cottony and Irregular	MoO1, MoO2, MoO3, MoO4, MoO5, MoO6, MoO7, MoO8, MoO9, MoO10, MoO11, MoO12, MoO13, MoO14, MoO17, MoO18, MoO19, MoO20.	18	90
	Blackish, Rough cottony and Irregular	MoO15, MoO16	2	10

In Table 4, two types of isolates character were shown. On PDA, PSA and OMA media the isolates characteristics were blackish white, rough cottony and irregular and blackish, rough cottony and irregular. On Water Agar media all the isolates characteristics was White, Rough and Irregular.

The results of the present study was supported by Nazifa *et. al*. (2021). They reported that among five different media highest mycelial growth was observed in Oat Meal Agar (OMA) and the lowest in Water Agar (WA) Media at 7 DAI. Colony color of all isolates was whitish grey to blackish with sufficient growth. Dutta (2017), stated in his study that, epiphytotic blast disease has caused substantial yield losses in numerous parts of India, including the Tanjore delta, Nellore, Hyderabad, Bombay, sections of Orissa, Kashmir, and Kerala. In the medium, the *Magnaporthe* pathogen isolated in PDA appears white at first, then blackish fungal growth.

The present study corroborate with the study of Mahdeih (2013) of PDA culture medium, may be the optimal medium for *M.oryz*ae vegetative growth regardless of light conditions. *M.oryzae*, on the other hand, could sporulate either continuously or intermittently when exposed to light. A combination of 16/8 hr light/dark intervals and the addition of rice ingredients to culture media induces *M.oryzae* abettor sporulation. Metz and Chandra (2011) looked at how five different medium affected vegetative mycelia growth,

Pigmented mycelia formation, and conidial production in *M.oryzae*: potato dextrose agar (PDA), oat meal agar (OMA), V8 agar (V8A), prune juice agar (PJA), and saint. Augustine grass agar (STAA). Following inoculation, varied media were stored at 26 degrees Celsius for 11 days under diurnal (12 hour) fluorescent light. He discovered that V8A, PDA, and OMA had higher vegetative mycelial growth, while OMA, STAA, and V8A had higher pigment mycelia growth. On STAA, conidial highest production was detected. Malviya (2014) investigated *M.grisea* mycelia growth in vitro using four different culturing media. After 168 hours of incubation, PDA media supported the most mycelia growth, followed by Richard's Agar medium. After 168 hours of incubation,

sporulation of *M.grisea* was observed in traces in PDA medium RAM. The czapek-Dox medium, on the other hand, was found to be ineffective for both vegetative growth and sporulation of the test pathogen. Priya *et al*. (2013) reported that colonies of *M. oryzae* appeared white on oat meal, rice polish, and malt extract agar, grey on potato dextrose agar, and whitish grey on rice agar after cultivation of several isolates of *Magnaporthe oryzae*. On maize stem pieces, spore induction was faster than on rice and panicum repens. Conidia of Pennisetum purpureum isolates were substantially larger than those of other isolates when spores of 11 M. oryzae isolates were analyzed. Rice isolates from Erode and geopichettipalayam have considerably shorter and narrower spores. Ram *et al*. (2007) stated that when the last node is attacked, sterility ranges from partial to complete. They also discovered that isolates of the fungus from different hosts had variable responses in mycelia growth and sporulation media. *Magnaporthe oryzae* radial mycelia growth and days of sporulation were investigated using three fungal isolates from rice, finger millet, and panicum sm. Prune agar (PA), oat agar meal (OMA), potato dextrose agar (PDA), finger millet leaf decoction agar, finger millet polish agar (FPA), and finger millet meal agar were tested on six different media. The isolates from finger and millet had the highest RMG, whereas the isolates from rice had the lowest. The isolates from rice had the least days of sporulation (1 week). Whereas the isolates from finger millet had the longest (>2 weeks). PA and OMA were shown to be the optimum media for mycelia growth and sporulation of the isolates, both in terms of the media and isolates used.

CHAPTER V
SUMMARY AND CONCLUSION

Magnaporthe oryzae-caused rice blast has become a limiting factor in rice yield in Bangladesh. The experiment was carried out both at the field and in the Laboratory of the Department of Plant Pathology, Sher-e Bangla Agricultural University following Complete Randomized Design (CRD) with three replications, and statistical analysis was carried out using Statistix 10 software.

The purpose of this study was to determine blast disease incidence and severity, isolation of causal organism and confirmation of etiology. A survey was conducted in three villages in the union of Sonatola Upazila under Bogura District in the northern portion of Bangladesh during Boro 2019-2020. Pakulla had the highest incidence of blasts (81.84), with a degree of severity value was 3. The opposing viewpoint the highest blast incidence in Charalkandi was 79.36, with a severity level of 7. The highest blast incidence in Balua hat was 70.20, with a severity level of 9.

Magnaporthe oryzae was inoculated on Water Agar (WA), Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), and Oat Meal Agar (OMA), and the highest growth was observed in Potato Dextrose Agar (65mm) and the lowest growth was observed in Water Agar (10.6 mm) at 7 DAI. On different culture conditions, the mycelial growth of *Magnaporthe oryzae* isolates differed significantly. On PDA, the highest mycelial growth was observed, which was statistically equivalent to that observed on OMA. Water Agar (WA) had the smallest amount of mycelial growth.

CHAPTER VI
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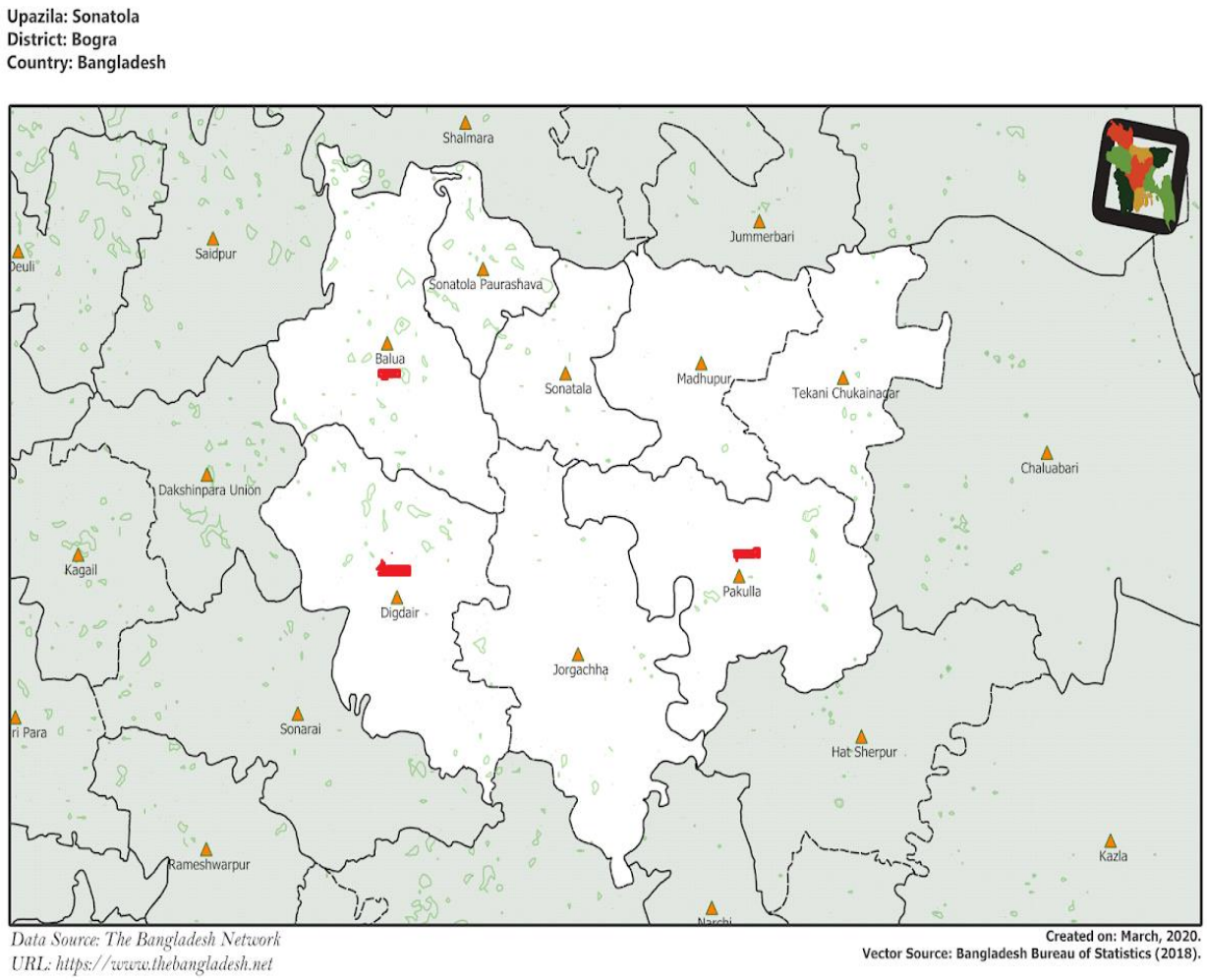
APPENDICES

Appendix I. Map of Sonatola Upazila of Bogura District, Indicating the Data Collecting area of this research.



Appendix II. Morphological characterization of *Magnaporthe oryzae* in Water Agar (WA) media.

No Of Isolates	Radial Mycelial Growth (3Days) (mm)	Colony Character	Surface Texture	Shape
MoO1	5.5	White	Rough	Irregular
MoO2	6	White	Rough	Irregular
MoO3	4.5	White	Rough	Irregular
MoO4	6.5	White	Rough	Irregular
MoO5	11.5	White	Rough	Irregular
MoO6	9	White	Rough	Irregular
MoO7	8.5	White	Rough	Irregular
MoO8	5	White	Rough	Irregular
MoO9	9.5	White	Rough	Irregular
MoO10	11	White	Rough	Irregular
MoO11	10.5	White	Rough	Irregular
MoO12	9	White	Rough	Irregular
MoO13	11	White	Rough	Irregular
MoO14	9	Whit	Rough	Irregular
MoO15	8	White	Rough	Irregular
MoO16	17	White	Rough	Irregular
MoO17	12.5	White	Rough	Irregular
MoO18	15	White	Rough	Irregular
MoO19	14	White	Rough	Irregular
MoO20	22.2	White	Rough	Irregular

Appendix III. Radial and Mycelial growth and cultural characteristics of the isolates on Potato Dextrose Agar (PDA) media.

No of Isolates	Radial Mycelial Growth (mm)			Colony Character	Surface Structure	Shape
	3 Days	7 Days	14 Days			
MoO1	12	31	42	Blackish White	Rough Cottony	Irregular
MoO2	7.5	23.5	41	Blackish White	Rough Cottony	Irregular
MoO3	21	35	47	Blackish White	Rough Cottony	Irregular
MoO4	8.5	28	35	Blackish White	Rough Cottony	Irregular
MoO5	19	36	51	Blackish White	Rough Cottony	Irregular
MoO6	20.5	37.5	49.5	Blackish White	Rough Cottony	Irregular
MoO7	16	33	52	Blackish White	Round Cottony	Regular
MoO8	18.5	42	74.5	Blackish White	Rough Cottony	Irregular
MoO9	15	32	65.5	Blackish White	Rough Cottony	Irregular
MoO10	19.5	36.5	67	Blackish White	Rough Cottony	Irregular
MoO11	15	28	62	Blackish White	Round Cottony	Regular
MoO12	14	30	62.5	Blackish White	Rough Cottony	Irregular
MoO13	15.5	31	62.5	Blackish White	Rough Cottony	Irregular
MoO14	14	29	61	Blackish White	Rough Cottony	Irregular
MoO15	13	25	52	Blackish White	Rough Cottony	Irregular
MoO16	24	55.3	72	Blackish	Rough Cottony	Irregular
MoO17	23.5	44	73.5	Blackish	Rough Cottony	Irregular
MoO18	22	56.5	75	Blackish	Rough Cottony	Irregular
MoO19	24.5	46	76	Blackish White	Rough Cottony	Irregular
MoO20	23.5	65	77.2	Blackish	Rough Cottony	Irregular

Appendix IV. Radial and Mycelial growth and cultural characteristics of the isolates on Potato Sucrose Agar (PSA) media.

No of Isolates	Radial Mycelial Growth (mm)			Colony Character	Surface Structure	Shape
	3 Days	7 Days	14 Days			
MoO1	5.5	11.5	28.5	Blackish White	Rough Cottony	Irregular
MoO2	6.5	15.5	32.5	Blackish White	Rough Cottony	Irregular
MoO3	9	28	48	Blackish White	Rough Cottony	Irregular
MoO4	5.6	10.6	20.5	Blackish White	Rough Cottony	Irregular
MoO5	11	23	45.5	Blackish White	Rough Cottony	Irregular
MoO6	12.5	20	42.5	Blackish White	Rough Cottony	Irregular
MoO7	12.5	23	48	Blackish White	Rough Cottony	Irregular
MoO8	18	20	38.5	Blackish White	Rough Cottony	Irregular
MoO9	17.5	18.5	39	Blackish White	Rough Cottony	Irregular
MoO10	14	29	53	Blackish White	Rough Cottony	Irregular
MoO11	12	26.5	52	Blackish	Rough Cottony	Irregular
Mo12	13.5	24	46	Blackish	Rough Cottony	Irregular
MoO13	9	16.5	35	Blackish	Rough Cottony	Irregular
MoO14	10	22.5	47	Blackish	Rough Cottony	Irregular
MoO15	16	28.5	51	Blackish	Rough Cottony	Irregular
MoO16	18	35.5	57.5	Blackish	Rough Cottony	Irregular
MoO17	11.5	23	41.5	Blackish	Rough Cottony	Irregular
MoO18	22	38.5	66.5	Blackish	Rough Cottony	Irregular
MoO19	8.5	18	44	Blackish	Rough Cottony	Irregular
MoO20	20	42.5	61.5	Blackish	Rough Cottony	Irregular

Appendix V. Radial and Mycelial growth and cultural characteristics of the isolates on Oat Meal Agar (OMA) media.

No of Isolates	Radial Mycelial Growth (mm)			Colony Character	Surface Structure	Shape
	3 Days	7 Days	14 Days			
MoO1	11	22	54.5	Blackish White	Rough Cottony	Irregular
MoO2	7.5	25	48	Blackish White	Rough Cottony	Irregular
MoO3	12	20.5	53.5	Blackish White	Rough Cottony	Irregular
MoO4	8.2	11.5	35	Blackish White	Rough Cottony	Irregular
MoO5	19.5	28	57.5	Blackish White	Rough Cottony	Irregular
MoO6	12.5	38.5	48	Blackish White	Rough Cottony	Irregular
MoO7	19	39	53.5	Blackish White	Rough Cottony	Irregular
MoO8	11.5	34.5	49.5	Blackish White	Rough Cottony	Irregular
MoO9	12.5	41.5	47	Blackish White	Rough Cottony	Irregular
MoO10	21.5	35.5	62.5	Blackish White	Rough Cottony	Irregular
MoO11	18	34	61	Blackish White	Rough Cottony	Irregular
MoO12	16.5	33.5	58.5	Blackish White	Rough Cottony	Irregular
MoO13	16	30.5	56	Blackish White	Rough Cottony	Irregular
MoO14	13.5	28	58	Blackish White	Rough Cottony	Irregular
MoO15	19	34	62	Blackish	Rough Cottony	Irregular
MoO16	26	56	66.5	Blackish	Rough Cottony	Irregular
MoO17	24.5	46.5	68	Blackish White	Rough Cottony	Irregular
MoO18	25	44.8	70	Blackish White	Rough Cottony	Irregular
MoO19	23	47	62.5	Blackish White	Rough Cottony	Irregular
MoO20	32.6	55	72.5	Blackish White	Rough Cottony	Irregular