EVALUATION OF RICE CULTIVARS AGAINST RICE BLAST AND *IN VITRO* MANAGEMENT OF *MAGNAPORTHE ORYZAE*

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This is to certify that the dissertation entitled **'EVALUATION OF RICE CULTIVARS AGAINST RICE BLAST AND *IN VITRO* MANAGEMENT OF *MAGNAPORTHE ORYZAE*'** was submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY in PLANT PATHOLOGY**, embodies the result of a piece of *bonafide* research work carried out by **LUTFUNNAHER LAILA**, Registration No.: **26214/00505** under my supervision and guidance. No part of the dissertation has been submitted for any other degree or diploma.

I further certify that any help or source of information received during the course of this investigation has duly been acknowledged.

Dated: 20-06-2022
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DEDICATED

TO

MY BELOVED PARENTS AND CHILDREN

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ABSTRACT

A set of investigation comprising four experiments was conducted in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, during three consecutive year 2016, 2017, 2018 from March to June in Bangladesh. The investigation was aimed to find out the pathogenic variability of rice blast pathogen *Magnaporthe oryzae*, its epidemic nature in rice growing areas in Bangladesh and finally its *in vitro* management including varietal screening against the disease. For the epidemic nature of rice blast and its pathogenic variability, a survey was conducted in different rice growing regions in Bangladesh from where the disease incidence and disease severity data and subsequently the blast infected leaves and stems samples were collected. Survey data revealed that the highest disease incidence (60%) was found in Bogura district, whereas the lowest (10%) was observed in Dhaka, Sunamgonj, and Moulvi Bazar districts. The highest (5%) disease severity was recorded in the Kishoregonj District, while the lowest (3.33%) was in the Dinajpur District. Twenty-six (26) isolates of *Magnaporthe oryzae* were isolated and identified from the sample collected from survey areas. The highest mycelial radial growth of *M. oryzae* (29.67 mm) was recorded for OMA, whereas the lowest (15.00 mm) in PR_SDA culture media. In 7 days after culture, the highest redial mycelial (51.50 mm) was recorded from the isolate of PBSL20, while the shortest (32.00 mm) was from the isolate of MNKL12. At 14 days after incubation, the longest redial mycelial growth (85.83 mm) was found from the isolates of DKRP19, while the shortest redial mycelial growth (60.33 mm) was found from the isolates of MBBL09. In case of *in vitro* management among the 12 fungicides, maximum growth inhibition (100%) of *M. oryzae* was found in Folicular 250 EC (Tebuconazole-10%), Seltima 100 CS (Pyraclostrobin-10%), Filia 525 SE (Propiconazole-12.5%) + Tricyclazole-40%)) and Difar 300 EC ((Difenoconazole-15%) + Propiconazole-15%)), whereas the lowest (0.00%) inhibition was recoded in Autostin 50 WDG. Among 8 botanicals Neem, Alamanda, and Aloe vera were performed best and significantly inhibited radial mycelial growth. *In vitro* radial mycelial growth of *M. oryzae* with bio-agent (*Trichoderma harzianum*) in PDA media trial the radial mycelial growth of *M. oryzae* was 0.00 mm irrespective of inoculation design. In contrast, the radial mycelial growth of bio-agent *Trichoderma harzianum* was 41.67 mm, 36.67, and 41.67 mm. In control condition, radial mycelial growth of test fungus and bio-agent was 12.33 mm and 41.67 mm, respectively. Among 17 rice germplasms tested, only two cultivars, Jeera Vog and BRRI dhan33, were found resistant against *M. oryzae* in the uniform rice blast nursery.

Key words: Rice blast, *Magnaporthe oryzae, in vitro,* fungicide, botanical, bio-agent

CONTENTS

LIST OF SOME ABBREVIATED FORMS AND THEIR ELABORATIONS

ABBREVIATE FORM	ELABORATION
AEZ	Agro-Ecological Zone
FAO	Food and Agriculture Organization
et al.	And others / Co-workers
BBS	Bangladesh Bureau of Statistics
BARI	Bangladesh Agricultural Research Institute
IRRI	International Rice Research Institute
CRD	Complete Randomized Design
DAI	Days after inoculation
No.	Number
%	Percentage
PDA	Potato Dextrose Agar
OMA	Oat Meal Agar
RFYA	Rice Flour Yeast Agar
DAA	Days After Application
LSD	Least Significant Difference
0C	Degree Centigrade
NS	Not significant
LSD	Least Significant Difference
CV	Coefficient of variance
ha	Hectare
Hr	Hour
mm	Millimeter
cm	Centimeter

ml	Milliliter
w/v	Weight/Volume
Max	Maximum
Min	Minimum
SAU	Sher-e-Bangla Agricultural University

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CHAPTER I

INTRODUCTION

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INTRODUCTION

Rice (*Oryza sativa* L.), belonging to the family Poaceae, is the principal staple food for more than 50% of the world's population (Jahan *et al.,* 2017) and about 163 million Bangladeshi people (BBS, 2021; Liu *et al*., 2017; Shelley *et al*., 2016). It is one of the world's most essential cereal food crops growing in at least 114 countries under diverse conditions (Anis *et al*., 2016). Rice contributes on an average 20% of the world's apparent total calorie intake and 30% of the Asian population (Hien *et al.,* 2006). Bangladesh has the highest per capita consumption of rice, which is about 170 kg annually, and its food security and economy are primarily dependent on good harvests year after year (BBS, 2019). Bangladesh ranks the 4th largest country in the world in both area and production and the 6th in the production of per hectare yield of rice (Sarkar *et al*., 2016).

The population in Bangladesh will swell progressively to 223 million by 2030, demanding more than 48 million tons of food grains (Bhuiyan *et al*., 2014). So, a continuous increase in rice production and the highest priority have been given (Ahmed *et al.*, 2014). Yearly increment of rice production needs to be sustained to feed the ever-increasing population, although there is minimal scope to increase rice area (Sarkar *et al*., 2016) rather agricultural land is declining @ 0.7% per annum (BBS, 2019). There are two general ways to increase rice production: increase productivity through improving management practices or increase cropping intensity. The average rice yield in Bangladesh is about 3.12 t ha^{-1} which is very low compared to other rice-growing countries, like 6.30 t ha^{-1} in China, 6.60 t ha^{-1} in Japan, and 6.30 t ha^{-1} in South Korea (FAO, 2018). However, the low yield is not an indication of the low yielding

potentiality of rice is but may be attributed to several biotic and abiotic factors (Singh *et al*., 2018; Mahbub *et al*., 2017).

Different diseases play an essential role in the growth, yield attributes, and rice yield among the biotic factors. More than 70% of diseases have been caused by fungi, viruses, bacteria, and nematodes (Zhang *et al*., 2009). Among various rice diseases, blast is the most destructive disease globally, and it occurred to the extent of 50% yield loss (Wilson and Talbot, 2009). Blast brings four typical disease symptoms such as leaf blast, nodal blast, neck blast, or panicle blast (Miah *et al*., 2013). Globally, it causes 70 to 80% rice yield loss (Nasruddin and Amin, 2013). Neck blast causes the highest yield loss since it directly affects the panicle compared to leaf blast. An area with high rainfall and a cooler climate is sternly affected by blast diseases (Ghatak *et al*., 2013). The leaf blast is characterized by the white to gray-green lesions or spots with darker borders produced on the leaf, and old lesions are elliptical or spindle-shaped and whitish to gray with necrotic border. The neck blast is characterized by the dark brown lesion on the panicle neck's basal that makes it unable to support the panicle (Kumar and Ashraf, 2019).

Blast disease is one of the most devastating diseases of both seasons caused by *Magnaporthe oryzae* and is reported from more than 85 countries of the world (Gilbert *et al*., 2004). *M. oryzae* is one of the most important plant pathogenic fungi having an exceptional capacity of rapidly changing its genetic makeup resulting in new pathogenic variants races (Dean *et al*., 2012; Khan *et al*., 2016). It is the causal agent of rice blast, one of the most devasting rice diseases observed in most rice-growing countries worldwide, including India, China, Korea, Vietnam, and the USA (Kihoro *et al*., 2013; Wang and Valent, 2017). The pathogen can cause infection on leaves, stems, peduncles, panicles, seeds, and even roots. This disease is a potential threat that may

cause crop failure and yield loss. *M. oryzae* is a hemibiotroph that establishes a biotrophic relationship with the host initially and necrotrophic association later. It weakens the plant defense system without producing visible symptoms during biotrophic association and promotes cell death when it shifts to the necrotrophic association (Fernandez and Orth, 2018).

The distribution of rice blast disease in almost all rice-growing areas and the broad host range of *M. oryzae* makes eradicating the disease difficult. Rice blast has never been eliminated from a region where rice is generally grown (TeBeest *et al*., 2012). The various methods used for managing rice disease include using resistant varieties, cultural practices, biological and chemical control. All these methods have varying degrees of success in managing rice diseases. Being rice blast pathogen seed-borne, it is not easy to manage (Hubert *et al*., 2015). Virulence diversity of blast pathogen also makes it difficult to breed resistance (Marangoni *et al*., 2013). Management of rice blast is complex because the pathogen is seed-borne, and management approaches have mainly focused on the use of synthetic chemicals (Mossini *et al*., 2004). Among the methods, fungicidal control is primarily practiced for blast disease in many temperate or subtropical rice-growing countries, primarily in Japan, China, South Korea, Taiwan, and Vietnam (Kumar *et al*., 2014).

Farmers depend on chemical pesticides for the management of blast. Mancozeb is effective against blast at 1000 ppm and 10,000 ppm (Jamal-u-ddin *et al*., 2012). A group researcher reported that foliar spray of Isoprothiolane at 1.5 ml/l decreased blast, followed by carpropamid and carbendazim (Varma and Santhakumari, 2012). With the application of isoprothiolane, both grain and straw yield were increased compared to other control. However, chemical management practices are neither practical nor environmentally familiar. Combination of Tricyclazole 22% + Hexaconazole 3% SC in

Thrice from booting stage at weekly interval showed highest disease control (87.03% and 79.62% in leaf and neck blast, respectively) and highest grain yield (4.23 t ha^{-1}) (Magar *et al*., 2015). Earlier study revealed that Captan and Acrobat controlled rice blast (Haq *et al*., 2015). The rest of fungicides are Benomyl, Carbendazim 12%+Mancozeb 63%, Iprobenfos, Capropamid, Hexaconazole, Tebuconazole, etc. (Gohel and Chauhan, 2015; Prasanna *et al*., 2011). Different scientists confirmed that strobilurin-derived fungicides were effective in controlling rice blast disease compared to other fungicides (Pramesh *et al.*, 2016; Dutta *et al.*, 2012).

Chemical practices are highly effective and low-cost management but harm the environment, and it also makes rice blast more drug-resistant (Slusarenko *et al*., 2008). The management of rice diseases using chemicals is standard (Pandey, 2015) as the risk of disease highly influences grain yield and requires judicious use of fungicides (Pandey, 2016). However, the use of chemicals resulted in environmental pollution and ill health to the biotic community as a whole, and this necessitates developing the natural product as an alternative to synthetic fungicides to control the disease (Hubert *et al.*, 2015). Nowadays, ecofriendly chemical methods like organomercuric, organophosphorus, copper fungicides, antibiotics like Kasugamycin/Blasticidin, and leaf extracts are used to effectively control the blast disease (Upadhyay and Bhatta, 2020). Botanicals and the bioagents play a significant role in preventing the germination of blast fungal spores. A recent study confirms the antifungal activity of some botanicals and bioagents against *M. oryzae* (Law *et al*., 2017; Singh *et al*., 2012). Botanicals that are target-specific, biodegradable, and relatively safe to non-target organisms would be the best alternative (Pandey, 2018). Natural compounds as economically accessible disease control methods are receiving increased attention due to their nontoxicity and biodegradability (Zarandi *et al*., 2009; Sukanya *et al*., 2011;

Hajano *et al*., 2012; Bhattacharji and Dey, 2014 and Ali and Nadarajah, 2014). Plant extracts have been known for their medicinal and antimicrobial properties since ancient times (Lalitha *et al*., 2010). They offer a greater scope than synthetic chemicals as they are relatively safe, easily biodegradable, and ecofriendly (Sukanya *et al*., 2011; Khan and Nasreen, 2010; Gurjar, 2012). Neem-based pesticides spray, plant dried commercial products like Achoole, Neemzal, Neem Gold, and Neem Azal are promising botanicals in reducing the severity of rice blast (Pandey, 2018). Extracts from *A. indica*, *A. vera*, *A. sativum*, *C. arabica*, *D. stramonium*, *C. sinensis*, *Z. officinalis*, and *N. tabacum* can be used to manage rice blast disease *in-vitro* and *in-vivo* (Hubert *et al*., 2015). Leaf extract of different medicinal plants has lowered the severity of blast disease of rice (Jyotsna *et al*., 2017).

Several microbes have been effective as biological control agents of plant diseases, i.e., *Bacillus*, *Dactylella*, *Gliocladium*, *Penicillium*, *Pseudomonas*, *Phasiliomyce*, *Trichoderma*, etc. (Ali and Nadarajah, 2014). *Trichoderma* has a broad spectrum biotypes that ranges from effective soil colonizers to non-plant symbionts (Meraj-ul *et al*., 2012) that live in the rhizosphere and can successfully colonize the plant epidermis, and they have been used as biocontrol agents against plant pathogens (Andrei *et al*., 2012; Harman *et al*., 2004; Sundaramoorthy *et al*., 2012). Several investigators have pointed out that *Trichoderma* is an appealing candidate for controlling blast disease in rice. Many studies have described using microorganisms like *Trichoderma* spp. to control rice blast in rice plants (Abeysingne, 2007; Andrei *et al*., 2012; Lixuan *et al*., 2008; Yang *et al*., 2009). *Trichoderma* secreting hydrolytic enzymes and causing mycoparasitism of fungal pathogens of plants. Bio-agents' growth promotion might be due to the direct involvement of some plant hormones such as auxins, cytokinins, etc. (Malleswari and Bagyanarayana, 2013; Sivakumar *et al.* 2012). The efficacy of

biocontrol agents used against the blast of paddy incidence and promoting plant growth of paddy in field conditions (Islam and Faruq, 2012; Razu and Hossain, 2015; Jha and Subramanian, 2013; Sharma, 2013; Ramezanpour, 2010 and Khorshidi *et al*., 2011). Several experiments have been conducted in different regions in the world regarding the screening of resistant rice genotypes(Ghazanfar *et al.*, 2009; Ghimire *et al.*, 2019; Challagulla *et al.*, 2015; Naik *et al.*, 2021; Qudsia *et al.*, 2017 and Puri *et al.*, 2009) but no experiment has been conducted yet about resistant rice screening in Bangladesh. Considering the above facts, the research has been conducted with the following objectives.

Objectives

i) To determine the incidence and severity of rice blast from different rice-growing regions of Bangladesh

ii) To isolate, identify morphological characterization and pathogenicity of the isolates of *Magnaporthe oryzae* causing rice blast collected from different rice-growing regions of Bangladesh

iii) To evaluate different fungicides, botanicals, and bioagents against *Magnaporthe oryzae* in *in vitro*

iv) To screen out resistant rice genotypes against *M. oryzae* in the rice blast nursery

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Occurrence and distribution

Rice blast disease is distributed in about 85 countries in all continents where the rice plant is cultivated in low land and upland conditions. Rice blast is present wherever rice is cultivated, but the disease occurs with variable intensities depending on climate and cropping system. Environments with frequent and prolonged dew periods and cool daytime temperatures are more favorable to blast (Liu *et al*., 2004).

During the last two decades, in Pakistan, rice blasts have been primarily found in Faisalabad, Toba Tek Singh, Vehari, and places like Gaggoo Mandi (Arshad *et al*., 2008). In addition, rice blast has been recorded in the Northern Territory (Stahl, 1955; Heaton, 1964), Brazil (Prabhu and Morais,1986), Queensland, Australia (Perrott and Chakraborty 1999; You *et al*., 2012), Sri Lanka (Senadhira *et al*., 1980), Colombia (Ahn and Mukelar, 1986), Philippines, Japan, South Korea (Ou, 1985; Pena *et al*., 2007), Egypt (Reddy and Bonman, 1987; Sotodate *et al*., 1991), China (Li *et al*., 2011).

Outbreaks of rice blast are a severe and recurrent problem in rice-growing regions worldwide. Rice blast is a widespread and damaging disease of cultivated rice caused by the fungus *M. grisea* (Rossman *et al.*, 1990). It is the most destructive pathogen of rice worldwide. Around 50% of production may be lost in a field moderately affected by infection (Devi and Sharma, 2010).

It was first reported as a rice fever disease in China by Soon Ying-shin in 1637 (Ou, 1985). In Japan, it was reported as Imochi-byo by Tsuchiya in 1704. In Italy, it was reported as brusone, and in India, it was first reported in the Thanjavur delta of Tamil Nadu in 1913 (Padmanabhan, 1965). It is a disease of immense importance in

temperate, tropical, subtropical Asia, Latin America, and Africa and is found in approximately 85 countries worldwide (Pooja and Katoch, 2014**).**

The disease is also a significant problem in Penna river belts and the Godavari in Andhra Pradesh. The blast fungus can attack more than fifty other species of grasses. It causes disease at seedling and adult plant stages on the leaves, nodes, and panicles. It appears in irrigated low land or rainfed upland rice and submerged or deepwater rice.

Rice blast is the most severe disease found in the extensive rice areas of Latin America, Africa, and Southeast Asia and is a global problem in rice production. As a result, rice blast disease is a significant constraint to global food security and agricultural trade (Leong, 2004).

In the West African sub-region, the blast is recognized as a primary constraint to rice production, causing 3.2-77.0% yield losses (Fomba and Taylor, 1994). In Ghana, rice production is constrained by several biotic factors, including diseases such as blast, brown spot, bakanae, stackburns, narrow leaf spot, and false smut. The most 12 prevalent among these diseases are blast and brown spot. Rice blast was listed as a significant disease in Ghana by Oduro (2000). In addition, various reports by Twumasi (1998), Twumasi and Adu-Tutu (1995), Nutsugah (1997), and Nutsugah and Twumasi (2001) have identified the blast disease of rice as a severe threat to rice production in Ghana.

2.2 Identification of rice blast

Rice blast can be identified based on the presence of lesions on plant parts, including leaf, leaf collars, necks, panicles, pedicles, seeds, etc. Recent findings show that roots of rice plants also have lesions. The most common and distinct symptoms of rice blast occurrence are ‘diamond-shaped lesions’ on the leaf surface of rice plants (Upadhyay and Bhatta, 2020).

Symptoms on leaves: Rice leaves are the most readily affected part of the rice plant. Symptoms of rice blast in the leaves vary with the agroclimatic and environmental state, plant age, and resistance level of the host cultivar. On susceptible cultivar, gray-green and water-soaked lesions appear initially with a darker green border which expands rapidly to several centimeters long. Older lesions on susceptible varieties become light with necrotic borders. Resistant varieties are characterized by small size (1-2 mm) lesions with brown to dark brown color.

Symptoms in rice collar: Area of necrosis at the union of the leaf blade and stem sheath are symptoms of rice collar infection. This infection can kill the entire leaf and expands to a few millimeters on the stem sheath.

Symptoms in rice neck and panicles: The rice neck is the stem that rises above the upper leaf that supports the panicle. *Magnaporthe oryzae* infects rice neck at the node, leading to a rotten neck or neck blast. Infection on the neck is disastrous that develops chaffy grains due to failure of seed to fill in the case of neck rot entire panicle falls. In panicle infection, gray-brown lesions can easily be found on panicle branches, spikes, and spikelets. Over time, panicle branches breaks at the lesion presence spot.

Symptoms on rice seeds: The seed surface of the infected rice panicle has brown spots, blotches, and sometimes diamond-shaped lesions, as seen in leaves. The fungus is present in the penicles of the seed resulting in a blank seed of rice, and the condition is known as blanking. Recent findings believe blast fungus can infect seeds through florets as they develop into seeds. However, the whole process and time of seeds infection by spores of the pathogen is still unknown.

2.3 Disease cycle and epidemiology

M. oryzae overwinters on crop debris, seeds, and weed hosts. The pathogen overwinters in mycelium in crop debris and as conidia on the living hosts. Although infected crop

debris is a significant source of primary inoculum, infested seeds are also considered a vital source (Thurston, 1995). Infested seeds are produced when the plant is inoculated at any stages after the flag leaf is fully developed. The infested seeds produce diseased seedlings, which die and serve as primary inoculum (Faivre-Rampant *et al*., 2013). Conidia are produced and released by overwintering fungus during a period of high relative humidity (>90%). The airborne conidia land on rice plants and adhere firmly through the mucilage they produce at their tip. In wet conditions, conidia germinate to form a germ tube which later produces specialized structures called an appressorium. Deposition of melanin and recycled conidium contents inside the appressorium creates internal turgor pressure of up to 8.0 MPa, which is enough to penetrate the rice cells by forming penetration peg (Fernandez and Orth, 2018). At optimum temperatures, lesions appear within 4 to 5 days. In wet weather, new conidia are produced within an hour from the appearance of lesions, and most of them are released between midnight and sunrise. These new conidia germinate and cause a secondary infection (Agrios, 2005). The secondary cycles can be repeated many times during the growing season, depending on environmental and growing conditions. Prolonged wetness, high humidity, high nitrogen application, and moderate temperature (24°C) favor disease development (TeBeest *et al*., 2012).

2.4 Growth of the pathogen in different media

Ravindra (2014) used four culture media to study the mycelial growth of *P. grisea* under *in vitro.* PDA media supported maximum mycelial growth followed by Richard's Agar medium after 168 hr of incubation. Then sporulation of *P. grisea* was observed in traces in Potato dextrose agar medium and Richard's Agar medium after 168 hrs of incubation. However, the Czapek-Dox medium was not effective for both vegetative growth and sporulation of the test pathogen.

Mahdieh (2013) reported that PDA culture medium could provide the best medium for *P. oryzae* vegetative growth, regardless of light condition. However, *P. oryzae* could sporulate when the light was continuously provided or at intervals. Therefore, combining 16/8 hr light/darkness intervals and adding rice materials to culture media could induce *P. oryzae* for better sporulation.

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

Culturing of different isolates of *Pyricularia oryzae* was studied by Vanaraj *et al.* (2013) and reported that P. oryzae appeared as white on oat meal, rice polish and malt extract, grey on potato dextrose agar, and whitish-grey on rice agar. Spore induction was hastened on maize stem pieces than on rice and *Panicum repens*. When spores of 11 isolates of *P. oryzae* were compared, conidia of the isolate from *Pennisetum purpureum* were significantly bigger than the other isolates. On the other hand, the spores of rice isolates from Erode and Gopichettipalayam were significantly smaller in length and width.

2.5 Forecasting blast disease

Disease forecasting is one of the essential tools to select prevention and control measures. Epidemiological study of disease helps to select the efficient disease management tool. Van der Plank emphasizes that epidemiological study is crucial for selecting effective disease management tools developed by plant breeders and chemical industries.

For the most devastating disease of rice, some blast forecasting models have been developed to predict its occurrence and severity to control the disease effectively. However, disease forecasting methods are not widely used due to uncertainties and inaccuracy in prediction. Disease forecasting focuses on predicting the possible outbreak of the pathogen or increasing the intensity of disease that allows when how and where a specific disease management practice should be done (Agrios, 2005). Forecasting methods rely on the host, pathogen, and environmental conditions for disease development; the host must be susceptible, pathogen virulent, and favorable.

Researchers have suggested that most epidemic models are either analytical or simulation. Analytical measures are simple and consider only a few variables, whereas simulation measures consist of equations. Berger observed that some researchers combined two approaches starting with analytical models and gradually increasing confidence with breaking down to the complex models in analytical forms.

2.6 Strategies to control rice blast disease

The distribution of rice blast disease in almost all rice growing areas and the broad host range of M. oryzae makes eradicating the disease difficult. Rice blast has never been eliminated from a region in which rice is grown (TeBeest *et al*., 2012). Cultural methods were the only tool to control the blast disease when chemicals and resistant varieties were not available. These methods effectively create a less favorable environment for the pathogen in the field and reduce the blast disease epidemics. In areas of low blast pressure, control of rice blast is primarily based on the use of resistant varieties (Agrios, 2005). Chemicals with specific fungicidal effects on the blast pathogen control the disease. Biological control agents are used as an eco-friendly strategy for rice blast management. Rice blast control strategies and techniques that have been most effectively utilized will be explored in this study. These strategies can

be broadly classified as cultural control, resistance, chemical control, and biological control.

2.6.1 Use of fungicides against blast disease of rice

Pal (2014) studied six fungicides like Kresoxim methyl, Azoxystrobin, Propiconiazole, Trifloxystrobin + Tebuconazole, Difeconazole, and Tricyclazole to evaluate the mycelial growth control of *Pyricularia grisea* under the laboratory conditions and found that Azoxystrobin & Tricyclazole were the most effective.

Haq *et al.* (2015) experimented with various fungicides like Captan, Acrobat, Bayeltan, Sunlet, Dithane M-45 Trimiltox, and Derosal in controlling the mycelial growth of *Pyricularia oryzae* under the laboratory conditions and found that Captan and Acrobat were the most effective fungicides.

Kunova *et al.* (2013) observed that *Pyricularia grisea* mycelium growth was inhibited at low concentrations of Azoxystrobin and relatively high concentrations of Tricyclazole, while sporulation was more sensitive to both fungicides and was affected at similarly low doses. Furthermore, infection efficiency of conidia obtained from mycelia exposed to Tricyclazole was affected to a greater extent than for conidia produced on Azoxystrobin-amended media, even though germination of such conidia was reduced after Azoxystrobin treatment.

Gohel *et al*. (2008) reported that tricyclazole, mancozeb, carbendazim, iprobenfos, propiconazole, and edifenphos were found fungi toxic with cent percent growth inhibition of Pyricularia oryzae highly. Therefore, Gohel *et al*. (2009) tested nineteen fungicides against *P. oryzae in vitro*. Among this tricyclazole (500, 1000, and 1500 ppm), mancozeb (1000, 2000 and 3000 ppm), carbendazim (500, 1000, and 1500 ppm), iprobenfos (500, 1000 and 1500 ppm), propiconazole (500, 1000, and 1500 ppm and

edifenphos (500, 1000 and 1500 ppm) were found fungi toxic with 90.0 % growth inhibition highly.

The efficacy of fungicides *viz*., thiophanate-methyl, carbendazim, fosetyl-aluminum, mancozeb, and copper oxychloride tested against *Magnaporthe oryzae*, only mancozeb appeared as the highly effective fungicide that completely inhibited the mycelial growth of the fungus. All other fungicides showed little effect at higher concentrations (Hajano *et al.,* 2012). The efficacy of five fungicides against rice blast pathogen *M. oryzae* was evaluated by Varma and Santhakumari (2012). Evaluation of isoprothiolane 40% EC (Fuji-one) (at 1ml l^{-1}, 1.5 ml l^{-1} and 2 ml l^{-1}), carpropamid 27.8% SC (Protiga) (at 0.5 ml l^{-1},1 ml l^{-1} and 2 ml l^{-1}), carbendazim 50% WP (Bavistin) (at 0.75 g l^{-1}, 1 g l^{-1} and 1.5 g l^{-1}), tricyclazole 75%WP (Beam) (at 0.1 g l^{-1}, 0.6 g l^{-1}, and 1 g l^{-1}) and propiconazole 25% EC (Tilt) (at 0.5 ml l^{-1}, 0.75 ml l^{-1} and 1 ml l^{-1}) done by poisoned food technique. The results showed that isoprothiolane at 1.5ml/l concentration showed maximum inhibition of mycelial growth (94.85%), followed by carpropamid at 1ml/l concentration (91.48%).

Mohan *et al.* (2013) screened fungicides *viz.*, Folicur (tebuconazole), Tilt (propiconazole), Score (difenconazole), Dithane-78 (zineb), Kasu-B (kasugamycin), Amistar top (azoxystrobin + difenoconazole), Baan (tricyclazole) and Merger (tricyclazole + mancozeb) under *in vitro* conditions each at a concentration of 0.1, 1, 10, 25, 50 and 100 ppm. Among tested fungicides, Tilt, Amistar top, Score, and Folicur were found significantly effective over other treatments. Tilt exhibited 100% growth inhibition at 10 ppm while Folicur, Amistar Top, and Score exhibited 100 percent growth inhibition at 25 ppm. Merger and Baan exhibited 50 percent growth inhibition at 10 and 25 ppm, respectively. However, Kasu-B registered the least (60%) growth

inhibition of fungal growth at 100 ppm. These studies revealed that Tilt followed by Amistar top, Score, and Folicur are the most promising fungicides.

Naik and Jamadar (2014) reported that among the non-systemic fungicides evaluated *in vitro* against *P. grisea*; mancozeb 75WP gave maximum inhibition (93.30%) of the mycelial growth of the pathogen. It was noticed that mancozeb was on par with another combi product; captan 70 + hexaconazole 5 (93.17%) as well as copper oxychloride (89.41%) whereas, it was found to be significantly superior over the combi product; tricyclazole18 + mancozeb 62 (87.38%) and chlorothalonil 75WP (83.81%) across different concentrations. Further, it was observed that there were no significant differences among the different concentrations tested and no interaction effect among the fungicides and concentrations. Among systemic fungicides evaluated against *P. grisea*, tricyclazole 75WP gave maximum inhibition of the mycelial growth (87.78%) of the pathogen followed by difenoconazole 25EC (86.91%), hexaconazole 5EC (85.33%), and propiconazole 25EC (75.92%) and were found to be on par with each other as well as significantly superior over carbendazim 50WP (54.23%) which was found to be the least efficient in inhibiting mycelial growth of the pathogen. However, there was no significant difference among the different concentrations tested as well as there was no interaction effect between the fungicides and concentrations.

Singh *et al*. (2014) reported that tebuconazole was most effective as it completely inhibited the colony growth of *P. grisea* at 10 µg ml^{-1} whereas azoxystrobin + difenoconazole, propiconazole, and difenoconazole completely inhibited the colony growth of the pathogen at 25 µg ml^{-1}. The remaining fungicides, *viz*., zineb, tricyclazole, kasugamycin, and azoxystrobin, proved least effective even at a concentration of 200 µg ml-1. Prasanna *et al.* (2011) screened various fungicides against blast (leaf and neck) and sheath blight disease of rice. Among them, Conika

50% WP (Kasugamycin 5% + Copper Oxychloride 45% WP), Dhanucop Team (Tricyclazole 75% WP) and RIL-068/F1 48 WG (Kresoxim methyl 40% + Hexaconazole 8% WG) were found effective against blast pathogen *Pryricularia oyrzae.*

In order to inhibit mycelial growth of *Pyricularia oryzae in-vitro*, two fungicides (Carbendazim 50% WP and Tricyclazole 75% WP) were evaluated against the fungus by poisoned food technique, and bioagent *Pseudomonas fluorescens* (2×108 cfu/ml) were evaluated against *Pyricularia oryzae* by dual culture technique under laboratory conditions. Carbendazim 50% WP observed the maximum inhibition (100%) at 0.1%, 0.2%, and 0.4% concentration at 4, 8, and after 12 days while Tricyclazole 75% WP also proved effective 100 % at 0.06 % and 0.12 % followed by 98.93 % inhibition at 0.03 % concentration at 4, 8 and 12 days and the bioagent *P. fluorescens* was also found to inhibit the mycelial growth of *Pyricularia oryzae* significantly (59.72 %) (Lal *et al.*, 2015).

Pal (2014) studied six fungicides like Kresoxim methyl, Azoxystrobin, Propiconiazole, Trifloxystrobin +Tebuconazole (Nativo), Difeconazole, and Tricyclazole to control the leaf blast of rice. Among them, Trifloxystrobin + Tebuconazole (Nativo) was highly effective.

Ravindra (2014) tested newly evolved fungicides viz; Trifloxystrobin 25% + Tebuconazole 50% (Nativo 75 WG), Kresoxim methyl (Ergon 44.3 SC), Thifluzamide 24 SC, Metaminostrobin 20 SC, Azoxystrobin 25 SC (Amistar), Tricyclazole 75 WP (Beam), Carbendazim 50WP (Bavistin), Propiconiazole 25EC (Tilt) against leaf blast of rice under natural conditions. Among them, Azoxystrobin and Tricyclazole show better results.

Singh and Prasad (2007) reported that Tricyclazole (beam) is the most effective fungicide for controlling rice blast and increasing the yield.

Netam *et al.* (2014) reported that among different fungicides used as a foliar spray against rice blast pathogen, Ediphenphos and Tricyclazole were significantly more effective.

Tirmali and Patil (2000) conducted a field experiment on susceptible rice cultivar E. K. 70 and 5 new fungicide formulations. Antaco 170, Carpromid 30 SC, Fliqiconazate 25 WP, Ocatve 50 WP and Opus 15.5 SC. These fungicides were sprayed at tillering, booting, and heading stages of the crop. The new formulations reduced neck blast incidence by 16.27% to 29.23%; Opus 15.5 SC was highly effective in controlling neck blast by 29.23% and increasing grain yield.

Tirmali *et al*. (2001) reported the efficacy of new fungicides in controlling rice neck blast caused by *Pyricularia oryzae* on rice cultivar Ek-70 (blast susceptible) treated with WIN 30 SC (Capropamid), Folicur 250, WE Swing 250 Ec, and Beam 75 WP at maximum tillering panicle initiation and heading stage of the crop and found that all these new fungicides resulted in significantly reduced neck blast.

Rabicide 30WP, Nativo SC, and Score 250 EC treatments were made with dose rates of 3 g/liter of water, 0.8 gm/liter of water, and 1.25 ml/liter of water and proved effective in all three weeks in reducing the disease (Prabhu *et al.* 2003; Kim *et al.* 2008; Ghazanfar *et al.* 2009).

Jamal-u-Ddin *et al.* (2012) recorded that Mancozeb appeared as the most effective fungicide that completely inhibited the mycelial growth of the *Magnaporthe oryzae*. Tricyclazole (0.06%), Kitazine (0.1%), and Ediphenphos (0.1%) were found significantly superior in controlling the disease and also resulted in a significant increase in yield in Tricyclazole sprayed plots (7783.33 kg ha^{-1}) followed by

Ediphenphos (6941.66 kg ha^{-1}.), Kitazine (6850.00 kg ha^{-1}) with B:C ratio 1:2.64, 1:2.39, 1:2.31, respectively (Ganesh *et al.,* 2012).

Ganesh *et al.* (2012) recorded that foliar spraying Isoprothiolane at 1.5 ml/l significantly decreased the disease incidence (78.3%) and intensity (89.7%), followed by carpropamid (67.5 and 80.5% disease incidence and intensity, respectively) and carbendazim (56.9 and 73.1% disease incidence and intensity, respectively) over the control. In addition, the highest increase in grain and straw yield over the control was also recorded with isoprothiolane (22.5 and 28.3%), followed by carpropamid (20.5 and 25.7%).

Joshi and Mandokhot (2002) conducted a field experiment in Maharashtra, India, during the seasons of 1998-2000 to determine the efficacy of Tricyclazole in controlling *Pyricularia grisea* causing blast disease in rice and evaluated its effect on rice yield. Beginning one month after planting, a 3-week-old seedling of rice cv. RTN-711 were sprayed with 0.05,0.06 and 0.12% Tricyclazole at fortnightly intervals, along with Mancozeb or Carbendazim and no spray as controls. All three concentrations of Tricyclazole were significantly superior to the control in reducing disease intensity. In addition, there was a linear relationship between disease intensity and yield.

Prajapati *et al.* (2004) found that Tricyclazole proved to be significantly superior in decreasing the leaf and neck blast by 62.9 and 64.1%, respectively, with a corresponding increase of 72.3% in grain yield over grain yield the control and was at par with Carbendazim 50 WP.

Tirmali *et al.* (2004) found that brine solution+ seed dressing with Carbendazim (3 g kg^{-1}) followed by spraying of Phosphomedon (0.05%) was found to be most effective in controlling the blast of rice.

Sundravadana *et al*. (2008) *in vitro* study reveals no phytotoxicity effect at different concentrations of Azoxystrobin. However, the reduction of blast incidence and yield increased curve obtained showed flattening between the range 125, 250, and 500 g a. i/ha rates; hence the optimum rate of Azoxystrobin was fixed to be at 125 g a.i/ha for the control of blast disease.

Nasruddin and Amin (2013) reported that Difenoconazole and Difenoconazole + Propiconazole were evaluated against the rice blast disease and found effective in suppressing blast and protecting yield compared to the other tested fungicides.

Tripathi (2000) reported that seed treatment with Carbendazim @ 4g./kg followed by one foliar spray with this fungicide @ 0.05 at tillering and Corotop 205 G @ 30 kg/ha at the panicle initiation stage was found to be the best for blast control (39.20%) and increasing the yield (31.81%). The efficacy of azoxystrobin and trifloxystrobin to tricyclazole and tricyclazole + propiconazole was compared when applied at the beginning of the stem elongation and late booting and concluded that two applications of azoxystrobin (250 g ha^{-1}) and trifloxystrobin (125 g ha^{-1}) were more effective than tricyclazole (225 g ha^{-1}). However, the efficacy between two treatments of strobilurins and one treatment of tricyclazole at 450 g ha^{-1} was not significant. Both strobilurins and tricyclazole were effective against leaf blast and neck blast and reduced incidence and disease severity by 90-100%, respectively. There was no significant improvement in the efficacy of tricyclazole (225 g ha^{-1}) in combination with propiconazole (125 g ha^{-1}) compared to tricyclazole alone.

The response of four upland rice cultivars to foliar fungicide application concerning panicle blast control was evaluated by Prabhu *et al.* (2003) and obtained differential disease control and yield response of cultivars. The losses in grain yield of cultivars

IAC 202, Caiapo, Rio Paraniba, and Araguaia due to panicle blast were 44.8, 27.4, 24.4, and 18.2%, respectively.

The efficacy of carpropamid, tricyclazole, thiophanate methyl, carbendazim, chlorothalonil, validamycin, and copper oxychloride was evaluated by Dubey (2005) against rice blast in the main field using the susceptible rice cultivar Birsa Dhan 202. He showed that carpropamid was the most effective fungicide for blast management with the minimum neck (1.1%) and node infections (1.7%), disease severity (3.8%), and the maximum grain yield (4.54 t ha^{-1}) followed by tricyclazole and thiophanate methyl.

Epoxiconazole 12.5 SC (2 ml l^{-1}) was the most effective fungicide against rice blast, followed by prochloraz 50 WP (1 g l^{-1}), propineb 70 WP (5 g l^{-1}), chlorothalonil 40 EC (2 ml l^{-1}), and chlorothalonil 75 WP (1 g l^{-1}). However, the efficacy of all the fungicides was next only to tricyclazole 75 WP (0.6%), which suppressed the neck blast incidence to 37.88 percent over the control. A maximum increase of 60.99 percent in grain yield was achieved with tricyclazole 75 WP followed by epoxiconazole 12.5 SC, which recorded an increase of 34.85 percent over the control (Kumbhar, 2005).

Groth (2006) applied azoxystrobin as a foliar spray to the naturally infected field plots at the rates of 0.11, 0.17, and 0.22 kg a.i. Per ha at boot (B) and heading (H) or only at H and growth stages and at 0.17 kg a.i per ha at 5 (H+5), 10 (H+10), and 15 (H+15) days after H and B with low or high blast pressure. Azoxystrobin application made at H, H+5, and B+H significantly reduced blast incidence with high and low disease pressure resulting in significantly higher grain and head rice milling yields than unsprayed plots with high blast pressure. On the contrary, with fungicide application made at B, H+10, and H+15 days post heading, rice has higher disease incidence resulting in lower grain and milling yields than rice receiving a heading application.

Effect of fungicides Armure, Rabicide, Score, Nativo, and Tilt on leaf and neck blast under field conditions and their ultimate effect on crop yield were studied by Ghazanfar *et al.* (2009). They showed that after the application of fungicides Armure (propiconazole + difenoconazole), Rabicide (tetrachlorophthalide), and Score (difenoconazole) showed the best results with disease percentages of 28.11%, 30.61%, and 30.92%, respectively. The fungicides like Nativo (tebuconazole + trifloxystrobin) and Tilt (propiconazole) showed intermediate results, and the disease percentage recorded was 31.44% and 32.63%. WSH004 was the least effective of all the fungicides in controlling the blast disease, and the disease percentage was recorded up to 38.11%.

Application of isoprothiolane and tricyclazole significantly reduced the blast severity by 19.5 and 20.06% compared to 66.6% in untreated control, but the grain yield was more with isoprothiolane (4.13 t ha^{-1}) compared to tricyclazole (3.91 t ha^{-1}) (Arun *et al.*, 2011).

Sharma *et al.* (2011) reported that application of isoprothiolane (1.01 ml l^{-1}) and tricyclazole (0.6 g l^{-1}) significantly reduced the blast severity by 19.5 and 20.06% as compared to 66.6% in the untreated control. In addition, the grain yield was higher in the plots sprayed with isoprothiolane (4.13 kg ha^{-1}) followed by tricyclazole (3.91 kg ha^{-1}) compared with untreated control (2.77 kg ha^{-1}).

The fungicides *viz.*, fenoxanil + isoprothiolane, isoprothiolane, metominostrobin, and tricyclazole were tested by Singh *et al*. (2011) against neck blast incidence. Out of the four fungicides, fenoxanil + isoprothiolane at 20 ml/l was found most promising fungicide in reducing the disease severity (12.8%) with grain yield of 2950 kg ha^{-1} followed by tricyclazole (9.8%) with grain yield of 3300 kg ha^{-1} and isoprothiolane (10.40%) with grain yield of 2950 kg ha^{-1}. On the other hand, Metominostrobin was

found least effective against neck blast with a disease incidence of 27.3% and grain yield of 2550 kg ha^{-1} compared with control (29.7% and 2780 kg ha^{-1}).

Dutta *et al.* (2012) tested various fungicides viz., Nativo 75WG, Gain 75 WP, Score 250 EC, Hexacon Super 5% SC, and Tilt 25 EC against rice blast on MTU 7029 rice variety and applied fungicides with dose rates of 0.4 g l^{-1}, 0.6 g l^{-1}, 1.25 ml l^{-1}, 1.5 ml l^{-1}, 1 ml l^{-1} water, respectively. All the fungicides proved to be effective in the management of rice blast disease but, Nativo, Gain, and Score proved effective in all three weeks in reducing the disease more in the third week with 10.15%, 12.85%, and 11.46%. The control of disease in case of neck blast was shown by Score, Tilt, and Nativo with 11.63%, 14.29%, and 18.98% disease, respectively. Tilt was proved the least effective in controlling leaf blast and Hexacone Super in controlling neck blast.

Barnwal *et al.* (2012) evaluated six new fungicide formulations for their efficacy to control rice blast in a separate field trial with susceptible variety CO 39, three sprays of RIL 0.13 SDC (fenoxanil+isoprothiolane) @ 0.2% was most effective in controlling the disease with leaf blast severity of 8.8% and neck blast incidence of 4.7%. This treatment also recorded a maximum grain yield of 26.6 q ha-1 with an increase of 66.4% over control. This fungicide was followed by three sprays of Baan 70 WP (tricyclazole) @ 0.06% in reducing blast disease severity to 11.9% and neck blast incidence to 6.2% with a concomitant grain yield of 24.8 q ha^{-1}.

Ahmad *et al.* (2020) carried out an investigation was aimed to evaluate the comparative efficacy of different fungicides for blast management in rice crops under agro-climatic conditions of Pakistan. Five fungicides were used for blast management in rice crops. The fungicides viz., Recado 32.5% SC @ 200 ml/acre, Thrill 20% WP @ 250 g per acre, Nativo 75% WP @ 65 g/acre, Recado Ultra 40% SC @ 200 ml/acre and Amistar Top 325 SC @ 200 ml/Acre. For each treatment, there were three replications. The

significantly affected parameters were percent disease intensity (PDI), plant height (cm), number of tillers (m^{-2}), number of grains (m^{-2}), and grain yield (kg ha^{-1}). The results showed that maximum yield was recorded for Nativo 75% WP whereas minimum yield was for control plot. The fungicide Nativo 75% WP @65 g a.i. Per acre was applied at performed well and exhibited effective blast control and better yield in rice. Minimum percent disease intensity (11.16%) was observed in Nativo 75% WP treatment, whereas maximum (58.50%) percent disease intensity was in the control treatment. Maximum plant height (88.83 cm) was recorded for Nativo 75% WP. Similarly, the maximum tillers recorded for Nativo 75% WP were 164 m^{-2}. The maximum value of grain yields of 4403.32 kg ha^{-1} was observed in Nativo 75% WP treated plots followed by Amistar Top 325 SC

Hossain et al. (2017) evaluated the occurrence the blast disease in rice in Bangladesh. Incidence and severity of blast disease of rice was recorded in ten agro-ecological zones (AEZs) of Bangladesh during Boro (November to May; irrigated ecosystem) and Transplanted Aman (July to December; rain fed ecosystem) seasons. Disease incidence and severity was higher in irrigated ecosystem (Boro season) (21.19%) than in rain fed ecosystem (Transplanted Aman season) (11.98%) regardless of locations (AEZs). It was as high as 68.7% in Jhalak hybrid rice variety followed by high yielding rice cultivar BRRI dhan47 (58.2%), BRRI dhan29 (39.8%), BRRI dhan28 (20.3%) during Boro and in BRRI dhan34 (59.8%) during T. Aman season. Maximum yield loss was noted in AEZ9 for both the seasons. Percent yield loss was higher in all the locations for Boro season (irrigated ecosystem) compared to T. Aman season (rain fed ecosystem). In the crop sequence1 (CS-1= Crop cycle with one rice followed by fallow/other crops) disease incidence was 16.7% and in crop sequence2 (CS-2= Crop cycle with two rice followed by fallow/other crops) it was 31.9%. Most popularly

adopted Boro rice was BRRI dhan28 (29.6%) followed by BRRI dhan29 (25.9%) and T. Aman rice was BRRI dhan34 (22.9%).

Singh *et al*. (2019) evaluated the efficacy of different foliar fungicides against blast disease of rice. The fungicides viz., azoxystrobin 18.2% + difenoconazole 11.4% (Amistar top 29.6% SC) @ 0.13% (T_1), tebuconazole 50% + trifloxystrobin 25% (Nativo 75% WG) @ 0.07% (T_2), carbendazim 12% + mancozeb 63% (Saaf 75% WP) @ 0.2% (T_3), tebuconazole 25.9% (Folicur 250 % EC) @ 0.2 % (T_4), hexaconazole 5% (Contaf plus 5% SC) @ 0.2% (T_5), difenoconazole (Score 250% EC) @ 0.06% (T_6) and control (Spray of plain water)- T_7, were applied first at just after occurrence of disease symptoms and second at 15 days after the first spray. Among the six fungicides, the minimum disease per cent intensity was recorded in T_2 i.e. tebuconazole 50% + trifloxystrobin 25% (WG) 11.46%, followed by T_1 i.e. azoxystrobin 18.2% + difenoconazole 11.4% (SC) with 12.85%, respectively. The highest per cent disease intensity of 69.40% was observed in Control (Spray of normal water) treatment. Significantly highest grain yield was recorded in Nativo (WG) sprayed treatment i.e. (4102.11 kg ha^{-1}), followed by azoxystrobin + difenoconazole 29.6% (SC) (3967.28 kg ha^{-1}) and the lowest yield of 2116.23 kg ha^{-1} was recorded with untreated control.

Rijal and Devkota (2020) reviewed the different methods of controlling blast diseases. Management of blast can be done through various methods, but ecofriendly, integration of various cultural, Nutrient, chemical biological and botanical is best. Recent research has been done in biological, botanical, Resistance development, and Nutritional management, but the development of variety and Biological are the best option. Isoprothiolane at 1.5 ml/l and Tricyclazole 22 % + Hexaconazole 3% SC (thrice from booting stage at weekly interval) are the best chemicals. In contrast, *Pseudomonas fluorescens* strain Pf1 @ 10g/kg, SPM5C-1 and SPM5C-2 (aliphatic compounds

obtained from *Streptomyces sp*), *Bacillus tequilensis* (GYLH001), and pseudomonad EA105 effectively inhibit the growth of M. oryzae. More than 100 R genes are identified as Resistance in Blast. Gene Pyramiding and the use of multiline varieties are efficient and can overcome pesticide hazards. Neem extract 4ml/15ml, Coffee arabica@25%, Nicotiana tabacum@10% are effective, but garlic extract @higher doses and neem extract @ 4ml/15 ml are best for complete control. 4 g Si/L in greenhouse conditions observed the most significant reduction of blast incidence. Several forecasting models predict potential disease outbreaks and reduce crop losses. Similarly, the burning of residues and flooding make an unfavorable pathogen condition.

2.6.2 Use of botanicals against blast disease of rice

Sahu *et al*. (2018) experimented with isolating, identifying, and characterizing the pathogen (using morphological, physiological, and biochemical methods). The blast appears every year in varying intensity and causes heavy losses in yield. Therefore, studies were conducted on the isolation, the pathogenicity of *Pyricularia oryzae* on paddy, botanicals against *Pyricularia oryzae* causes a blast of paddy. Two botanical viz. neem, tulsi, and carbendazim, used in present studies, were evaluated in vitro against Pyricularia oryzae by poisoned food technique at 10.00% incubation concentration. The maximum percent inhibition of mycelial growth was recorded T_3-Carbendazim (89.67%), T_1-Neem *(Azadirachta indica)* (57.48%), T_2-Tulsi (leaf) (45.00%), as compared to control (00.00%), in botanical whereas the *Pyricularia oryzae* maximum disease intensity (%) was recorded in T_1 Neem Leaf Extract @ 10% FS (*Azadirachta indica)* (24.57) as compared to treated (13.12) T_0 untreated check (30.31).

Field experiments were undertaken by Pandey (2018) under irrigated ecosystem to evaluate the efficacy of botanicals along with standard fungicides for assessing percent disease incidence, plant height, number of tiller per plant, number of spikelet per panicle, panicle length, 100-grain weight, and grain yield against the blast of rice. Pooled data of two years suggest that neem-based commercial biopesticides with azadiractin as active ingredients were found effective in reducing disease severity and improving the yield attribute of the crop and proves promising products compared to standard fungicides. Among the botanicals, the spraying of Achook, Neem Azal T/S, Neem gold, and Tricure shows a significant reduction in disease severity and improves yield attributes, increasing the 100-grain weight and grain yield.

Kumar *et al.* (2017) were evaluated biocontrol agents *Trichoderma viride, T. harzianum, Pseudomonas fluorescens,* and botanicals neem oil and neem oil + neem leaf extract (*Azadirachta indica*) their efficacy against the blast of rice (Pusa Basmati 1121). The results concluded that neem oil (26.20%) and neem oil + neem leaf extract (24.15%) inhibits the blast, respectively.

Hubert *et al.* (2015) experimented to determine the effect of aqueous extracts of *Aloe vera, Allium sativum, Annona muricata, Azadirachta indica, Bidens pilosa, Camellia sinensis, Chrysanthemum coccineum,* processed *Coffee arabica, Datura stramonium, Nicotiana tabacum,* and *Zingiber officinalis* for control of rice blast disease (*Pyricularia grisea*) *in-vitro* and *in-vivo*. The results indicate that processed *C. arabica* at 10% and 25% (v/v) had the highest (81.12%) and (89.40%) inhibitory effect, respectively, against *P. grisea*. Aqueous extract from *N. tabacum* at 10% concentration ranked third (80.35%) in inhibiting *P. grisea*. These were followed by extracts from 25% *A. vera* (79.45%) and 25% *C. coccineum* flower (78.83%). The results also indicate that extracts from *A. indica, A. vera, A. sativum, C. arabica, D. stramonium, C.*

sinensis, Z. officinalis, and *N. tabacum* did not have any phytotoxic effect on seed germination, shoot height, root length, dry weight, seedling growth, and seedling vigor index. These plant extracts can thus be used for rice seed treatment to manage rice blast disease. Rice blast disease, caused by a seed-borne fungus *Pyricularia grisea*, is an essential and severe disease of rice (*Oryza sativa* L.) worldwide. The disease has been reported to cause yield losses of up to 40% in Tanzania. Studies were conducted to determine the effect of aqueous extracts of *Aloe vera*, *Allium sativum*, *Annona muricata*, *Azadirachta indica*, *Bidens pilosa*, *Camellia sinensis*, *Chrysanthemum coccineum*, processed *Coffee arabica*, *Datura stramonium*, *Nicotiana tabacum*, and *Zingiber officinalis* for control of rice blast disease (*Pyricularia grisea*) *in-vitro* and *in-vivo*. The results indicate that processed *C. arabica* at 10% and 25% (v/v) had the highest (81.12%) and (89.40%) inhibitory effect, respectively, against *P. grisea*. Aqueous extract from *N. tabacum* at 10% concentration ranked third (80.35%) in inhibiting *P. grisea*. These were followed by extracts from 25% *A. vera* (79.45%) and 25% *C. coccineum* flower (78.83%). The results also indicate that extracts from *A. indica*, *A. vera*, *A. sativum*, *C. arabica*, *D. stramonium*, *C. sinensis*, *Z. officinalis,* and *N. tabacum* did not have any phytotoxic effect on seed germination, shoot height, root length, dry weight, seedling growth, and seedling vigor index. These plant extracts can thus be used for rice seed treatment to manage rice blast disease.

2.6.3 Use of bioagents against blast disease of rice

Effect of biocontrol agents (BCAs) evaluated by Kumar and Ashraf (2019) against pathogen *Pyricularia oryzae* causing blast disease of rice *in vitro* and field condition. The result revealed that after three days of inoculation, *Trichoderma harzianum was* found most effective against *Pyricularia oryzae* which inhibited mycelial growth around 63%. Meanwhile, *Trichoderma viride* inhibited 60% radial growth of

Pyricularia oryzae. The best result shown by *Trichoderma harzianum* inhibited pathogen by 63%, 56%, and 41% at consecutive intervals of three, five, and seven days respectively. Dry shoot weight increased by *T. harzianum* (48.24%) followed by *T. viride* (45.98%) concerning seed treated with a pathogen. Among treatment of different bioagents, the best yield was found 15.01 q/hac and 14.88 q/hac treatment with *T. harzianum* and *T. viride* against crops infected with the pathogen. Seed treated with *T. harzianum* and *T. viride*, the disease severity decreased 51.33% and 48.52%, respectively. The application of biocontrol agents reduced the disease severity, promoted plant growth, and ultimately increased the grain yield significantly compared to control without any hazardous effect on the environment.

Kumar *et al*. (2017) reported that the biological method, an ecofriendly and economical approach seems to be an alternative to chemotherapy in managing virulent strains like *P. oryzae* causing a blast of rice. Biocontrol agents *Trichoderma viride, T. harzianum, Pseudomonas fluorescens,* botanicals; neem oil, neem oil, + neem leaf extract (*Azadirachta indica*) were evaluated for their efficacy against blast in Pusa Basmati 1121; a wide yielding variety of rice. Carbendazim was used for standard check fungicides for comparison. The result concludes that *T. viride* is the best bio-control (24.53%) which was followed by *T. harzianum* (23.08%), neem oil (26.20%), and neem oil + neem leaf extract (24.15%) inhibit the blast respectively, while *P. fluorescens* inhibit the blast (21.99%) as compared to treated and untreated control (18.57% and 34.15 %, respectively).

Kumar *et al.* (2017) were evaluated biocontrol agents *Trichoderma viride, T. harzianum, Pseudomonas fluorescens,* and botanicals neem oil and neem oil + neem leaf extract (*Azadirachta indica*) their efficacy against the blast of rice (Pusa Basmati 1121). The results concluded that the *P. fluorescens* inhibit the blast (21.99%) as

compared to treated and untreated control (18.57% and 34.15%, respectively) when *T. viride* is the best bio-control (24.53%) which was followed by *T. harzianum* (23.08%).

An experiment was conducted by Ghimire *et al*. (2017) to check the efficacy against neck blast disease of rice variety "DY-69" (a Chinese variety) with Hexaconazole 5 SC, Tricyclozole 76 WP, and Kasugamycin 3% SL as chemical fungicides while with *Trichoderma viridae* as a biological agent during June to November 2014 in an experimental plot with four replications at Plant Pathology Division of Nepal Agricultural Research Council, Lalitpur. The treatments were applied two times in the field, i.e., at tillering stage, i.e., 35 days after transplanting (DAT), and at the booting stage, i.e., 65 DAT. Disease incidence, disease index, test weight, and total yield were calculated and mean computed. Disease scoring of neck blast was done following the standard scoring system developed by SES (2002). Tricyclazole appeared better to control the neck blast disease, followed by Hexaconazole determined disease incidence, disease index, test weight, and total yield. However, *T. viridae* appeared quite comparable to tricyclazole. So, the use of T. viridae as an option of bio-agent to control a disease will be an ecofriendly measure and more study in its dose and application should be tested in the field to verify the results and control the blast of rice. Thus, using appropriate fungicide or bio-agents (alternative to fungicide) help in reducing health hazard by minimizing adverse impact on the environment.

From the above-cited literature, it is observed that rice blast disease is distributed in almost all rice-growing countries and can be identified on the basis of presence of lesions on plant parts that include leaf, leaf collars, necks panicles, pedicles, seeds, etc. Furthermore, prolonged wetness, high humidity, high nitrogen application, and moderate temperature (24°C) favor the disease development. Therefore, chemical, botanical, and bioagents effectively control blast disease.

2.7 Screening of resistant rice genotype

Ghazanfar *et al.* (2009) evaluated the disease screening nursery of rice germplasm consisting of course and fine varieties were established during the Kharif 2007 to determine the source of resistance in rice germplasm against *Pyricularia oryzae*, the cause of rice blast disease at Rice Research Institute Kala Shah Kaku by artificial inoculum with an aqueous spore suspension of the pathogen. The screening revealed that none of the test lines was immune or highly resistant. One line IR70181-1-1-1 of course type was found to be resistant. Nine lines of the course type displayed a moderately resistant response, while none of the fine type lines showed this response. Seventy-seven lines of both course (35) and fine (42) rice were found to be moderately susceptible. Twenty-four lines of fine rice showed susceptible to the highly susceptible response. However, the prevalence of the resistance against rice blast pathogen is more common in the course as compared to the fine germplasm lines of rice.

Ghimire *et al.* (2019) conducted a field experiment to detect the response of different rice genotypes against rice blast disease under DSR conditions at Mid hill during the rainy season of 2017. Screening of different genotypes was carried out in a field against rice blast disease and checked in one-factor RCBD with 3 replication and 9 genotypes. The experiment was conducted to impart knowledge about the response of different genotypes against rice blast disease. The disease severity, AUDPC was found high in Shankharika genotype while found low in Sabitri genotype. Thus the use of the Sabitri genotype provides proper resistance against rice blast disease in rice under the hill region of Baitadi district under Direct Seeded Rice (DSR) condition.

Challagulla *et al.* (2015) assisted with rapid screening for rice blast resistance as a precursor in a breeding program, the susceptibility to rice blast of 13 rice genotypes from Australia was evaluated in May to June 2013 using three distinct inoculation

methods (spot, filter paper and standard methods) at seedling, vegetative and reproductive stages. The results revealed that the spot and filter paper inoculation methods were successful in discerning susceptibility to the rice blast disease (P 0.05). Disease susceptibility declined significantly from the vegetative to reproductive stages. The standard method was conducted at three different stages for pot plants grown inside the mist house. However, low temperatures did not produce disease symptoms except in a few genotypes. Among the 13 rice genotypes screened, AAT9 expressed a highly resistant response, and AAT4, AAT6, AAT10, AAT11, AAT13, AAT17 and AAT18 expressed resistance at various stages. The results will be useful for selecting elite genotypes for disease tolerance where rice blast is prevalent. In addition, the resistant genotypes can serve as a gene pool used in breeding programmes to develop new resistant genotypes .

Naik *et al.* (2021) evaluated the screening of rice germplasm against blast disease for the identification of resistant sources. To find the leaf blast resistance sources in rice accessions, an open field investigation was carried out in natural and artificial epiphytotic forms during rabi seasons in 2018 and 2019. A total of 97 rice genotypes including resistant check (Tetep) and susceptible check (NLR34242 and BPT5204) were grown, in a uniform blast nursery (UBN). Rice Leaf blast disease severity assessment was scored according to 0-9 scale. Among rice genotypes,21.6 % were resistant, 29.8 % moderately resistant, 21.6 % moderately susceptible, 29.8 % susceptible and 16.4 % were highly susceptible during rabi 2018 whereas only 18.5 % resistant 29.8 % moderately resistant,15.4 % moderately susceptible and 23 % were susceptible and 12.37 % to rice leaf blast disease during rabi 2019. As per result, these resistant accessions with required agronomical traits can be used in the leaf blast

resistance breeding program as donor parents for the development of leaf blast-resistant varieties in rice.

Qudsia *et al.* (2017) evaluated rice germplasm for resistance against *Pyricularia oryzae* the Cause of rice leaf blast. Fifty-two rice genotypes including one susceptible check, Basmati C-622, were evaluated to find out new sources of resistance and assess their diversity based on the reactions against *P. oryzae*. The test genotypes were evaluated against leaf blast after three weeks of inoculation by following the standard evaluation system for rice introduced by the International Rice Research Institute, Philippines. The diversity of the 52 genotypes was also assessed based on blast symptoms. Moderately resistant reactions were observed with genotypes KSK-470, KSK-463, KSK460, PK 8685-5-1-1-1, KSK-462, KSK-474, PK 3810-30-1, KSK-471 and KSK-472. The 52 genotypes were grouped in 4 clusters. The grouping of some genotypes in the same cluster is based on their similar reaction against leaf blast. The results of this study can be useful for selecting suitable genotypes for the development of blast-resistant rice varieties through hybridization.

Puri *et al.* (2009) investigated leaf and neck blast resistance reactions in tropical rice lines under greenhouse conditions. The leaf and neck blast resistance of 182 rice breeding lines were assessed for leaf and neck blast and classified relative to a susceptible check-Masuli and resistant check-Laxmi, from a greenhouse experiment in 2005 and 2006. The test plants were inoculated with 10^5 conidial suspension/ml of *M. oryzae* at 21 days old seedlings for the leaf blast, and at neck base for the neck blast. Among them, for leaf blast, 77 rice lines were resistant, 43 were moderately resistant, 39 were moderately susceptible and 23 were susceptible. While among the selected 31 rice lines evaluated for neck blast, 4 lines were resistant, 4 were moderately resistant, 16 were moderately susceptible and 7 were susceptible.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

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Consequently, four experiments were conducted to evaluate the morphological and pathogenic variation and *in vitro* management *Magnaporthe oryzae* causing rice blast through fungicides, botanicals, and bio-agents. The details of the materials and methods that were followed for conducting these experiments have been presented under the following headings and sub-headings:

3.1 Description of the experimental site

3.1.1 Location of the experiment

Firstly, the surveys were conducted in different rice-growing regions of Bangladesh to determine blast incidence and severity (Table 1 and Fig. 1), and subsequently, blast-infected leaves and stems were collected from farmers' rice fields BRRI dhan28 grower for different laboratory studies. Then the morphological and pathogenic variation of rice blast disease-causing pathogens *Magnaporthe oryzae* and their management using fungicides, botanicals, and bio-agents were carried out at Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The location of this experimental site is $23^0 46' 19.9''$N latitude and $90^0 22' 15.1''$ E longitude with an elevation of 9.0 meters from sea level.

3.1.2 Experimental period

The surveys and laboratory experiments were conducted from April 2016 to August 2018.

3.1.3 Climatic condition

The geographical location of the experimental site was under the subtropical climate, and its climatic conditions are characterized by high temperature, heavy rainfall during

Kharif-1 season (March-June), and scanty rainfall during Rabi season (October-March) associated with moderately low temperature.

3.2 Experimental details

3.2.1 Experiment-1: Survey on the incidence and severity of rice blast in BRRI dhan28 and collection of disease samples from different rice-growing regions of Bangladesh

3.2.1.1 Experimental period

The survey study was conducted in the BRRI dhan28 rice field from April to August 2016.

3.2.1.2 Survey location

The survey was conducted in the following locations (Table 1 and Fig. 1).

Table 1. The survey area on rice blast

Blast survey areas		
District	Upazila	Village/Union
Kishoregonj	Hossainpur	Araibaria
		Madkhola
		Dangri
	Itna	Sadar
	Mitamoin	Sadar
Mymensingh	Phulpur	Chalk deula
		Bahadurpur
	Tarakanda	Kakni
	Bhaluka	Birunia
	Gafargaon	Datter bazar
		Moshakhali
	Netrokona	Kolmakanda
Bogura	Dupchachia	Dupchachia
	Kahalu	Kalai
Dinajpur	Birganj	Palashbari
	Kaharol	Rasulpur
Panchagar	Buda	Sepaipara

Dhaka	Savar	Birulia
Narsingdi	Narsingdi	Belabo
Cumilla	Chouddagram	Kalikapur
Cox’s Bazar	Ramu	Rajarkul
Meherpur	Gangni	Raipur
Chuadanga	Alamdanga	Baradi
Barisal	Babuganj	Chadpasa
Sunamganj	Satok	Satok sadar
Moulvi Bazar	Kulaura	Kulaura

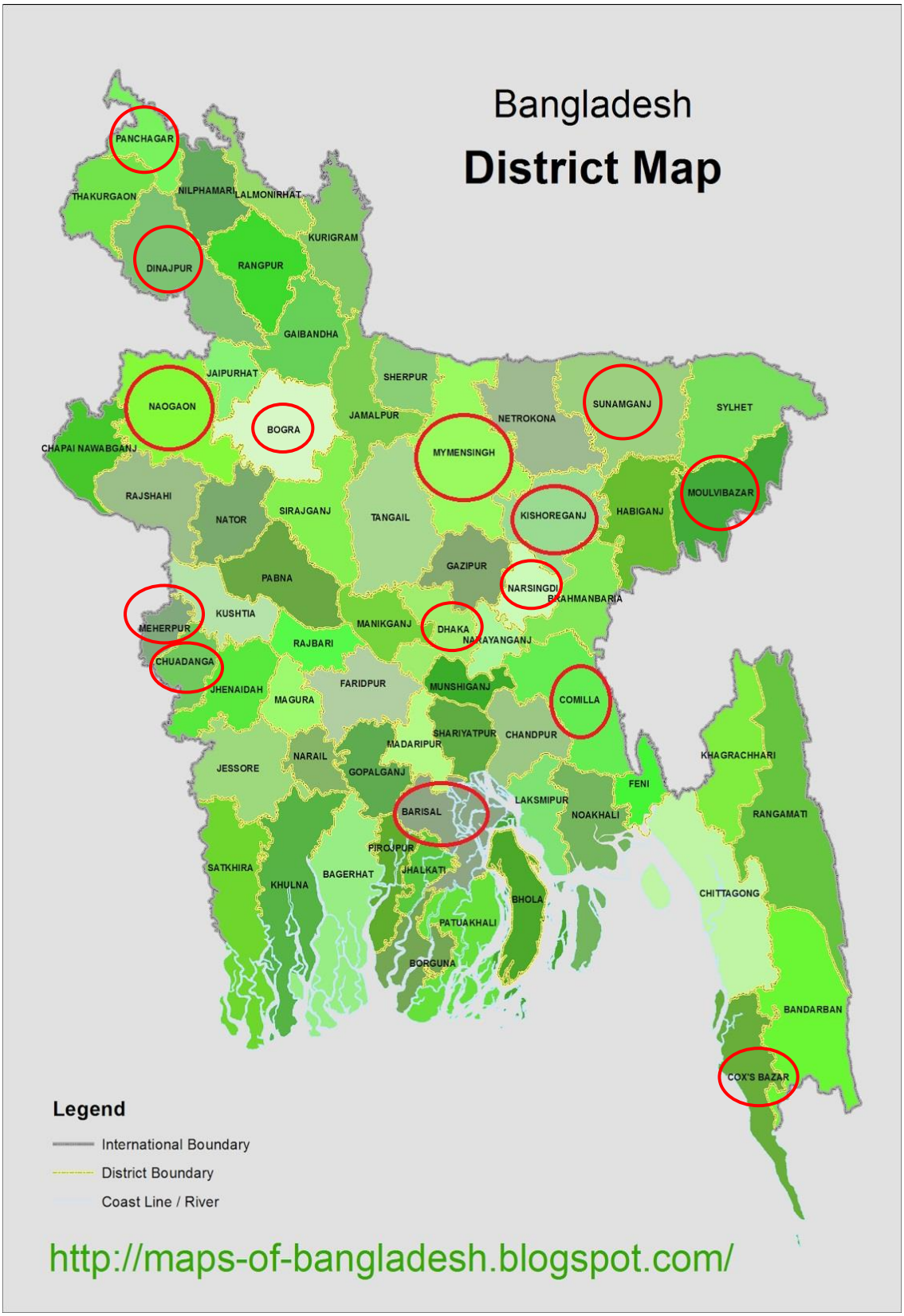


Figure 1. Rice blast survey and sampling sites on Bangladesh.

3.2.1.3 Collection of blast disease sample

The survey on blast disease of rice was conducted in the farmer's fields of selected areas of Bangladesh. The survey was conducted at the grain filling stage of the rice plant and observed panicle blast of rice. Soil type, cropping pattern, and cropping intensity were taken into consideration for the selection of locations, and fields from each location were selected randomly to estimate rice blast disease prevalence and incidence and, finally, severity score. Twenty samples from each selected village/union were identified, following zigzag sampling pattern (Savary *et al*., 1996).

3.2.1.4 Data Collection

For survey data collection from farmer's rice field grower BRRI dhan28 varieties, 20 infected rice fields were selected in a specific location of Bangladesh during the *Boro* season (April to August; irrigated ecosystem) season. For data collection, 1 m^2 was randomly selected area from the sample location, and the number of healthy and infected rice plants was counted and recorded accordingly, and the disease incidence of rice blast was estimated as stated by Dar *et al*., 2018 by using the following formula:

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

The severity of the blast disease was examined visually on the whole plants within the quadrants and recorded as the percentage of plant parts (tissue) affected (percentage of blast infection of the plant).

The disease severity of panicle blast in rice plants was recorded based on the percent infected leaves as present in the field. Disease severity was recorded using the scale of 0 to 9 (Table 2) developed by International Rice Research Institute (IRRI, 2014).

Table 2. Scale for panicle blast disease severity based on symptom

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

3.2.2 Experiment-2: Isolation, identification morphological characterization and pathogenicity test of the *Magnaporthe oryzae* isolates causing rice blast collected from different rice-growing regions of Bangladesh

3.2.2.1 Experimental period

The experiment was conducted from April to August, 2016.

3.2.2.2 Isolation of *Magnaporthe oryzae* from infected leaf

Samples of typical blast symptoms on rice leaves were collected from selected regions of Bangladesh. The infected portion was cut into small pieces, and the surface was sterilized by dipping in 10% Clorox for 1 minute and rinsed three times with sterile distilled water. Then the cut pieces were placed in moist filter paper in petridish and incubated at 25^0C for 48 hours. After sporulation the conidia were transferred on water agar by observing the plate under the stereomicroscope. The plates were incubated in +/- 25^0 C, and subsequently, hyphal tips were transferred on PDA and OMA.

3.2.2.3 Culture of *Magnaporthe oryzae*

Different culture media, namely Water Agar, Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Potato Sucrose Agar (PSA), Rice flour Yeast Agar (RfYA), and Potato-Rice straw Dextrose Agar (PRsDA), were used for the culture of *M. oryzae* (Ravindra, 2014; Mahdieh, 2013; Mijan, 2000 and Vanaraj *et al.*, 2013). The composition of the used media is presented below:

Water Agar

Composition	Quantities (g/litter)
Agar	20

Potato Dextrose Agar (PDA)

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

Oat Meal Agar

Composition	**Quantities (g/litter)**
Oat meal	60
Agar	12.5
Chloramphenicol	0.05

Potato Sucrose Agar (PSA)

Composition	Quantities (g/litter)
Potato (peeled and sliced)	200
Sucrose	20
Agar	20
Chloramphenicol	0.05

Rice Flour Yeast Agar

Composition	Quantities (g/litter)
Complete rice flour	15
Yeast extract	04
Agar	15
Chloramphenicol	0.05

Potato-Rice straw Dextrose agar (PRSDA)

Composition	Quantities (g/litter)
Potato	200
Dextrose	20
Agar	20
Rice straw	80

3.2.2.4 Isolation of *M. oryzae* from the infected panicle

The necrotic patches of rotten neck regions were cut with healthy portion and surface sterilized by dipping in mercuric chloride solution (1:1000) for one minute and washed with sterilized water several times. The cut neck region was inoculated in a sterilized Petri dish containing previously water-soaked blotter paper in aseptic condition and kept in an incubator at 25 ± 2^0C for the development of fungal growth. Newly developed fungal conidia were observed under a stereomicroscope, transferred on water agar, and incubated at 25 ± 2^0C for two days. The hyphal tips were then transferred on PDA and OMA and incubated at 25 ± 2^0C for 30 days.

3.2.2.5 Preparation of pure culture

The marginal mycelial growth that developed subsequently was picked up aseptically for sub-culturing. Then again transferred the mycelial structure in PDA (Potato dextrose agar) and OMA (oatmeal agar), and subculturing was done at an interval of 15 days and preserved at low temperature (5 ± 1^0C) in the refrigerator.

3.2.2.6 Identification of *M. oryzae*

For sporulation of the fungus, oatmeal agar media was used. First, oatmeal agar media was sterilized in a conical flask in an autoclave at 15 psi (121^0C) for 20 minutes. Then poured in petri dishes and solidified and the pathogen was inoculated in the sterilized oatmeal agar media, kept at 25 ± 2^0C. After 30 days of inoculation, the spores was formed. The conidia produced on OMA collected from leaf samples and neck region were observed under a microscope. In addition, photographs of the spores were taken with a binocular microscope fitted with Moticam 3.0 MP camera, and accordingly, the number of spores was counted. The organism was identified based on two septate they are called Pyriform conidia.

3.2.2.7 Designation of collected isolates of *M. orayzae*

The isolates of *M. oryzae* were designated following Aminuzzaman *et al.* (2010). The collected isolates were designated based on their location and sources. For example, an isolate designated by KHAL01 represents that this isolate was collected from District Kishoregonj (K), Upazila Hossainpur (H), Village/Union Araibaria (A), and isolate from leaf (L). 01 denotes collection number.

3.2.2.8 Cultural characteristics and morphology of *M. oryzae*

Isolates of *M. oryzae* collected from rice-growing areas were cultured on PDA, OMA, PSA, RfYA, and PRsDA and incubated for 30 days. Mycelial growth rate and colony characteristics, viz color, surface texture, and shape, were determined.

3.2.2.9 Pathogenicity test of isolates of *M. oryzae*

A pot culture technique was used to prove the pathogenicity of the test organism. The paddy seeds were sown in sterilized earthen pots containing sterilized soil plus FYM (1:1). The rice seedlings with vigorous growth were selected for artificial inoculation of pathogen. Spore suspension of 30 days old culture was prepared by pouring 10 ml sterile water on the colony surface in Petri plate and gently scraped with the help of sterilized needle and collected in 100 ml sterilized conical flask for inoculating the test fungus. The spore concentration of 26 isolates was maintained at 1x 10^5 conidia/ml. The homogenous spore suspension prepared in sterile water was sprayed on the upper and lower surfaces of the leaves. The plants were kept in a moist chamber for 48 hours in darkness and then placed under 12 hr light and 12 hr dark conditions for disease development at 25^0c. The development of leaf blast symptoms was recorded.

3.2.3. Experiment-3: *In vitro* evaluation of fungicides, botanicals, and bioagents against *Magnaporthe oryzae*

3.2.3.1 *In vitro* evaluation of different fungicides against *M. oryzae*

Poisoned Food Technique (Nene and Thapliyal, 1979) was applied in present assay to determine the efficacy fungicides. Each fungicide along with control was tested against *Magnaporthe oryzae*. PDA was used as basal medium and distributed in 100 ml aliquots in each 250 ml Erlenmeyer flasks, which was sterilized at 15 lb psi pressure for 15 minutes. The quantity of fungicide per treatment was calculated for 100ml medium separately. The requisite quantity of test fungicide was added to each flask at 45^0C. The fungicides were thoroughly mixed before solidification and poured immediately into sterilized petri plates. The mycelial disc of 5 mm diameter of fifteen days old culture was cut with the help of a sterile cork borer. Each disc was transferred aseptically to the center of each petri plate with the poisoned medium. The PDA plates without fungicide (with water only) were also inoculated and maintained as a control. The plates were incubated at 27 ± 1^0C for seven days. Four replications per treatment were maintained. The observations on colony growth were recorded until the petri plate in the control treatment was entirely covered with mycelial growth.

The following chemical fungicides, namely Folicular 250 EC, Amister Top 250 SC, Nativo 75 WP, Trooper 75WP, Blastin 75 DG, Azonil 56 SC, Seltima 100 CS, Autostin 50 WDG, Filia 525 SE, Difar 300 EC, Dithane M-45, and Knowing were tested for their efficiency against *Magnaporthe oryzae* following poisoned food technique (Nene and Thapliyal, 1979). The details of the fungicides are presented in Table 3 and Fig. 2.

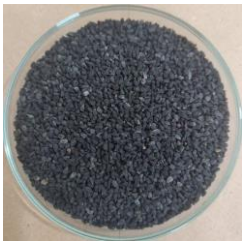
Figure 2. Fungicides tested against *Magnoporthe oryzae in vitro*

Table 3. The details of the fungicides used in the experiment

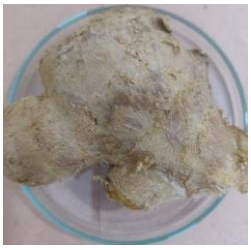
Sl. No.	Trade name	Common name	Manufacturer	Dose rate	Percentage
1	Folicure 250 EC	Tebuconazol	Bayer Crop Science Limited	1 ml/L	0.10
2	Amistar Top 250 SC	Azoxystrobin (20%) + Difenoconazole (12.5%)	Syngenta Bangladesh Limited	1 ml/L	0.10
3	Nativo 5 WP	Tebuconazol (50%) + Trifloxystrobin (25%)	Bayer Crop Science Limited	0.6 g/L	0.06
4	Trooper 75 WP	Tricyclazole	Auto Crop Care Limited	0.8 g/L	0.08
5	Blastin 75 DG	Tebuconazole (50%) + Trifloxystrobin (25%)	ACI Crop Care Limited	0.6 g/L	0.06
6	Azonil 56 SC	Azoxystrobin (6%) + Clorothalonil (50%)	Haychem Bangladesh Limited	0.1 ml/L	0.01
7	Seltima 100 CS	Pyraclostrobin (10%)	Shetu Pesticide Limited	20 ml/L	0.20
8	Autostin 50 WDG	Carbendazim	Auto Crop Care Limited	2 g/L	0.20
9	Filia 525 SE	Propiconazole (12.5%) + Tricyclazole (40%)	Syngenta Bangladesh Limited	0.2 ml/L	0.02
10	Difar 300 EC	Difenoconazole (15%) + Propiconazole (15%)	ACI Agrochemicals Limited	1.52 ml/L	0.15
11	Dithane M-45	Mancozeb	Bayer Crop Science Limited	4.4 g/L	0.44
12	Knowin 50 WP	Carbendazim	McDonald Bangladesh (Pvt.) Limited	1 g/L	0.10

3.2.3.2 *In vitro* control of *Magnaporthe oryzae* by botanicals

The botanicals, namely Kalijira, Turmeric, Ginger, Garlic, Onion, Neem, Allamanda, Aloevera, etc. were evaluated against *Magnaporthe oryzae in-vitro* following food poison technique of Nene and Thapliyal (1979) (Table 4 and Fig. 3).


Kalijira


Turmeric Powder


Ginger


Garlic


Onion


Neem Leaves


Alamonda Leaves


Aloe vera Leaves

Figure 3. Botanical used for *in vitro* evaluation against *Magnaporthe oryzae*

The particulars of plant species used in this study are presented in Table 4.

Table 4. Botanicals used for in vitro evaluation against *M. oryzae*

Common Name	English Name	Scientific Name	Plant parts used
Kalijira	Black cumin	*Nigella sativa*	Seed
Turmeric	Turmeric	*Curcuma longa*	Rhizome
Ginger	Ginger	*Zingber officinale*	Rhizome
Garlic	Garlic	*Allium sativum*	Clove
Onion	Onion	*Allium cepa*	Bulb
Neem	Margosa tree	*Azadirachta indica*	Leaf
Allamanda	Allamanda	*Allamanda cathertica* L.	Leaf
Aloe vera	Aloe vera	*Aloe vera*	Leaf

3.2.3.2.1 Preparation of botanical extracts

Kalijira Seed, Turmeric Rhizome, Ginger Rhizome, Garlic Clove, Onion Bulb, Neem Leaves, Alamanda Leaves were washed with tap water and then grinded in pestle mortar and sewed through a muslin cloth. Botanical extracts mixed with water in different concentrations were used to determine the efficiency of the botanical extracts those are 1:4 (w/v) = 25 g botanical in 100 ml water, 1:2 (w/v) = 50 g botanical in 100 ml water, 1:1 (w/v) =100 g botanical in 100 ml water. Botanical extracts mixed with ethanol in different concentration were also used to determine the efficiency of the botanicals those are 1:4 (w/v) = 25 gm botanical in 100 ml ethanol, 1:2 (w/v) = 50 g botanical in 100 ml ethanol, 1:1 (w/v) =100 g botanical in 100 ml ethanol. These mixtures were kept overnight in beakers to increase their efficiency. The next day, they were applied in PDA (Potato Dextrose Agar) culture plates, and then mycelial discs were placed in a disc cutter. Streptomycin sulphate at 1 ml/L^{-1}medium was used as an antibiotic to avoid bacterial contamination. PDA medium without plant extract was kept as control. There were three replications of each treatment. After solidifying of culture medium, one cm disc of pure culture of the test fungus was placed in the center of each Petri dish and incubated at 25±2^{0}C. The diameter of growing colonies of the fungus was recorded in mm after every 24 hours till the control plates were filled with mycelial growth.

3.2.3.3 *In vitro* control of *M. oryzae* by bio-agents

Two bio-control agents, *Trichoderma harzianum* and *Purpureocillium lilacinum* were tested under laboratory conditions against rice blast causing fungus *Magnaporthe oryzae* following the dual culture method.

3.2.3.4 Collection of bio-control agents

Bio-control agents *Trichoderma harzianum* Th4 and *Purpureocillium lilacinum* PLSAUI were collected from the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University. The fungal antagonists were cultured in Potato Dextrose Agar (PDA) medium for 10 days (Fig. 4)

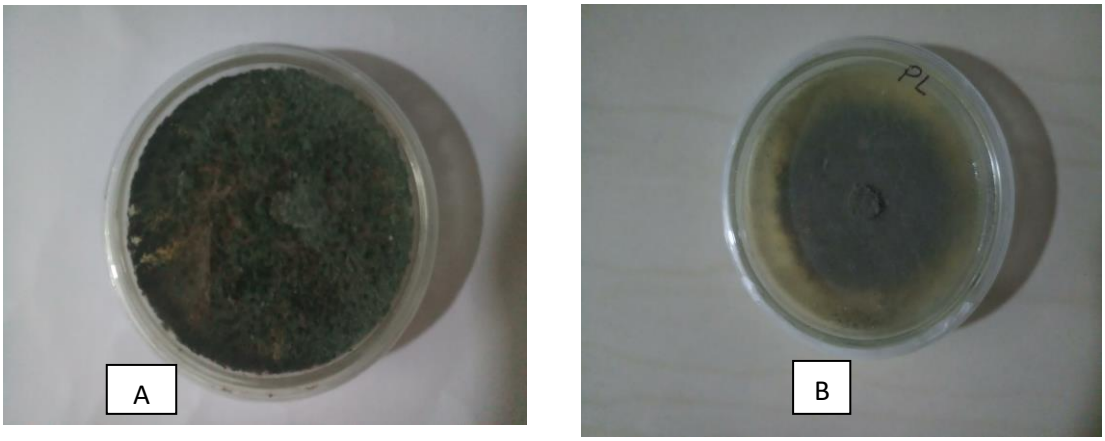


Figure 4. Pure culture of *Trichoderma harzianum (A) and Purpureocillium lilacinum (B)*

This experiment was performed to test the *Trichoderma harzianum* and *Purpureocillium lilacinum* in separate dual culture methods against the *M. oryzae.* In five separate designs containing sterilized PDA medium, four mm disc of test fungus and biocontrol agents at 2 DAI were placed on opposite sides in Petri dishes. There were 3 replicates of each design containing biocontrol agents, and *M. oryzae* was incubated at 25 ± 2^0 C. Biocontrol agents and test fungus inoculated separately and incubated at 25 ± 2^0 C were served as control. The colony diameter of both the biocontrol agent and the test fungus were recorded every 48 hours by giving a straight line in the center of both colonies with a permanent marker, and the antagonism of both fungi was determined.

In the first design, one disc of *M. oryzae* was set against one disc of *Trichoderma harzianum,* and one disc of *M. oryzae* was set against one disc of *Purpureocillium lilacinum.* In the second design, three discs of *Trichoderma harzianum* and three discs of *Purpureocillium lilacinum* were set separately on the periphery of the separate Petri dishes against one disc of *M. oryzae* on the center of the petri dishes following dual culture technique. In the third design, four discs of *Trichoderma harzianum* and four discs of *Purpureocillium lilacinum* were set separately on the periphery of the separate Petri dishes against one disc of *M. oryzae* on the center of the petri dishes following dual culture technique. In the fourth design, three discs of *M. oryzae* were set on the periphery of the separate Petri dishes surrounding one disc of *Trichoderma harzianum* and one disc of *Purpureocillium lilacinum* on the center of the Petri dishes following dual culture technique. In the fifth design, four discs of *M. oryzae* were set on the periphery of the separate Petri dishes surrounding one disc of *Trichoderma harzianum* and one disc of *Purpureocillium lilacinum* on the center of the Petri dishes following dual culture technique. Two different Petri dishes were set as control conditions; both tested fungus *M. oryzae,* bioagent *Trichoderma harzianum,* and *Purpureocillium lilacinum.*

3.2.4 Experiment 4. Screening of rice genotypes against *Magnaporthe oryzae* in rice blast nursery

Seventeen rice genotypes were collected (Table 5) and evaluated in the uniform blast nursery (UBN), BRRI Gazipur to screen for resistant rice genotype (s) against *Magnaporthe oryzae.* Collected seeds were soaked in water in petri dishes for 24 hrs, and germinated seeds were sown in the soil of the blast nursery in line. Each line of tested genotypes was surrounded by two lines of susceptible check US2 with four replication for each tested genotypes. Twenty-days-old seedlings were inoculated with Lal-1, a virulent race of *Magnaporthe oryzae*, maintaining a conidia concentration of $1x10^5$ conidia/ml suspension. Blast reaction was recorded up to 15 days after inoculation following a scale of IRRI, 2014.

Table 5. Sources of rice genotype tested against *M. oryzae*

Sl. No.	Rice genotype	Source of collection
1.	BADC BRRI Dhan 28	BADC
2.	BRRI Dhan 34	BRRI
3.	BRRI Dhan 71	BRRI
4.	Chinigura	Local
5.	Jeera Vog	Local
6.	Badsha Vog	Local
7.	Chini Atop - 2	BRRI
8.	Begun Bichi	Local
9.	Katari Vog	Local
10.	BADC BRRI Dhan 29	BADC
11.	NeelSagar Seed Jholok Hybrid Dhan	Local
12.	LalTeer Tia Hybrid Dhan	Local
13.	Kalijeera	Local
14.	Tej Gold BAYER Hybrid	Local
15.	BINA 7	Bogura
16.	BRRI dhan 34 (Atap)	Bogura
17.	BRRI dhan33	Bogura

3.3 Experimental design and statistical analysis

The experiment was laid out in Complete Randomized Design (CRD) with three replications. The mean values of all recorded parameters were evaluated, and analysis of variance was performed by the 'F' (variance ratio) test using MSTAT-C software. The significance of the difference among the treatment means under the experiment was estimated by Duncan's Multiple Range Test (DMRT) at 1% level of probability (Gomez and Gomez, 1984).

CHAPTER 4

RESULTS AND DISCUSSION

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Rice blast (*Magnaporthe oryzae*) is a key concern in combating global food insecurity, given that the disease is responsible for approximately 30% of rice production losses globally, and it was the equivalent of feeding 60 million people. Yield loss from blast infections depends on varietal susceptibility, the degree of infection, and fungicide application timing. Finally, it varied from location to location due to environmental conditions.

The study was conducted to determine the severity of rice blast disease through a survey program, isolation, identification, and pathogenicity of *M. oryzae*, *in-vitro* evaluation of chemicals, bio-agent *Trichoderma* sp. and botanicals against *M. oryzae*. The results are presented and discussed with the help of tables, graphs, and possible interpretations were given under the following headings and sub-headings.

4.1 Survey of rice blast disease

A survey was carried out to collect the rice blast sample in different regions of Bangladesh from April to August 2016-2018 (Figure 5).

Figure 5. Pictorial view of blast infected rice field in survey areas

4.2 Rice blast disease incidence and severity score in surveyed areas

Survey data revealed that rice blast disease prevalence was 100 percent for all the surveyed areas (Table 6). However, disease incidence varied from 10 percent to 70 percent for individual village/union, but it varies from 10 percent to 60 percent district-wise. Among which, the highest disease incidence (60%) was found in the Bogura district, whereas the lowest (10%) was observed in Dhaka, Sunamgonj, and Moulvi Bazar districts. Suprapta and Khalimi (2012) recorded rice blast disease incidence around 21-37% in Bali, Indonesia.

The severity score of rice blast disease varied from 0-7. The highest severity score (5) was recorded in Kishoregonj district with a 25% disease incidence, while the lowest (2) was found in Dhaka, Cox's Bazar, and Sunamganj districts, respectively with the disease incidence of 10%, 30%, and 10%, respectively. Hossain *et al*. (2017) reported that blast disease incidence and severity of rice was higher (21.19%) in Boro season and transplanted Aman season it was 11.98 percent regardless of locations. The present study's findings were also supported by Nazifa *et al.,* 2021. They found that Gobindogonj had the highest incidence (84.26%) of blasts, with a severity score of 7. The maximum blast severity score of 9.00 (65%) was achieved in Mohimagonj, where the blast incidence was only 29.12 percent. Regardless of location, disease incidence and severity was higher in irrigated ecosystems (Boro season) (21.19%) than in rainfed ecosystems (Transplanted Aman season) (11.98%) (AEZs). The spread of disease is affected by the weather and climatic conditions of various places (Hossain *et al.,* 2017; Rayhanul *et al.,* 2019; Shahriar *et al.,* 2020 and Bevitori and Ghini, 2020). Mahmud *et al.* (2021) discovered that rice blast disease (leaf and neck blast diseases) is more widespread in northern Bangladesh, acting as a hotspot for the disease due to the disease's excellent predisposing factors.

Table 6. Incidence and severity of blast disease in rice cultivated farmers' fields at different districts of Bangladesh

Blast survey areas				Incidence (%)	Severity (Score)
District	Upazila	Village/Union	Variety		
Kishoregonj	Hossainpur	Araibaria	BR28	25	7
		Madkhola	BR29	30	5
		Dangri	BR29	20	0
	Itna	Sadar	BR59	30	5
	Mitamoin	Sadar	BR63	20	3
Average				25	5
Mymensingh	Phulpur	Chalk deula	BR28	30	7
		Bahadurpur	BR63	20	3
	Tarakanda	Kakni	BR29	30	7
	Bhaluka	Birunia	BR28	20	4
	Gafargaon	Datter bazar	BR29	50	3
		Moshakhali	BR28	10	3
	Netrokona	Kolmakanda	BR29	70	7
Average				33	4.85
Bogura	Dupchachia	Dupchanchia	BR28	70	5
	Kahalu	Kalai	BR28	50	3
Average				60	4
Dinajpur	Birganj	Palashbari	BR28	20	3
	Kaharol	Rasulpur	BR58	30	5
Average				27	3.33
Panchagar	Boda	Sepaipara	BR28	30	4
Dhaka	Savar	Birulia	BR28	10	2
Narsingdi	Narsingdi	Belabo	BR29	30	5
Cumilla	Chouddagram	Kalikapur	BR28	20	5
Cox's Bazar	Ramu	Rajarkul	BR28	30	2
Meherpur	Gangni	Raipur	BR28	50	3
Chuadanga	Alamdanga	Baradi	BR29	40	5
Barisal	Babuganj	Chadpasa	BR74	30	3
Sunamganj	Satok	Satok sadar	BR29	10	2
Moulvi Bazar	Kulaura	Kulaura	BR58	10	3
Average				**30**	**4**

4.3 Isolation and identification of *Magnaporthe oryzae*

The isolates of *M. oryzae* were identified based on morphological and cultural characteristics. After confirming microscope examination, one monoconidial culture from each isolate was prepared and maintained on Potato Dextrose Agar (PDA) and Oat Meal Agar (OMA) for further study (Figure 6 and 7).

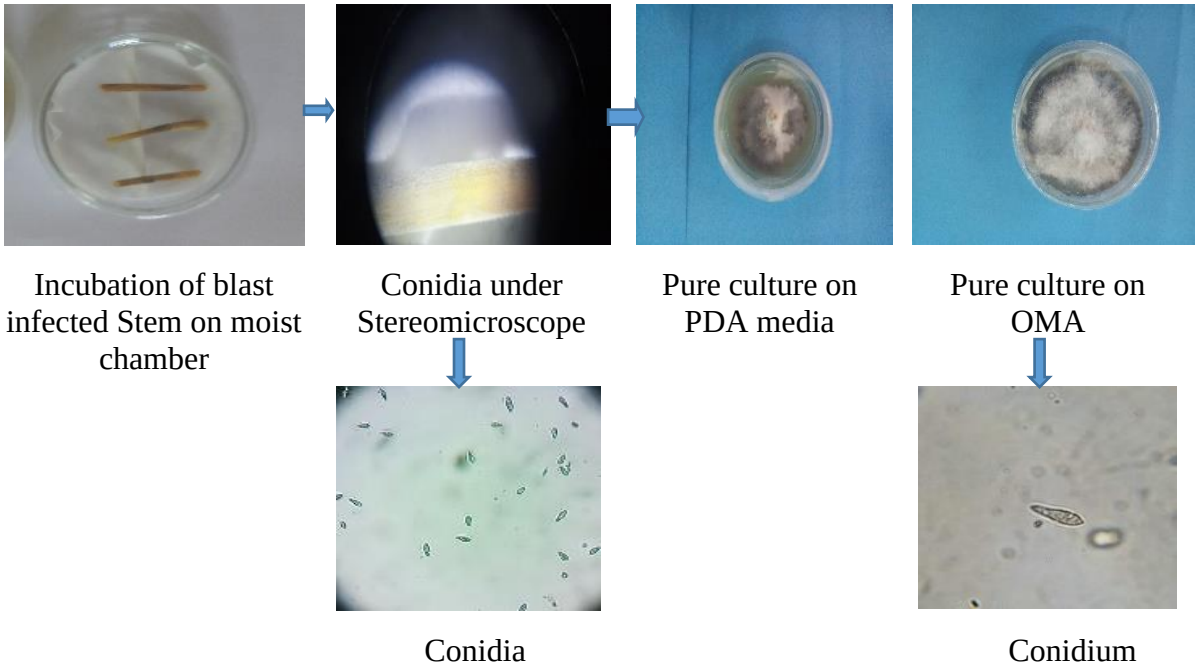


Figure 6. Flow chart of isolation, identification, and culture of *Magnaporthe oryzae* from the infected stem on PDA and OMA media

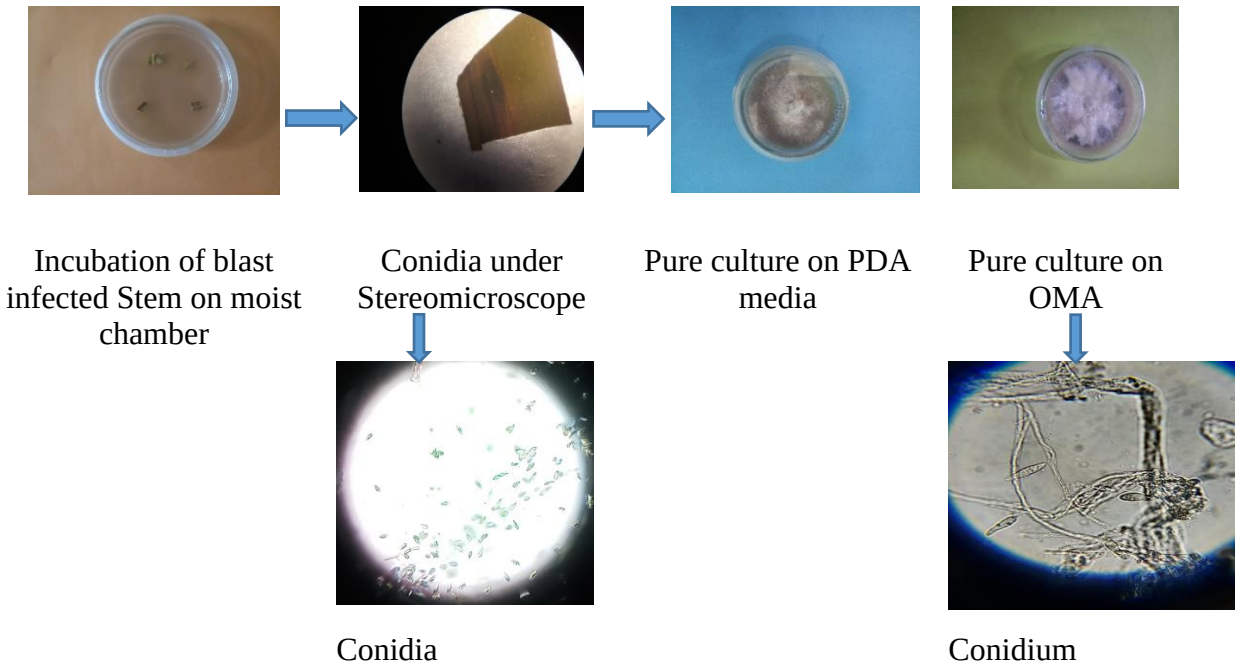
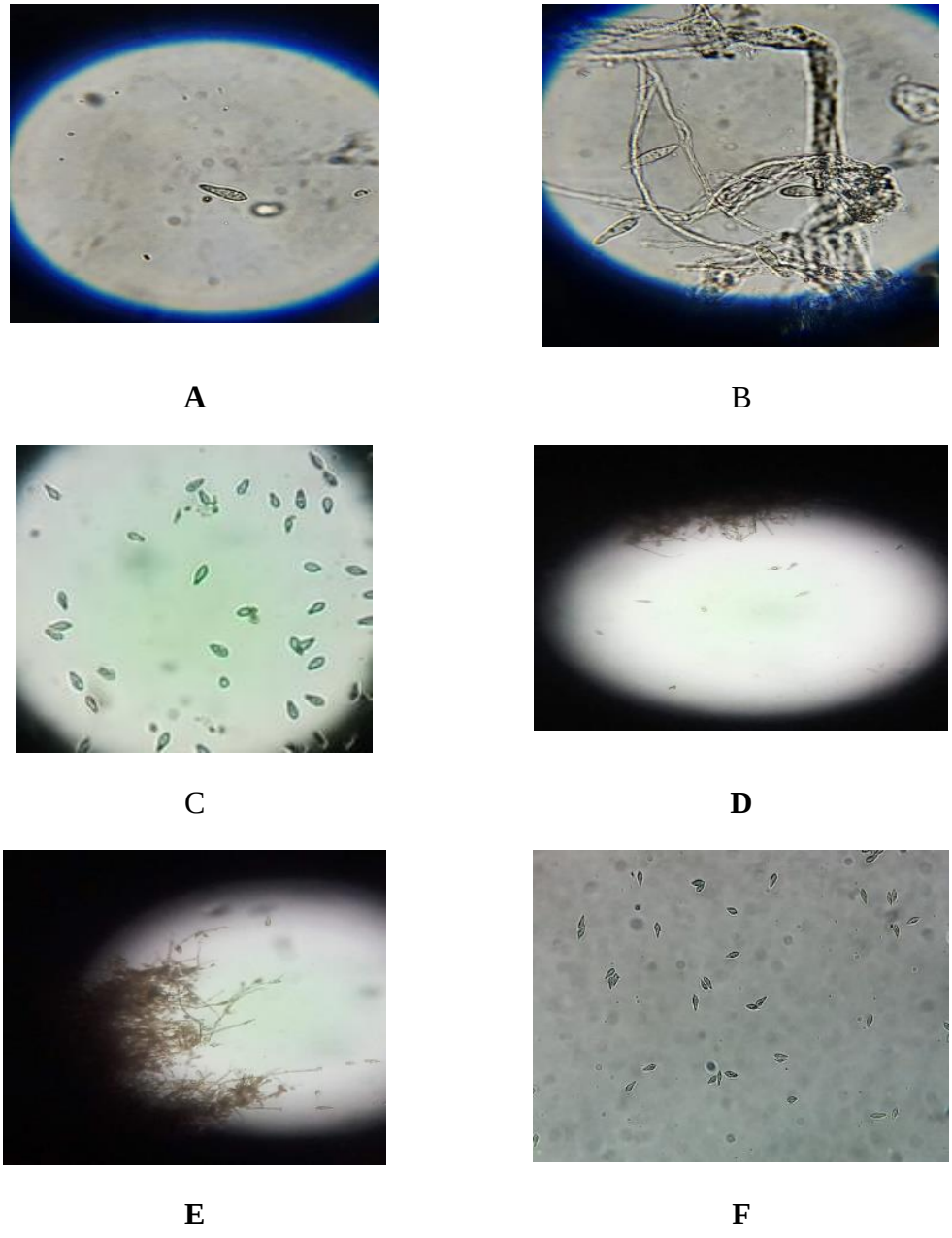


Figure 7. Flow chart of isolation, identification, and culture of *Magnaporthe oryzae* from the infected leaf on PDA and OMA media

4.4 Confirmation of *Magnaporthe oryzae*

A fine tip needle was used to pick up the conidial masses and placed in the glass slide. Then the slide was observed under a compound microscope with a coverslip. Typical two septate, three celled pyriform conidia were observed (Figure 8). This findings illustrated the confirmation of fungus was M. oryzae.



A B

C **D**

E **F**

Figure 8. Conidia of *Magnaporthe oryzae* under the compound microscope (X 20X)

4.5 *In vitro* mycelial growth of *Magnaporthe oryzae* on different culture media

Mycelial radial growth of *Magnaporthe oryzae* was observed in Water Agar (WA), Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Rice Flour Yeast Agar (RFYA), Oat Meal Agar (OMA), and Potato-Rice straw Dextrose agar (PR_SDA) culture media and statistically significant variation were recorded (Table 7). Furthermore, it was revealed that the mycelial radial growth of *M. oryzae* varied 15.00 to 29.67 mm in different culture media.

Table 7. Mycelial radial growth of *Magnaporthe oryzae* in different growing media at 7 DAI

Culture media	Radial mycelial growth (mm) at 7 DAI
Water Agar (WA)	15.33 c
Potato Dextrose Agar (PDA)	22.67 b
Potato Sucrose Agar (PSA)	20.00 b
Rice Flour Yeast Agar (RFYA)	19.33 b
Oat Meal Agar (OMA)	29.67 a
Potato-Rice straw Dextrose agar (PR_SDA)	15.00 c
$LSD_{(0.01)}$	4.360
Level of significance	0.01
CV(%)	8.60

The highest mycelial radial growth of *M. oryzae* (29.67 mm) was recorded on OMA followed by PDA, PSA, and RFYA (22.67, 20.00, and 19.33 mm and they were statistically similar. In contrast, the lowest mycelial radial growth of *M. oryzae* (15.00 mm) was observed in PR_SDA culture media (Table 7 and Figure 9).

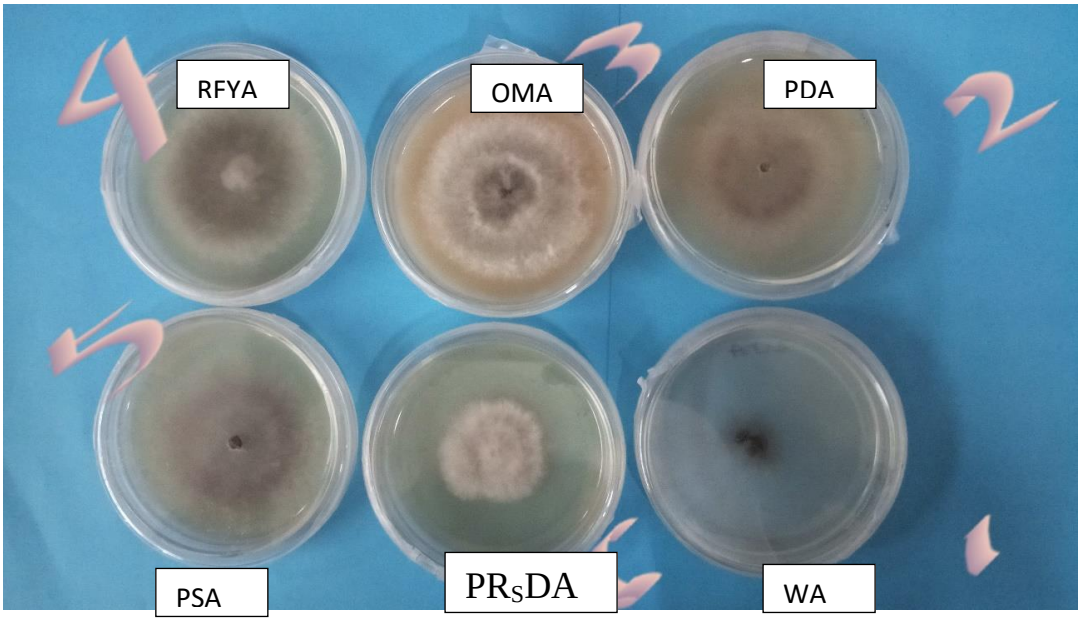


Figure 9. Growth of *Magnaporthe oryzae* on different growth media at 7 DAI

Kulmitra *et al*. (2017) also evaluated on different solid media viz., potato dextrose agar, potato carrot agar, Kirchoff's, medium, Richard's medium, Sabourad's medium, Takahashii's medium, rice leaf extract agar, and oatmeal agar and liquid media viz., potato dextrose broth, potato carrot broth, Kirchoff's broth, Richard's broth, Sabourad's dextrose broth, Takahashii's broth, and rice leaf extract broth. Among all the solid media, the highest mean mycelial growth of the fungus *Pyricularia oryzae* (Cav.) was recorded on oatmeal agar (77.6 mm) followed by rice leaf extract (75.9 mm) and least mean mycelial growth of the *P. oryzae* (Cav.) on Sabourad's media (44.7 mm) followed by Takahashi's media (52.5 mm). The maximum mean dry mycelial weight of fungus recorded in Kirchoff's broth (211.56 mg) followed by Richard's broth (206.3 mg) and the least mean dry mycelial weight of fungus recorded in Takahashii's broth (178.0 mg) followed by Sabourad's broth (179.7 mg). In general, among all solid media, the Oat Meal Agar media, and all liquid media, the Kirchoff's broth is more appropriate for the cultural study of rice blast fungus *P. oryzae* (Cav.). Nazifa *et al.*

(2021) evaluated five different growth media against rice blast caused by *Magnaporthe oryzae*. They showed that the highest mycelial growth was observed in Oat Meal Agar (20 mm) and lowest in Water Agar (10mm) at 7 DAI. The result is also supported by Aremu *et al.* (2019); Mior *et al.* (2017) and Gayatonde *et al.* (2016). Manjunatha and Krishnappa (2019) found that host extract agar (4.08 cm) had the highest mean mycelial growth of the fungus *Pyricularia oryzae*, followed by Oat meal agar (3.83 cm), and Richard's agar medium had the lowest mean mycelial growth of the *P. oryzae* (3.21cm). In general, the Potato Dextrose Agar media is better for cultural and morphological studies of the rice blast fungus *M. oryzae* than any other solid media.

4.6 Cultural and morphological characterization of *Magnaporthe oryzae* isolates

Statistically significant variation among the different isolates' colony characteristics was recorded in culture plates collected from different localities (Figure 10 and Table 8). The isolates KHDL03, KMSL05, MPCP06, MTKS08, MGML11, DSBL13, DNBS14, DKRP19, SSSL25, and MKKP26 were on the PDA medium Grayish Black color (Figure 10). The isolates KHAL01, KHMP02, KISL04, MGDL10, MNKL12, BDDS15, and BBCL24, were buff colors; on the other hand, the isolates of BKKL16, DBPL18, and PBSL20 were whitish Color. The black color was shown on the MPBS07, MBBL09, and CABL23 isolates (Table 8).

The smooth, cottony surface texture and the regular shape were found in the isolates KHAL01, KHDL03, KMSL05, MPCP06, MPBS07, MTKS08, MBBL09, MGDL10, MGML11, MNKL12, DSBL13, DNBS14, BKKL16, CRRL17, DKRP19, CCKL21, MGRL22, CABL23, BBCL24, SSSL25, and MKKP26. On the other hand, the isolates KHMP02, KISL04, BDDS15, DBPL18, and PBSL20, had a rough, velvety surface texture and irregular shape.

Good mycelial growth appeared in the isolates KHMP02, KISL04, MPCP06, MBBL09, MNKL12, DSBL13, BDDS15, DKRP19, PBSL20, SSSL25, and MKKP26. On the other hand, medium mycelial growth observed in the isolates KHAL01, KHDL03, KMSL05, MPBS07, MTKS08, MGML11, DNBS14, BKKL16, CRRL17, MGRL11, BBCL24, and poor mycelial growth recorded in the isolates MGDL10, DBPL18, CCKL21, and CABL23 simultaneously.

Mew and Gonzales (2002) stated that Mo isolates' colony color appeared grey on the PDA medium. Mew and Misra (1994) reported that the colonies of Mo isolates on PDA medium showed blackishly. Another 15 isolates of *Magnaporthe oryzae* from different villages/unions were cultured on Potato Dextrose Agar and Oat Meal Agar; their mycelial growth rate was recorded (Table 8 and 9). The effect of culture media on the growth and colony character of M. oryzae isolates was investigated using two different media, potato dextrose agar, and oatmeal agar. Results demonstrated significant heterogeneity among *M. oryza*e isolates in growth pattern, medium and mycelia color, texture, and sporulation (Jagadeesh and Devaki, 2020). On potato dextrose agar, morphological identification of the blast fungus revealed a grayish colony with a circular smooth edge and a concentric ring (Kulkarni & Peshwe, 2019) which was similar to my research. On different media, the fungus can be isolated. Isolation on Oat Meal Agar (OMA) revealed differences in the properties of the isolates. The colonies' colors ranged from brownish-black to dark grayish-green. They have darker colors than isolates cultivated on potato dextrose agar (PDA) (Mohammadpourlima *et al.*, 2017). Sahu *et al.* (2018) classified twenty isolates of *M. oryzae* into three categories according to colony color (grayish blackish, grayish, and white) and two groups according to colony texture (smooth and rough). The present investigation demonstrated considerable variation in mycelial color and texture between blast

isolates. Panda *et al.* (2017) classified twenty *M. oryzae* isolates into three categories according to colony color and smooth and rough colonies according to colony texture. The present study corroborated the findings of Srivastava *et al.* (2014), where the variation in colony color varied from buff to black color with rough and smooth colonies. Meena (2005) also demonstrated the blast isolates' grayish-black and raised mycelial growth. Gowrisri *et. al.,* (2019) indicates that the cultural characteristics of M. oryzae isolates vary greatly depending on their origin. This is contingent upon the lesions' age, varietal resistance, and the prevailing environmental circumstances. The colony color varied between light grey and white in six isolates. The culture's border was uneven to complete, and the coloring ranged from brown to black. The present study corroborated the findings of Mior *et al.* (2017), Srivastava *et al.* (2014), Longya *et al.* (2020), and Nazifa *et al.* (2021).

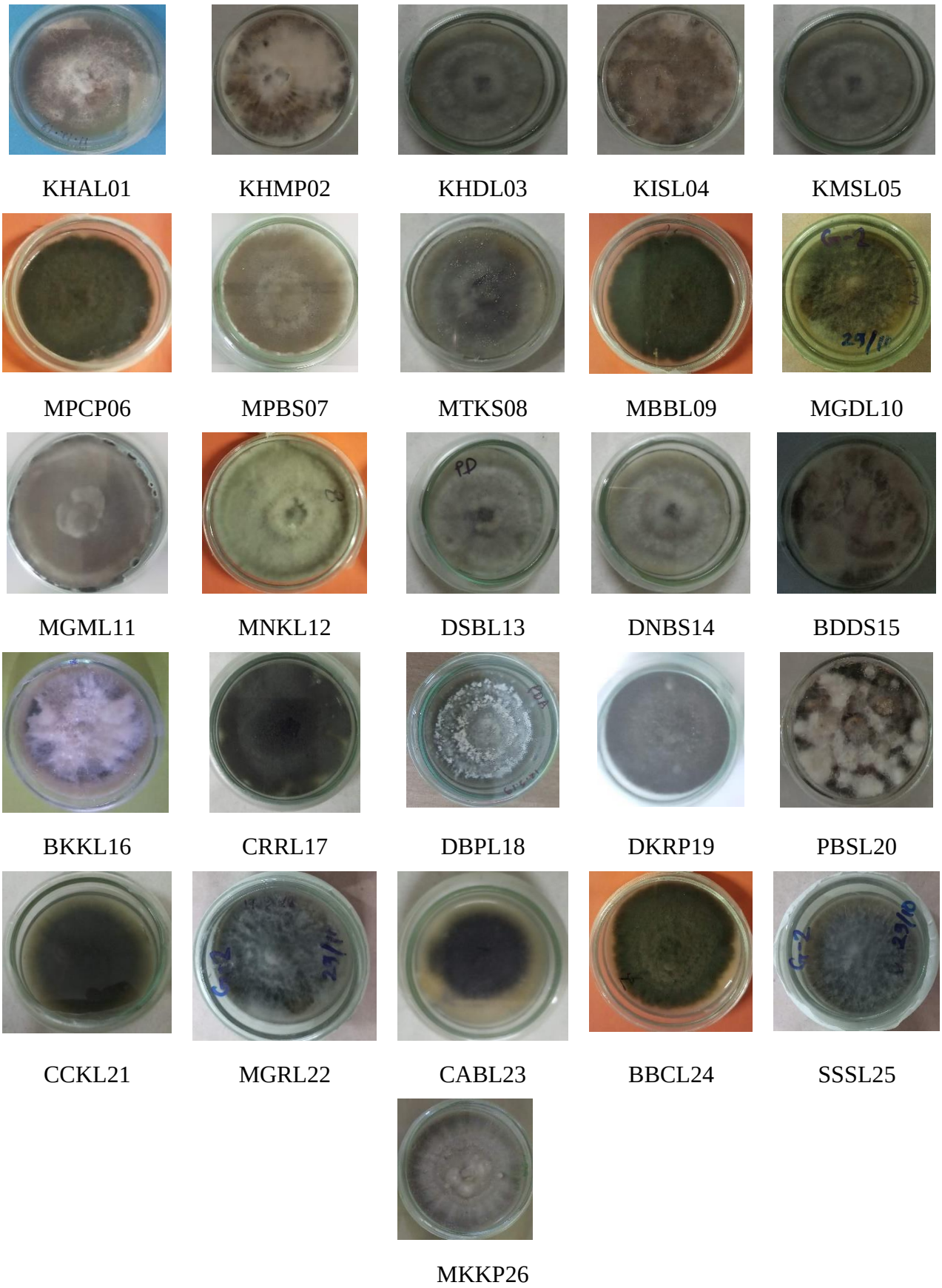


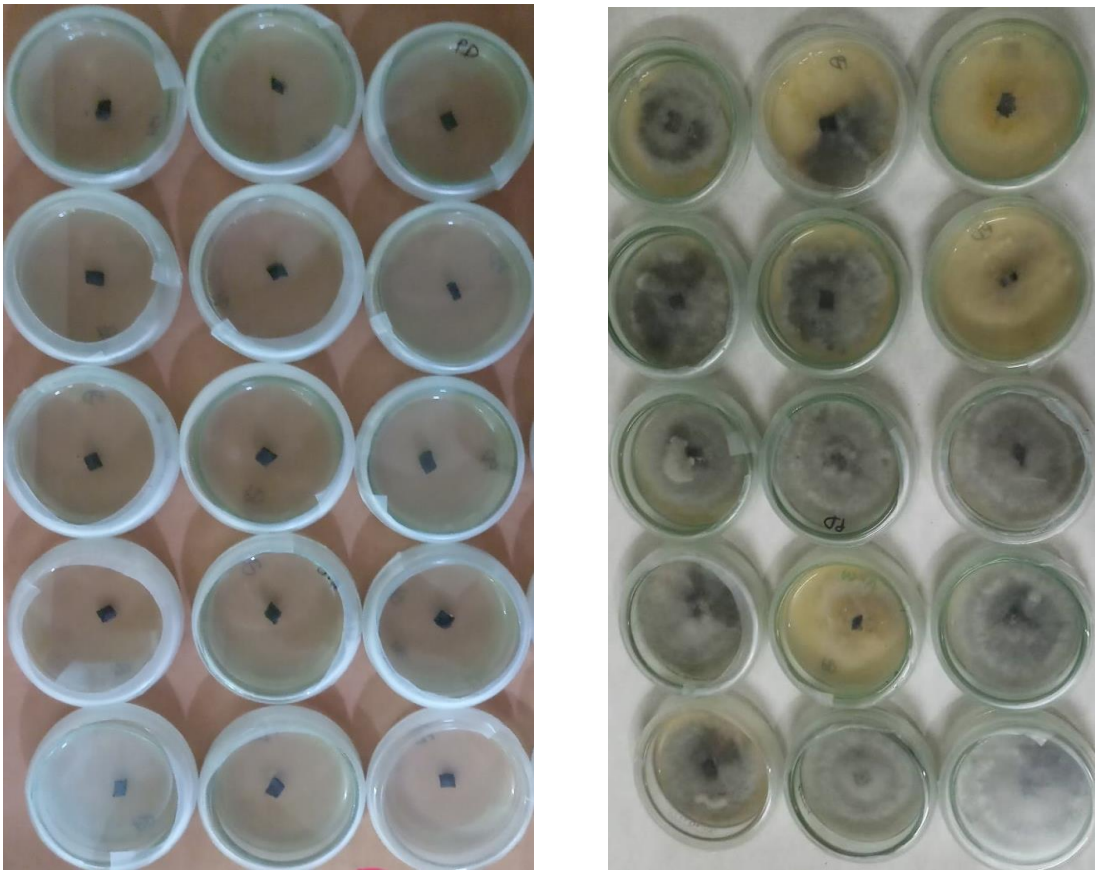
Figure 10. Mycelial growth of 26 isolates of *M. oryzae* on PDA media at 30 days after inoculation

Table 8. Cultural characteristics of different isolates of *Magnaporthe oryzae* collected from farmer's rice fields of different rice-growing regions of Bangladesh

Isolates	Sources of Isolates			**Colony**			Mycelial growth
	District	Upazila	Village/Union	Color	Surface Texture	Shape	
KHAL01	Kishoregonj	Hossainpur	Araibaria	Buff Color	Smooth, cottony	Regular	Medium growth
KHMP02			Madkhola	Buff Color	Rough, velvety	Irregular	Good growth
KHDL03			Dangri	Grayish Black	Smooth, cottony	Regular	Medium growth
KISL04		Itna	Sadar	Buff Color	Rough, velvety	Irregular	Good growth
KMSL05		Mitamoin	Sadar	Grayish Black	Smooth, cottony	Regular	Medium growth
MPCP06	Mymensingh	Phulpur	Chalk deula	Grayish Black	Smooth ,cottony	Regular	Good growth
MPBS07			Bahadurpur	Black Color	Smooth, cottony	Regular	Medium growth
MTKS08		Tarakanda	Kakni	Grayish Black	Smooth, cottony	Regular	Medium growth
MBBL09		Bhaluka	Birunia	Black Color	Smooth ,cottony	Regular	Good growth
MGDL10		Gafargaon	Datter bazar	Buff Color	Smooth, cottony	Regular	Poor growth
MGML11			Moshakhali	Grayish Black	Smooth, cottony	Regular	Medium growth
MNKL12	Netrokona	Kolmakanda	Kolmakanda	Buff Color	Smooth, cottony	Regular	Good growth
DSBL13	Dhaka	Savar	Birulia	Grayish Black	Smooth, cottony	Regular	Good growth
DNBS14		Narsingdi	Belabo	Grayish Black	Smooth, cottony	Regular	Medium growth
BDDS15	Bogura	Dupchachia	Dupchachia	Buff Color	Rough, velvety	Irregular	Good growth
BKKL16		Kahalu	Kalai	Whitish Color	Smooth, cottony	Regular	Medium growth
CRRL17	Cox's bazar	Ramu	Rajarkul	Black Color	Smooth, cottony	Regular	Medium growth
DBPL18	Dinajpur	Birganj	Palashbari	Whitish Color	Rough, velvety	Irregular	Poor growth
DKRP19		Kaharol	Rasulpur	Grayish Black	Smooth, cottony	Regular	Good growth
PBSL20	Panchagar	Buda	Sepaipara	Whitish Color	Rough, velvety	Irregular	Good growth
CCKL21	Cumilla	Chouddagram	Kalikapur	Blackish	Smooth, cottony	Regular	Poor growth
MGRL22	Meherpur	Gangni	Raipur	Grayish Black	Smooth, cottony	Regular	Medium growth
CABL23	Chuadanga	Alamdanga	Baradi	Black Color	Smooth, cottony	Regular	Poor growth
BBCL24	Barisal Sadar	Babuganj	Chadpasa	Buff Color	Smooth, cottony	Regular	Medium growth
SSSL25	Sunamganj	Satok	Satok sadar	Grayish Black	Smooth, cottony	Regular	Good growth
MKKP26	Moulvi Bazar	Kulaura	Kulaura	Grayish Black	Smooth, cottony	Irregular	Good growth

4.6.1 Radial Mycelial growth at 7 DAI and 14 DAI for 15 isolates on PDA plates

Average radial mycelial growth of collected total 15 isolates of *M. oryzae* was measured, and statistically significant variation was observed for 7 and 14 days after cultured in PDA plates at room temperature, and cultural characteristics are presented in Table 9. In 7 days after cultured, the longest redial mycelial (46.00 mm) was recorded from the isolate of KHAL and KHMP, while the shortest radial mycelial (35.00 mm) was observed from the isolate of KMSL. At 14 days after culture, the longest radial mycelial growth (82.50 mm) was found from the isolates of KHMP, KHML, and KHDL, whereas the shortest (61.00 mm) was from MPBS.



A B

Figure 11. Mycelial growth of *Magnaporthe oryzae* at A. Zero days B. at 7 days in PDA plates

Table 9. Growth of the isolates of *Magnaporthe oryzae* on PDA plates at 25^0C

Isolates	7 days growth (mm)	14 days growth (mm)	Colony Characters		
			Colony Color	Margin	Surface Texture
KHAL01	46.00 a	80.00 a	Buff Color	Irregular	Cottony
KHAL02	46.00 a	80.00 a	Buff Color	Entire	Cottony
KHMP03	46.00 a	82.50 a	Grayish Black	Entire	Cottony
KHML04	45.50 a	82.50 a	Buff Color	Irregular	Cottony
KHDL05	45.00 a	82.50 a	Grayish Black	Entire	Cottony
KISL06	40.00 ab	62.50 b	Black	Entire	Cottony
KISL07	41.00 ab	66.50 b	Black	Entire	Cottony
KISP08	36.00 b	66.00 b	Buff Color	Entire	Cottony
KMSL09	40.00 ab	66.00 b	Grayish Black	Irregular	Cottony
KMSL10	35.00 b	67.00 b	Buff Color	Entire	Cottony
MPCP11	35.50 b	66.00 b	Grayish Black	Irregular	Cottony
MPCS12	35.50 b	66.00 b	Grayish Black	Entire	Cottony
MPBS13	35.50 b	61.00 b	Buff Color	Irregular	Cottony
MTKS14	43.00 a	62.50 b	Grayish Black	Entire	Cottony
MTKS15	42.50 a	67.00 b	Buff Color	Irregular	Cottony
$LSD_{(0.01)}$	5.919	8.401			
Level of significance	0.01	0.01			
CV(%)	6.46	5.30			

4.6.2 Radial Mycelial growth at 7 and 14 days for 15 isolates on Oat Meal Agar plates

Average radial mycelial growth of collected total of 15 isolates of *M. oryzae* was measured, and statistically, significant variation was observed for 7 and 14 days after cultured in OMA plates at room temperature, and cultural characteristics are presented in Table 10. In 7 days after cultured, the longest redial mycelial (45.00 mm) was recorded from the isolate of KHAL, while the shortest redial mycelial (20.00 mm) was observed from the isolate of KHML and KISL. At 14 days after culture, the longest redial mycelial growth (86.00 mm) was found from the isolates of MTKS, whereas the shortest (47.50 mm) from KISL.

A **B**

Figure 12. Mycelial growth of *Magnaporthe oryzae*: A. Zero days B. at 7 days in Oat Meal Agar plates

Table 10. Growth of the isolates of *Magnaporthe oryzae* on Oat Meal Agar plates at 25^0C

Isolates	7 days growth (mm)	14 days growth (mm)	Colony characters		
			Colony color	Margin	Surface texture
KHAL01	45.00 a	60.00 bc	Black Color	Entire	Velvety
KHAL02	37.50 bc	60.00 bc	Buff Color	Entire	Velvety
KHMP03	37.50 bc	65.00 b	Buff Color	Entire	Velvety
KHML04	20.00 f	60.00 bc	Buff Color	Entire	Velvety
KHDL05	35.00 bc	61.00 bc	Grayish Black	Entire	Velvety
KISL06	28.50 de	80.00 a	Buff Color	Entire	Velvety
KISL07	20.00 f	47.50 d	Buff Color	Entire	Velvety
KISP08	25.00 ef	52.50 cd	Black Color	Entire	Velvety
KMSL09	37.50 bc	65.00 b	Grayish Black	Entire	Velvety
KMSL10	33.50 cd	60.00 bc	Buff Color	Entire	Velvety
MPCP11	36.50 bc	65.00 b	Buff Color	Entire	Velvety
MPCS12	37.50 bc	82.50 a	Grayish Black	Entire	Velvety
MPBS13	34.00 b-d	82.50 a	Buff Color	Entire	Velvety
MTKS14	36.00 bc	66.00 b	Black Color	Entire	Velvety
MTKS15	40.00 ab	86.00 a	Black Color	Entire	Velvety
$LSD_{(0.01)}$	5.439	8.831			
Level of significance	0.01	0.01			
CV(%)	7.22	5.94			

4.6.3 Radial Mycelial growth of 26 isolates of *M. oryzae* at 7 and 14 days after inoculation

Average radial mycelial growth of collected total 26 isolates of *M. oryzae* was measured, and statistically, significant variation was observed for 7 and 14 days after cultured in PDA media, and cultural characteristics are presented in Table 11. In 7 days after

cultured, the longest redial mycelial growth (51.50 mm) was recorded from the isolate collected from Sepaipara union under Boda Upazila of Panchagar district. In contrast, the shortest redial mycelial growth (32.00 mm) was observed from the isolate of Kolmakanda union under Kolmakanda Upazila of Netrokona district. At 14 days after culture, the longest radial mycelial growth (85.83 mm) was found from the isolates collected from Rasulpur union under Kaharol Upazila of Dinajpur, whereas the shortest radial mycelial growth (60.33 mm) of Birunia union under Bhaluka Upazila of Mymensingh district. Radial growth rates vary substantially depending on the growth medium and the sample source. Consequently, the isolates' growth rates differed significantly on these two growth media, and there were some variance in colony features (Mohammadpourlima *et al.,* 2017). The present study supported the findings of Gowrisri *et al.* (2019), where the radial mycelial growth rate of the six isolates differs substantially depending on their origin. Manjunatha and Krishnappa (2019) also demonstrated that among all solid media, the Potato Dextrose Agar media is more appropriate for cultural and morphological study of rice blast fungus *P. oryzae*. Similar results were found by Nazifa *et. al.* (2021). She found that the highest mycelial growth was observed in Oat Meal Agar (20 mm) and lowest in Water Agar (10mm) at 7 DAI among the five different growth media. The findings of our study were similar with others who studied on *Pyricularia oryzae* (Po) that was isolated from infected leaf and panicle and identified based on cultural characteristics and conidia morphology and recorded that mycelial growth of four Po isolates varied significantly with fair to excellent sporulation ability (Rayhanul *et al.,* 2019).

Table 11. Radial mycelial growth of different isolates of *Magnaporthe oryzae* collected from farmer's rice fields of different rice-growing regions of Bangladesh

Isolates	Radial Mycelial growth (mm)	
	7 DAI	14 DAI
KHAL01	42.83 a-c	77.00 a-d
KHMP02	44.33 a-c	82.50 ab
KHDL03	46.33 ab	84.83 a
KISL04	40.50 a-c	70.67 a-d
KMSL05	38.67 bc	72.67 a-d
MPCP06	38.83 bc	63.33 d
MPBS07	38.33 bc	62.00 d
MTKS08	42.17 a-c	64.83 cd
MBBL09	43.17 a-c	60.33 d
MGDL10	34.17 bc	62.50 d
MGML11	42.33 a-c	64.50 cd
MNKL12	32.00 c	62.83 d
DSBL13	40.00 a-c	60.83 d
DNBS14	42.17 a-c	65.83 b-d
BDDS15	37.17 bc	75.83 a-d
BKKL16	36.83 bc	70.50 a-d
CRRL17	37.33 bc	72.67 a-d
DBPL18	38.83 bc	60.67 d
DKRP19	46.33 ab	85.83 a
PBSL20	51.50 a	82.67 ab
CCKL21	45.17 ab	81.67 a-c
MGRL22	43.17 a-c	77.00 a-d
CABL23	42.33 a-c	61.33 d
BBCL24	39.67 a-c	65.83 b-d
SSSL25	41.50 a-c	71.67 a-d
MKKP26	42.83 a-c	73.33 a-d
$LSD_{(0.01)}$	10.40	14.65

Isolates	Radial Mycelial growth (mm)	
	7 DAI	14 DAI
Level of significance	0.01	0.01
CV(%)	11.59	9.51

4.7 Pathogenicity study of collected isolates

All the collected isolates were found pathogenic to susceptible rice variety US2. Rayhanul *et al.* (2019) used the pot culture technique to prove the pathogenicity of the test organism. The same procedure regarding the pathogenicity test was performed by Aslam *et. al.* 2019 for confirmation of virulent of *M. oryzae*. Pathogenicity assays confirmed that *M. oryzae* was the causal pathogen of blast disease. Nazifa *et. al.,* (2021) used the conidial suspension that was harvested, filtered, and centrifuged at 5000 rpm. The conidial spore suspension was sprayed on rice leaves cv. BRRI dhan28 and US2 in pots at the 3-4 leaf stage, days 20 and the seedlings were housed in a glasshouse at 25°C. Under *in vitro* conditions, sterile water was used instead of spore suspension as a control. After seven days of inoculation, seedlings were assessed. Gowrisri *et al. (2019)* conducted a pathogenity test with spore induction on maize stem bits, resulting in the maximum spore production quantity on the 15th day after inoculation. Panda *et al.* (2017) found that all the isolates were virulent on the HR12 cultivar, similar to my findings. Srivastava *et al.* (2014) found a similar result of the pathogenicity test that all the isolates were pathogenic and produced blast symptoms.

Figure 13. Pathogenicity test of collected isolates of *M. oryzae*. A and B: rice seedling after spraying spore suspension, C, D, and E: rice seedling covered with moistened polythene bag.

4.8 *In vitro* efficiency of chemicals against *Magnaporthe oryzae*

Different fungicides in different groups were tested *In-vitro* for estimated their efficiency against *Magnaporthe oryzae*, by employing a poisoned food technique and using Potato Dextrose Agar (PDA) as basal medium. The data was obtained as an effect of different fungicides *In-vitro* conditions on the vegetative growth and inhibitions of the pathogen at 5, 10, and 15 DAI, and significant variations were recorded in terms of mycelial growth (Table 12).

4.8.1 Effect of different fungicides on *in vitro* mycelial growth and % inhibition of mycelia growth of *Magnaporthe oryzae* at 5 days after inoculation

The mycelial growth and percent inoculation of *Magnaporthe oryzae* showed a different trend in response to different fungicides used. In vitro, the mycelial growth of *M. oryzae* was significantly different, shown in 5 DAI at different fungicidal treatments (Table 12). Among the 12 fungicides, maximum growth inhibition (100%) of *Magnaporthe oryzae* was found in Folicular 250 EC (Tebuconazole) (10%), Seltima (100 CS) [Pyraclostrobin (10%)], Filia (525 SE) [Propiconazole (12.5%) plus Tricyclazole (40%)] and Difar (300 EC) [Difenoconazole (15%) plus Propiconazole (15%)] which was significantly different and superior rest of the treatments (Table 12). The other fungicides according to their merit were Azonil (56 SC), Amister Top (250 SC), Dithane M-45, Trooper (75 WP), Blastin (75 DG), Knowing, Nativo (75 WP) with 87.51%, 85.00%, 73.50%, 68.01%, 66.51%, 62.50% and 60.00% inhibition of *Magnaporthe oryzae*, respectively compared to the control. The minimum inhibition was recorded in Autostin (50 WDG) (3.00%), the least effective fungicide for that test fungi. Mycelial growth (mm) at 5 days after inoculation (DAI) of fungicides were recorded in control treatment which was similar to Autostin (50 WDG), whereas no Mycelial growth was recorded for Folicular 250 EC, Seltima (100 CS), Filia (525 SE) and Difar (300 EC), Dithane M-45.

4.8.2 Effect of different fungicides on *in vitro* mycelial growth and % inhibition of mycelia growth of *Magnaporthe oryzae* at 10 days after inoculation

Mycelial growth and percent inoculation of *Magnaporthe oryzae* showed different trends in response to the different fungicides used. In vitro, the mycelial growth of *M. oryzae* was significantly different, shown in 10 DAI at different fungicidal treatments (Table 12). Among the 12 fungicides maximum growth inhibition (100%) of *Magnaporthe oryzae* was found in Folicular 250 EC (Tebuconazole) (10%), Seltima (100 CS) (Pyraclostrobin (10%), Filia (525 SE) (Propiconazole (12.5%) plus Tricyclazole (40%) and Difar (300 EC) (Difenoconazole (15%) plus Propiconazole (15%) which was significantly different and superior rest of the treatments (Table 12). The other fungicides according to their merit were Amister Top (250 SC), Blastin (75 DG), Azonil (56 SC), Dithane M-45, Nativo (75 WP), Trooper (75 WP), Knowing with 85.71%, 80.47, 70.00%, 69.04%, 58.10%, 62.39% and 46.19% inhibition of *Magnaporthe oryzae*, respectively compared to the control. The lowest inhibition was recorded in Autostin (50 WDG) (7.14%), the least effective fungicide for that test fungi. Mycelial growth (mm) at10 days after inoculation (DAI) of fungicides were recorded in control treatment which was similar to Autostin (50 WDG), whereas no Mycelial growth was recorded for Folicular 250 EC, Seltima (100 CS), Filia (525 SE) and Difar (300 EC), Dithane M-45.

4.8.3 Effect of different fungicides on *in vitro* mycelial growth and % inhibition of mycelia growth of *Magnaporthe oryzae* at 15 days after inoculation

Different fungicides showed significant differences in terms of radial mycelial growth and percent inhibition of *M. oryzae* at 15 DAI (Table 12). Among the 12 fungicides maximum growth inhibition (100%) of *Magnaporthe oryzae* was found in Folicular 250 EC (Tebuconazole) (10%), Seltima (100 CS) (Pyraclostrobin (10%), Filia (525 SE) (Propiconazole (12.5%) plus Tricyclazole (40%) and Difar (300 EC) (Difenoconazole (15%) plus Propiconazole (15%) which was significantly different and superior rest of

the treatments (Table 12). Amister Top (250 SC), Azonil (56 SC), Blastin (75 DG), Nativo (75 WP), Dithane M-45, Trooper (75 WP), Knowing inhibited 85.71%, 78.57%, 71.90%, 54.76%, 49.53%, 30.96% and 24.29% growth of *M. oryzae*, respectively compared to the control. On the other hand, Autostin (50 WDG) did not work against *M. oryzae* (0.00%) fungi. Mycelial growth (mm) at 15 days after inoculation (DAI) of fungicides were recorded in control treatment which was similar to Autostin (50 WDG), whereas no Mycelial growth was recorded for Folicular 250 EC, Seltima (100 CS), Filia (525 SE) and Difar (300 EC), Dithane M-45.

The present findings also confirmed the finding of Anwar *et al.* (2002) who reported that Mancozeb exhibited excellent control of rice blast disease caused by *M. oryzae*. Similarly, Gohel *et al.* (2008) evaluated 19 fungicides against *M. oryzae* and found Tricyclazole, Mancozeb, Carbendazim, Iprobenfos, Propiconazole, and Edifenphos were highly effective against the test fungus. Udayakumar *et al.* (2019) used a new combination molecule of Filia 525 SE (Tricyclazole 34.2% + Propiconazole 10.7%) against leaf and neck blast of rice under field condition followed by Fillia 525 SE @ 1.0 ml/l (72.80%), Tricyclazole 75 WP @ 0.8 g/l (71.02%). Raj and Pannu (2017) found that the fungicide Tricyclazole (Baan 75 WP) at 0.06% was most effective and provided disease control of 67.9%. Pramesh *et.al*. (2016) agreed that Pyraclostrobin 100 g/l CS (Seltima 100 g/l CS @ 75-100 g a.i./h can used for effective management of leaf blast diseases. Hegde (2015) also agreed to the statement that Tebuconazole @ 0.2% significantly reduced the blast (17.72%).

Table 12. Efficacy of different fungicides against in-vitro radial mycelial growth of *Magnaporthe oryzae*

Treatment	Mycelial growth (mm) at different days after innoculation (DAI) of fungicides					
	5 DAI		10 DAI		15 DAI	
	Mycelial growth (mm)	% Inhibition	Mycelial growth (mm)	% Inhibition	Mycelial growth (mm)	% Inhibition
Folicular 250 EC	0.00 f	100	0.00 i	100.00	0.00 h	100.00
Amister Top (250 SC)	10.00 e	85.00	10.00 h	85.71	10.00 g	85.71
Nativo (75 WP)	26.67 b	60.00	29.33 e	58.10	31.67 d	54.76
Trooper (75 WP)	21.33 cd	68.01	33.33 d	52.39	48.33 c	30.96
Blastin (75 DG)	22.33 c	66.51	13.67 g	80.47	19.67 e	71.90
Azonil (56 SC)	8.33 e	87.51	21.00 f	70.00	15.00 f	78.57
Seltima (100 CS)	0.00 f	100.00	0.00 i	100.00	0.00 h	100.00
Autostin (50 WDG)	64.67 a	3.00	65.00 ab	7.14	70.00 a	0.00
Filia (525 SE)	0.00 f	100.00	0.00 i	100.00	0.00 h	100.00
Difar (300 EC)	0.00 f	100.00	0.00 i	100.00	0.00 h	100.00
Dithane M-45	17.67 d	73.50	21.67 f	69.04	35.33 d	49.53
Knowing	25.00 bc	62.50	37.67 c	46.19	53.00 b	24.29
Control	66.67 a	0.00	70.00 a	0.00	70.00 a	0.00
$LSD_{(0.01)}$	3.687		3.249		4.596	
Level of significance	0.01		0.01		0.01	
CV(%)	8.04		6.17		7.46	

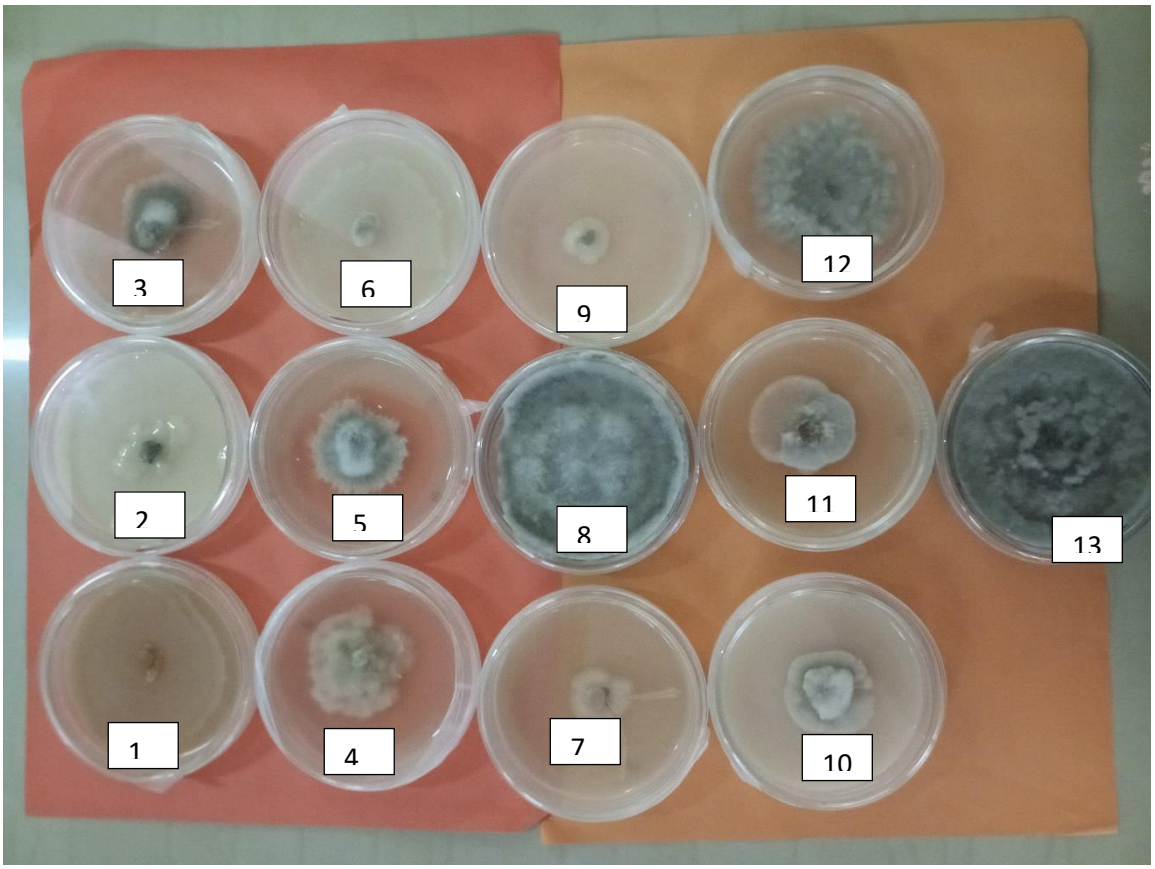


Figure 14. Efficacy of fungicides in controlling radial mycelial growth of *M. oryzae* at 15 DAI

The present study is similar to the findings of Wasimfiroz *et al.* (2018) where seven fungicides were evaluated. Among the fungicides, Carbendazim and Propiconazole showed inhibition of 84.07 percent and 74.25 percent, respectively, at 500 ppm. Sharma *et al.* (2018) demonstrated that the disease could be effectively managed with three sprays of tebuconazole + trifloxystrobin (Nativo) or propiconazole (Tilt). The fungicides chlorothalonil, tricyclazole, hexaconazole, kasugamycin, benomyl, carbendazim, tebuconazole + trifloxystrobin, and propiconazole have been reported to manage blast disease in rice crops effectively (Hegde *et al.*, 2000; Kumbhar, 2005; Groth, 2006; Ghazanfar *et al.*, 2009; Narayana Swamy *et al.*, 2009; Vahid *et al.*, 2011; Dutta *et al.*, 2012; Ganesh Naik *et al.*, 2012). Development of resistance to

carbendazim has been reported in the rice blast fungus *M. grisea* (Mohammad *et al.,* 2011). Similarly, another fungicide, tricyclazole, which is reported to be very effective against rice blast (Sood and Kapoor, 1997; Prajapati *et al.,* 2004; Kunova *et al.,* 2013) and pearl millet blast in Gujarat (Joshi and Gohel, 2015) was also ineffective against pearl millet blast. Differential sensitivity to tricyclazole in the rice blast isolates collected from different areas in China has also been reported (Yuan and Yang, 2003). Among five fungicides *viz*., Thiophanate-methyl, Carbendazim, Fosetyl-aluminium, Mancozeb and Copper oxychloride, used against the *Magnaporthe oryzae*, only Mancozeb appeared as the highly effective fungicide that completely inhibited the mycelial growth of the fungus(Khanzada & Shah, 2012). Different workers studied a wide range of fungicides and found many effective for rice blast fungus, *M. oryzae* (Verma *et al.*, 1985; Misra & Dharam, 1990; Prajapati *et al*., 2004). Our findings confirm those reported by Aslam *et al.* (2019), who observed that Difenoconazole is the most promising fungicide for the management of rice blast *M. oryzae*. Similarly, Gohel *et al*., (2008) evaluated 19 fungicides against *M. oryzae* and found that Tricyclazole, Mancozeb, Carbendazim, Iprobenfos, Propiconazole and Edifenphos were highly effective against the test fungus. These results agree with those reported by (Rayhanul *et al.,* 2019; Hossain and Kulkarni, 2001; Bhojyanaik and Jamadar, 2014). Surapu *et al.,* (2017), revealed that no resistance was developed at optimal doses for Tricyclazole and Carbendazim. Kavanashree *et al.,* (2019), disclosed that tricyclazole + tebuconazole (36% SC), tebuconazole 25% SC, hexaconazole 5% EC, zineb 68% + hexaconazole 4% WP and tebuconazole 50% + trifloxystrobin 25% WG completely inhibited the growth of fungus and germination of fungal spores in all concentration when compared with the control which was similar to Kongcharoen *et al.,* 2020; Kunova *et al.,* 2013 and our research.

4.9 Efficacy of botanicals in controlling radial mycelial growth of *Magnaporthe oryzae in vitro*

There were eight botanical extracts from different families, namely Kalijira (*Nigella sativa*), Turmeric (*Curcuma longa*), Ginger (*Zingber officinale*), Garlic (*Allium sativum*), Onion (*Allium cepa*), Neem (*Azadirachta indica*), Allamanda (*Allamanda cathertica L*.) Aloe vera (*Aloe vera*) for controlling the rice blast pathogen (*Magnaporthe oryzae) in-vitro* by poisoned food technique method (Nene and Thapliyal, 1979).

4.10 *In vitro* efficiency of botanicals with ethanol against *Magnaporthe oryzae*

Different botanicals were tested *In-vitro* with ethanol for estimated their efficiency against *Magnaporthe oryzae*, by employing poisoned food technique and using Potato Dextrose Agar (PDA) as basal medium. The data obtained as an effect of different botanical in *In-vitro* condition on the vegetative growth and inhibitions of the pathogen at 7 and 14 DAI and significant variation was recorded in terms of mycelial growth in the concentration of 1:1, 1:2, and 1:4 ml with ethanol (Table 13; 14; 15 and 16). The different concentration was 1:4 (w/v) = 25 g botanicals in 100 ml ethanol, 1:2 (w/v) = 50 g botanicals in 100 ml ethanol, 1:1 (w/v) = 1000 g botanicals with 100 ml ethanol.

4.11 *In vitro* efficiency of botanical with ethanol against *Magnaporthe oryzae* at 7 DAI inoculation.

The antimicrobial efficacy of eight botanicals with a particular concentration was assessed, and the findings were presented in Table 13. The result shows that botanicals were important in suppressing mycelia growth at higher concentrations over untreated regulation of fungal pathogens. A significant difference was found in In-vitro mycelial growth of *Magnaporthe oryzae*, on different botanical treatments with ethanol extracts at 7 days after inoculation. The result showed that minimum mycelial growth and

maximum growth inhibition of *M. oryzae* were recorded in Neem (*Azadirachta indica*), Allamanda (*Allamanda cathertica L.*) Aloe vera (*Aloe vera*) in all concentrations was significantly different and superior to the rest of the botanical treatment (Figure 16). The rest of the botanicals with their competence Garlic (*Allium sativum*) 1:2 (w/v), 1:4 (w/v) with 92% growth inhibition compared to control. The next botanicals in order to their capability were garlic 1:1 (w/v), turmeric 1:4 (w/v), ginger 1:2 (w/v), 1:4 (w/v) with 88%, 84%, 80% 80% mycelial growth inhibition respectively of the test fungus as compared to control. Kalijira, turmeric, and onion extract failed to suppress the radial mycelial growth of the test fungus with all concentrations. Control plates of all concentrations were always observed the highest radial mycelial growth and showed the lowest percent inhibition (Table 13)

4.12 *In vitro* efficiency of botanicals with ethanol against *Magnaporthe oryzae* at 14 DAI inoculation.

The antimicrobial efficacy of eight botanicals with a particular concentration was assessed, and the findings were presented in Table 13. The result shows that botanicals were essential in suppressing mycelia growth at higher concentrations over untreated regulation of fungal pathogens. A significant difference was found in vitro mycelial growth of *Magnaporthe oryzae*, on different botanical treatments with ethanol extracts at 14 days after inoculation. The result showed that minimum mycelial growth and maximum growth inhibition of *M. oryzae* were recorded in Aloe vera (*Aloe vera*) Neem (*Azadirachta indica*) in all of their concentration which was significantly different and superior to the rest of the botanical treatment (Figure 16). The rest of the botanicals with their competence Alamanda (*Allium sativum*) 1:4 (w/v) with 66.67% growth inhibition compared to control. The following botanicals to their capability were garlic 1:2 (w/v), Alamanda 1:2 (w/v) 63.33%, 51.67%, mycelial growth inhibition of the test

fungus compared to control. Kalijira, onion, turmeric, and ginger extract showed the least percent inhibition of the test fungus with all concentrations. Control plates of all concentrations always observed the highest radial mycelial growth and lowest percent inhibition (Table 13). The highest concentration of botanical extracts was more pronounced than the low concentration in reducing the fungus radial growth found in blast pathogens. The mycelial growth of *M. oryzae* decreases with an increasing concentration of all the botanical extracts tested compared to control. The findings are similar to Amadioha (2000), who reported that the cold-water extract of neem compared favorably with Cardendazim at 0.1 percent a.i. in controlling the rice blast *in-vivo*. In an experiment (Sireesha and Venkateswarlu, 2013), plant parts extracted from Neem seed kernel, Neem oil, and Pongamia spp effectively control fungal growth. Sandeep (2015) found a familiar pattern of growth suppression in blast and brown spot pathogens using leaf extracts of Neem (*Azadirachta indi*ca), Emblica (*Emblica officinalis*), Karanj (*Pongamia glabra*), and Babool (*Pongamia glabra*) (*Acacia nilotica*). Panchagavya and Asafoetida spp. extracts were tested against rice blast fungus in descending order. The mycelial development of *M. oryzae* was inhibited by a higher dose of garlic, according to Hajano *et al*. (2012). Neem leaf extract was found to be effective but not as effective as common fungicides and bio-agents in reducing leaf blast strength in rice, according to Gohel and Chauhan (2015). Hubert *et al*. (2015) observed that extracts from *C. arabica, N. tabacum, A. vera,* and *A. indica* were significant in managing in-vitro and in-vivo rice blast disease. According to Khoa *et al*. (2011), it was revealed that the foliar sprays of aqueous extracts of herbal plants successfully minimize rice blast intensity. The suppression of *M. oryzae* was significant at varying concentrations of plant extracts. When added at the highest concentration, Al-Hazmi (2013) found that Neem leaf extract was most effective in inhibiting the

growth of *Helminthosporium sp*. fungi (1:1, v/v) according to Pandey (2018), spraying Achook, Neem Azal T/S. Neem gold and tricure show a substantial reduction in disease severity against rice blast and enhance yield attributes, such as increasing the 100-grain weight and grain yield. Rajappan *et al*. (2001) found that neem extract inhibited *M. oryzae* mycelial development. Water and leaf extracts of seed (*Azadirachta indica*) have decreased the radial growth of *M. grisea* mycelium in vitro and the production and spread of blast disease in a greenhouse, according to Amadioha (2000). This result agrees with Nazifa *et al.* (2021), who reported that all the botanicals significantly reduced the radial growth of the tested pathogen. Maximum mycelia growth inhibition of MoO was achieved with water extract of turmeric (1:1 w/v) and ethanol extracts of neem (1:4 w/v) with 86.57% and 92.62% mycelia growth inhibition at 14 DAI, respectively. Agbowuro *et al.* (2020) also reported similar results with aqueous extracts of Apple of Sodom (*Calotropis procera*), Neem tree (*Azadirachta indica*), Thorn Apple/Angel's trumpet (*Datura metel*), Aleo plant (*Aleo vera*) and Siam weed (*Chromolaena odorata*)) at different concentrations (25, 50, and 100%) against rice blast disease (*Magnaporthe oryzae*) in-vitro and in-vivo for the control of rice blast disease (*Magnaporthe oryzae*) *in-vitro* and on the field. Hubert *et al*. (2015) also reported similar results with aqueous extracts of *Aloe vera, Azadirachta indica,* and *Datura stramonium* to control rice blast disease (*Magnaporthe oryzae*) *in-vitro* and on the field. Pandey (2014) reported a similar result on the effectiveness of plant extracts on rice blast disease when treated. Among the botanicals, the spraying of Achook, Neem Azal T/S, Neem gold, and Tricure shows a significant reduction in disease severity and improves yield attributes, increasing the 100-grain weight and grain yield. The results were supported with findings of Wasimfiroz *et al.* (2021), who reported that the extracts of *Lantana camara* (55.80%), Neem (53.06%), and Nilgiri (51.83%)

were showed maximum inhibition of pathogen at higher concentrations. The results are also similar to the findings of Kulmitra and Sanathkumar (2017), who reported the maximum inhibition of growth of *P. oryzae* in Neem (41.53%) and Eucalyptus (42.80%) at 15% concentration (Kumar et al., 2017). Jamal-U-Ddin *et al*. (2012) Reported that a higher concentration of neem extract was highly influential in checking the mycelial growth of *P. oryzae*. Chakraborty *et al.* (2021); Abed-Ashtiani *et al.* (2018), and Taiga and Friday (2009) reported that various secondary metabolites, such as alkaloids, phenolics, and terpenoids of plant and microbial origin, significantly inhibit fungal growth and may also effectively manage blast diseases. Standard modes of action of microbial biocontrol agents include antibiosis, production of lytic enzymes, induction of systemic resistance in the host plant, and competition for nutrients or space. Sahu *et al.* (2018) reported that the maximum percent inhibition of mycelial growth was recorded T1-Neem *(Azadirachta indica)* (57.48%), T2-Tulsi (leaf) (45.00%), as compared to control (00.00%).

Table 13. Efficacy of ethanol extracts of botanicals against radial mycelial growth of *Magnaporthe oryzae in vitro*

Botanicals	Treatment concentration (w/v)	7 DAI		14 DAI	
		Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
Kalijira	1:1	20.00 e	60.00	56.00 ab	6.67
	1:2	38.00 c	24.00	56.00 ab	6.67
	1:4	38.00 c	24.00	50.00 c	16.67
Turmeric	1:1	42.00 b	16.00	53.00 bc	11.67
	1:2	18.00 e	64.00	40.00 e	33.33
	1:4	8.00 fg	84.00	32.50 f	45.83
Ginger	1:1	31.00 d	38.00	53.00 bc	11.67
	1:2	10.00 f	80.00	53.00 bc	11.67
	1:4	10.00 f	80.00	39.00 e	35.00
Garlic	1:1	6.00 gh	88.00	45.00 d	25.00
	1:2	4.00 h	92.00	22.00 g	63.33
	1:4	4.00 h	92.00	39.00 e	35.00
Onion	1:1	38.00 c	24.00	55.00 b	8.33
	1:2	30.00 d	40.00	50.00 c	16.67
	1:4	18.00 e	64.00	45.00 d	25.00
Neem	1:1	0.00 i	100.00	6.00 h	90.00
	1:2	0.00 i	100.00	7.00 h	88.33
	1:4	0.00 i	100.00	5.00 h	91.67
Allamanda	1:1	0.00 i	100.00	55.00 b	8.33
	1:2	0.00 i	100.00	29.00 f	51.67
	1:4	0.00 i	100.00	20.00 g	66.67
Aloe Vera	1:1	0.00 i	100.00	6.00 h	90.00
	1:2	0.00 i	100.00	4.00 h	93.33
	1:4	0.00 i	100.00	4.00 h	93.33
Control		50.00 a	--	60.00 a	--
$LSD_{(0.01)}$		2.382	--	4.180	--
Level of significance		0.01	--	0.01	--
CV(%)		6.36	--	5.15	--

DAI= Days After Inoculation

4.13 *In vitro* mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 100% botanical with ethanol extract

All the eight botanicals mixed with ethanol in 1:1 (w/v) were tested against the pathogen 7 and 14 days after inoculation. All botanicals were found significantly effective in reducing the mycelial growth of the pathogens compared to control. The result revealed that neem, Alamanda, and Aloe vera were performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after inoculation, which is 0.00 mm radial mycelial growth and 100% growth inhibition. The maximum radial mycelial growth was found in turmeric (42.00 mm) compared to control (50.00 mm) at 7 days after inoculation.

Among the eight botanicals mixed with ethanol in 1:1 (w/v) at 14 DAI, the highest radial mycelial growth was found in Kalijira (56.00 mm) compared to the control. On the other hand, no significant difference was found among Kalijira (56.00 mm), Turmeric (53.00 mm), Ginger (53.00 mm), Onion (55.00 mm), and Alamanda (55.00 mm) compared to control at 14 DAI of test fungus (Table 14).

Table 14. Efficacy of botanicals with ethanol (1:1 w/v) againsti *in vitro* mycelial radial growth of *Magnaporthe oryzae*

Treatment	Treatment concentration (w/v)	7 DAI		14 DAI	
		Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
Kalijira	1:1	20.00 e	60.00	56.00 a	6.67
Turmeric	1:1	42.00 b	16.00	53.00 a	11.67
Ginger	1:1	31.00 d	38.00	53.00 a	11.67
Garlic	1:1	6.00 f	88.00	45.00 b	25.00
Onion	1:1	38.00 c	24.00	55.00 a	8.33
Neem	1:1	0.00 g	100.00	6.00 c	90.00
Allamanda	1:1	0.00 g	100.00	55.00 a	8.33
Aloe Vera	1:1	0.00 g	100.00	6.00 c	90.00
Control		50 a	--	60.00 a	--
$LSD_{(0.01)}$		2.073	--	6.460	--
Level of significance		0.01	--	0.01	--
CV(%)		4.25	--	6.36	--

DAI= Days After Inoculation

4.14 *In vitro* mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 50% botanical with ethanol extract

All the eight botanicals mixed with ethanol in 1:2 (w/v) were tested against the pathogen 7 and 14 days after inoculation. All botanicals were found significantly effective in reducing the mycelial growth of the pathogens compared to control. The result revealed that neem, Alamanda, and Aloe vera were performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after inoculation, which is 0.00 mm radial mycelial growth and 100% growth inhibition. The maximum radial mycelial growth was found in Kalijira (38.00 mm) compared to control (50.00 mm) at 7 days after inoculation.

Among the eight botanicals mixed with ethanol in 1:2 (w/v) at 14 DAI, the highest mycelial radial growth was also found in Kalijira (56.00 mm) compared to control (60.00mm). On the other hand, the minimum mycelial radial growth was also found in Aloe vera (4.00 mm), which is 93.33% of % growth inhibition. (Table 15).

Table 15. Efficacy of botanicals with ethanol (1:2 w/v) against *in vitro* mycelial radial growth of *Magnaporthe oryzae*

Treatment	Treatment concentration (w/v)	7 DAI		14 DAI	
		Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
Kalijira	1:2	38.00 b	24.00	56.00 ab	6.67
Turmeric	1:2	18.00 d	64.00	40.00 d	33.33
Ginger	1:2	10.00 e	80.00	53.00 bc	11.67
Garlic	1:2	4.00 f	92.00	22.00 f	63.33
Onion	1:2	30.00 c	40.00	50.00 c	16.67
Neem	1:2	0.00 g	100.00	7.00 g	88.33
Allamanda	1:2	0.00 g	100.00	29.00 e	51.67
Aloe Vera	1:2	0.00 g	100.00	4.00 g	93.33
Control		50.00 a	--	60.00 a	--
$LSD_{(0.01)}$		3.415	--	4.954	--
Level of significance		0.01	--	0.01	--
CV(%)		8.74	--	5.91	--

DAI= Days After Inoculation

4.15 *In vitro* mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 25% botanical with ethanol extract

All the eight botanicals mixed with ethanol in 1:4 (w/v) were tested against the pathogen at 7 and 14 days after inoculation. All botanicals were found significantly effective in reducing the mycelial growth of the pathogens compared to control. The result showed that neem, Alamanda, and Aloe vera were performed best and significantly inhibited the radial mycelial growth of *M. oryzae* at 14 days after inoculation, which is 0.00 mm radial mycelial growth 100% growth inhibition. The maximum radial mycelial growth was found in Kalijira (38.00 mm) compared to control (50.00 mm) at 7 days after inoculation.

Among the eight botanicals mixed with ethanol in 1:4 (w/v) at 14 DAI, the highest mycelial radial growth was also found in Kalijira (50.00 mm) compared to control

(60.00mm). On the other hand, the minimum mycelial radial growth was also found in Aloe vera (4.00 mm), which is 93.33% of % growth inhibition. (Table 16).

Table 16. Efficacy of botanicals with ethanol (1:4 w/v) against *in vitro* mycelial radial growth of *Magnaporthe oryzae*

Treatment	Treatment concentration (w/v)	7 DAI		14 DAI	
		Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
Kalijira	1:4	38.00 b	24.00	50.00 b	16.67
Turmeric	1:4	8.00 d	84.00	32.50 e	45.83
Ginger	1:4	10.00 d	80.00	39.00 d	35.00
Garlic	1:4	4.00 e	92.00	39.00 d	35.00
Onion	1:4	18.00 c	64.00	45.00 c	25.00
Neem	1:4	0.00 f	100.00	5.00 g	91.67
Allamanda	1:4	0.00 f	100.00	20.00 f	66.67
Aloe Vera	1:4	0.00 f	100.00	4.00 g	93.33
Control	1:4	50.00 a	--	60.00 a	--
$LSD_{(0.01)}$		2.598	--	4.071	--
Level of significance		0.01	--	0.01	--
CV(%)		7.79	--	5.29	--

DAI= Days After Inoculation

4.16 *In vitro* efficiency of water extract of botanicals against *Magnaporthe oryzae*

Different botanicals were tested *In-vitro* with water for estimated their efficiency against *Magnaporthe oryzae*, by employing a poisoned food technique and using Potato Dextrose Agar (PDA) as basal medium. The data obtained as an effect of different botanicals in *in vitro* condition on the vegetative growth and inhibitions of the pathogen at 7 and 14 DAI. Significant variation was recorded in mycelial growth in the different water concentrations (Table 17; 18; 19 and 20).

The highest radial mycelial growth was recorded in a control condition in 7 and 14 DAI for all the concentrations. At 7 DAI, no radial mycelial growth was recorded for Neem and Allamanda botanicals in all concentrations with water. Similarly, at 14 DAI, the

lowest radial mycelial growth was recorded for both the Kalijira, Ginger, Neem, and Aloe Vera botanicals in all concentrations with water.

4.17 *In vitro* efficiency of water extract of botanical against *Magnaporthe oryzae* at 7 DAI inoculation.

The antimicrobial efficacy of eight botanicals with a particular concentration was assessed, and the findings were presented in Table 17. The result shows that botanicals were essential in suppressing mycelia growth at higher concentrations over untreated regulation of fungal pathogens. A significant difference was found *in vitro* mycelial growth of *Magnaporthe oryzae*, on different botanical treatments with water extracts 7 days after inoculation. The result showed that minimum mycelial growth and maximum percent growth inhibition of *M. oryzae* were recorded in Kalijira, Neem (*Azadirachta indica*), and Allamanda (*Allamanda cathertica L*.) with the concentration of 1:4 (w/v), 1:1 (w/v) and 1:1 (w/v) and 1:4 (w/v) simultaneously. The rest of the botanicals with their competence Garlic (*Allium sativum*) 1:1 (w/v), 1:4 (w/v) with 88.89% growth inhibition compared to control. The following botanicals to their capability were ginger 1:1 (w/v) with 84.44% mycelial percent growth inhibition respectively of the test fungus compared to control. Turmeric, Aloe vera, and onion extract failed to suppress the radial mycelial growth of the test fungus with all concentrations. Control plates of all concentrations were always observed the highest radial mycelial growth and showed the lowest percent inhibition (Table 17).

4.18 *In* vitro efficiency of water extract of botanical against *Magnaporthe oryzae* at 14 DAI inoculation.

The antimicrobial efficacy of eight botanicals with a particular concentration was assessed, and the findings were presented in Table 17. The result showed that botanicals are important in suppressing mycelia growth at higher concentrations over

untreated regulation of fungal pathogens. A significant difference was found in vitro mycelial growth of *Magnaporthe oryzae*, on different botanical treatments with ethanol extracts at 14 days after inoculation. The result showed that minimum mycelial growth and maximum percent growth inhibition of *M. oryzae* were recorded in Kalijira 1:4 (w/v) (87.59%) Ginger 1:1 (w/v) (87.59%), which was significantly different and superior to the rest of the botanical treatment (Figure 20). The rest of the botanicals with their competence Garlic 1:1 (w/v) (87.59%), Garlic 1:4 (w/v) (84.83%), Neem 1:1 (w/v) (86.21%), Neem 1:4 (w/v) (55.17%), Alamanda (*Allium sativum*) 1:4 (w/v) 1:4 (w/v) with 86.21% growth inhibition compared to control. Onion, turmeric, and Aloe vera extract showed the least percent inhibition of the test fungus with all concentrations. Control plates of all concentrations always observed the highest radial mycelial growth and lowest percent inhibition (Table 17).

Table 17. Efficacy of different concentrations of botanicals (with water) against *in vitro mycelial radial growth of Magnaporthe oryzae*

Treatment	Treatment concentration (w/v)	7 DAI		14 DAI	
		Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
Kalijira	1:1	23.00 e	48.89	52.00 c	28.28
	1:2	25.00 e	44.44	30.00 e	58.62
	1:4	0.00 i	100.00	9.00 g	87.59
Turmeric	1:1	24.50 e	45.56	50.00 c	31.03
	1:2	20.00 f	55.56	30.00 e	58.62
	1:4	20.00 f	55.56	30.00 e	58.62
Ginger	1:1	7.00 h	84.44	9.00 g	87.59
	1:2	25.50 e	43.33	30.50 e	58.62
	1:4	24.00 e	46.67	30.00 e	58.62
Garlic	1:1	5.00 h	88.89	10.00 g	86.21
	1:2	9.50 g	78.89	18.00 f	75.17
	1:4	5.00 h	88.89	11.00 g	84.83
Onion	1:1	25.00 e	44.44	30.00 e	58.62
	1:2	25.50 e	43.33	35.00 d	51.72
	1:4	23.50 e	47.78	30.00 e	58.62
Neem	1:1	0.00 i	100.00	10.00 g	86.21
	1:2	20.50 f	55.44	30.50 e	58.62
	1:4	25.50 e	43.33	32.50 de	55.17
Allamanda	1:1	0.00 i	100.00	10.00 g	86.21
	1:2	30.50 d	32.22	35.00 d	51.72
	1:4	0.00 i	100.00	10.00 g	86.21
Aloe Vera	1:1	20.00 f	55.56	30.00 e	58.62
	1:2	20.00 f	55.56	35.50 d	51.03
	1:4	25.00 e	44.44	32.50 de	55.17
Control		45.00 a	--	72.50 a	--
$LSD_{(0.01)}$		2.382	--	3.356	--
Level of significance		0.01	--	0.01	--
CV(%)		5.47	--	4.89	--

DAI= Days After Inoculation

Curcumin, a polyphenolic compound derived from turmeric and turmeric oil, is antifungal, so turmeric powder can be used as an antifungal agent in its raw form

(Prajapati *et al*. 2021). According to Kim *et al*. (2003), the fungicidal behavior of turmeric rhizome-derived materials was checked using an in vivo whole-plant approach *against Botrytis cinerea, Erysiphe graminis, Phytophthora infestans, Puccinia recondita, Pyricularia oryzae,* and *Rhizoctonia solani*. The present study were in line with Damalas (2011), who investigated the satisfactory potential of turmeric as a natural pesticide for possible use in crop protection and thus a promising future in this direction, that is, the possibility of effective control of some agriculturally important pests using turmeric products as a less expensive and more environmentally friendly alternative to chemical pesticides. Sukanya *et al*. (2011) isolated and examined the essential oil and oleoresin from *Piper nigrum, Coriander sativum,* and *Curcurma demestica* on *Magnaporthae oryzae*. The present findings are also consistent with those of Punja (2005) and Slusarenko *et al*. (2008), who discovered that garlic juice and the compound obtained from it (Allicin) were highly effective against the rice blast fungus M. oryzae. Allicin successfully blocked the development and infection of M. oryzae, according to Fry *et al*. (2005). Amadioha (2000) and Rajappan *et al*. (2001) also reported that neem extract reduced the mycelial growth of *M. oryzae.*

4.19 *In vitro* mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 100% botanical with water extract

All the eight botanicals mixed with ethanol in 1:1 (w/v) were tested against the pathogen 7 and 14 days after inoculation. All botanicals were found significantly effective in reducing the mycelial growth of the pathogens compared to control. The result revealed that neem and Alamanda were performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after inoculation, which was 0.00 mm radial mycelial growth and 100% growth

inhibition. The maximum radial mycelial growth was found in Onion (25.00 mm) compared to control (45.00 mm) at 7 days after inoculation.

Among the eight botanicals mixed with ethanol in 1:1 (w/v) at 14 DAI, the highest mycelial radial growth was found in Kalijira (52 mm) compared to control (72.50 mm). On the other hand, no significant difference was found among Kalijira (52.00 mm) and Turmeric (50.00 mm), compared to control at 14 DAI of test fungus (Table 18).

Table 18. Efficacy of water extract of botanicals (1:1 w/v) against *in vitro* radial mycelial growth of *Magnaporthe oryzae*

Treatment	Treatment concentration (w/v)	7 DAI		14 DAI	
		Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
Kalijira	1:1	23.00 b	48.89	52.00 b	28.28
Turmeric	1:1	24.50 b	45.56	50.00 b	31.03
Ginger	1:1	7.00 d	84.44	9.00 d	87.59
Garlic	1:1	5.00 d	88.89	10.00 d	86.21
Onion	1:1	25.00 b	44.44	30.00 c	58.62
Neem	1:1	0.00 e	100.00	10.00 d	86.21
Allamanda	1:1	0.00 e	100.00	10.00 d	86.21
Aloe Vera	1:1	20.00 c	55.56	30.00 c	58.62
Control		45.00 a	--	72.50 a	--
$LSD_{(0.01)}$		2.598	--	4.291	--
Level of significance		0.01	--	0.01	--
CV(%)		6.66	--	5.99	--

DAI= Days After Inoculation

4.20 *In* vitro mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 50% botanical with water extract

All the eight botanicals mixed with water in 1:2 (w/v) were tested against the pathogen 7 and 14 days after inoculation. All botanicals were found significantly effective in reducing the mycelial growth of the pathogens compared to control.

The result revealed that Garlic was performed best and significantly inhibited the radial mycelial growth of *M. oryzae* at 7 days after inoculation, 9.50 mm radial mycelial growth, and 78.89% growth inhibition. The maximum radial mycelial growth was found in Alamanda (30.50 mm) compared to control (45.00mm) at 7 days after inoculation.

Among the eight botanicals mixed with water in 1:2 (w/v) at 14 DAI, the highest mycelial radial growth was also found in Aloe vera (35.50 mm) compared to control (72.50 mm). On the other hand, the minimum mycelial radial growth was also found in Garlic (18.00 mm) which is 75.15% of percent growth inhibition (Table 19). Azadirachtin, found in neem, has antimicrobial properties. In addition, almost all segments of neem have been shown to have antifungal effects (Kumari *et al*., 2013; Kazmi *et al*., 1995).

Table 19. Efficacy water extract of botanicals (1:2 w/v) against *in vitro* mycelial radial growth of *Magnaporthe oryzae*

Treatment	Treatment concentration (w/v)	7 DAI		14 DAI	
		Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
Kalijira	1:2	25.00 c	44.44	30.00 b	58.62
Turmeric	1:2	20.00 d	55.56	30.00 b	58.62
Ginger	1:2	25.50 c	43.33	30.50 b	58.62
Garlic	1:2	9.50 e	78.89	18.00 c	75.15
Onion	1:2	25.50 c	43.33	35.00 b	51.72
Neem	1:2	20.50 d	54.44	30.50 b	58.62
Allamanda	1:2	30.50 b	32.22	35.00 b	51.72
Aloe Vera	1:2	20.00 d	55.56	35.50 b	51.03
Control		45.00 a	--	72.50 a	--
$LSD_{(0.01)}$		4.309	--	5.077	--
Level of significance		0.01	--	0.01	--
CV(%)		7.45	--	6.12	--

DAI= Days After Inoculation

4.21 *In vitro* mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 25% botanical with water extract

All the eight botanicals mixed with water in 1:4 (w/v) were tested against the pathogen 7 and 14 days after inoculation. All botanicals were found significantly effective in reducing the mycelial growth of the pathogens compared to control. The result showed that Kalijira and Alamanda were performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after inoculation, which is 0.00 mm radial mycelial growth and 100% growth inhibition. The maximum radial mycelial growth was found in neem and Aloe vera (25.50 mm) and (25.00) compared to control (45.00 mm) at 7 days after inoculation.

Among the eight botanicals mixed with water in 1:4 (w/v) at 14 DAI, the highest mycelial radial growth was also found in Aloe vera (32.50 mm) compared to control (72.50mm). On the other hand, the minimum mycelial radial growth was found in Kalijira (9.00 mm), which is 87.59% growth inhibition (Table 20).

Kalijira (*N. sativa*) contains thymoquinone (TQ), which is one of the most active constituents and has antimicrobial properties, according to Forouzanfar *et al.* (2014). TQ and various *N. sativa* extracts have a broad antimicrobial repertoire, including Gram-negative and gram-positive bacteria, viruses, parasites, Schistosoma, and fungi. The efficacy of *N. sativa* seeds and TQ varies depending on the target microorganism organisms.

Table 20. Efficacy water extract of botanicals (1:4 w/v) against *in vitro* mycelial radial growth of *Magnaporthe oryzae*

Treatment	Treatment concentration	7 DAI		14 DAI	
		Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
Kalijira	1:4	0.00 e	100.00	9.00 c	87.59
Turmeric	1:4	20.00 c	55.56	30.00 b	58.62
Ginger	1:4	24.00 b	46.67	30.00 b	58.62
Garlic	1:4	5.00 d	88.89	11.00 c	84.83
Onion	1:4	23.50 b	47.78	30.00 b	58.62
Neem	1:4	25.50 b	43.33	32.50 b	55.17
Allamanda	1:4	0.00 e	100.00	10.00 c	86.21
Aloe Vera	1:4	25.00 b	44.44	32.50 b	55.17
Control		45.00 a	0.00	72.50 a	0.00
$LSD_{(0.01)}$		2.598	--	4.145	--
Level of significance		0.01	--	0.01	--
CV(%)		5.93	--	6.15	--

DAI= Days After Inoculation

Yadav *et al.* (2018) showed that garlic and eucalyptus, out of seven plant extracts, showed maximum percent growth inhibition followed by neem and karanj. Tulsi was found least effective on blast of rice, followed by onion where Aslam *et al.* (2019) reported that among the five botanical extracts (black pepper, clove, aloe vera, neem, and ginger), black pepper showed the most promising botanical for the management of rice blast in Punjab province, Pakistan. Gopi *et al.* (2016) observed that garlic bulb extract and neem oil @ 3% were found effective in reducing blast incidence. Ibrahim *et al.* (2020) reported that The biosynthesized AgNPs extracted from onion at a 40g/mL concentration had intense antifungal activity against rice blast pathogen *Magnaporthe oryzae* with an inhibition rate of 88% in mycelial diameter. Iqbal *et al*. (2014) explained that a direct relationship exists between concentrations and pathogen growth inhibitions. The pathogen growth inhibitions increase, and the rate of blast severity

reduces on rice plants as the concentration of the plant extracts increases, evidence that toxic metabolites are present in these aqueous plant extracts; because as the concentration increases, the metabolites also increases. Higher concentrations indicated more toxic components than lower concentrations. This is in conformation with the report Akintobi *et al*. (2016), who concluded that the effectiveness of plant extracts depends on their concentration. The differences recorded among the five different plant extracts might result from the different chemical makeup of each plant. Liu *et al*. (2013) and Chen *et al*. (2014) explained that the mechanisms of disease suppression by plant extracts could be that the phytochemicals present in the plant parts may either act and suppress the pathogen directly or it activates the systemic resistance in the plants, thereby increasing the plant immunity; hence the disease development is reduced.

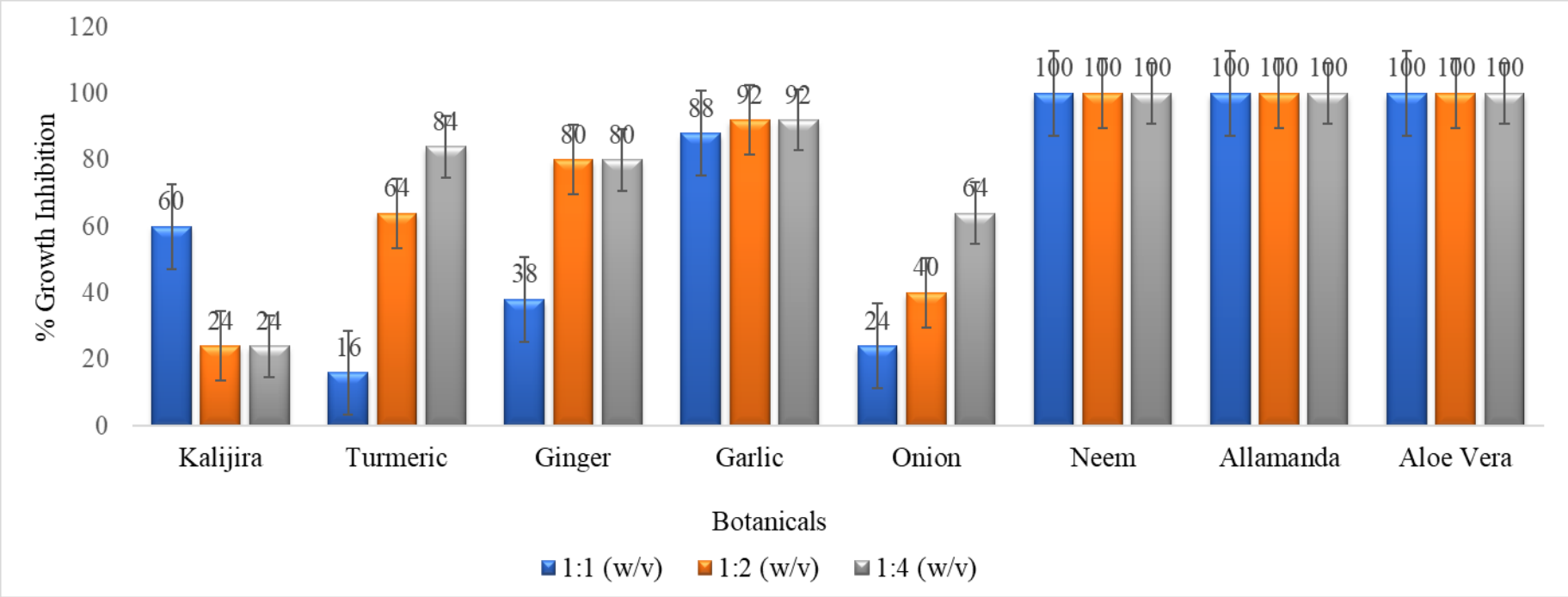


Figure 15. Percent inhibition of radial mycelial growth of *Magnaporthe oryzae in-vitro* against different concentration of botanical extracts with ethanol at 7 DAI

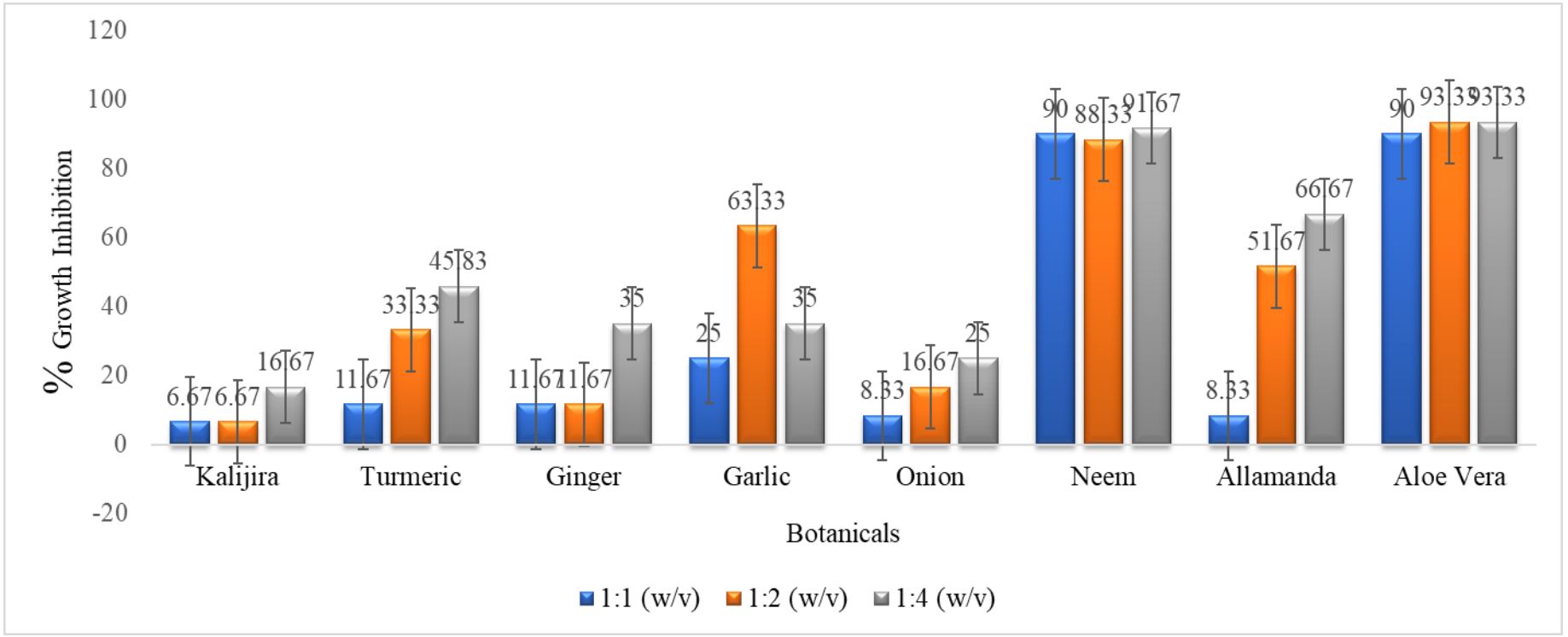


Figure 16. Percent inhibition of radial mycelial growth of *Magnaporthe oryzae in-vitro* against different concentration of botanical extracts with ethanol at 14 DAI

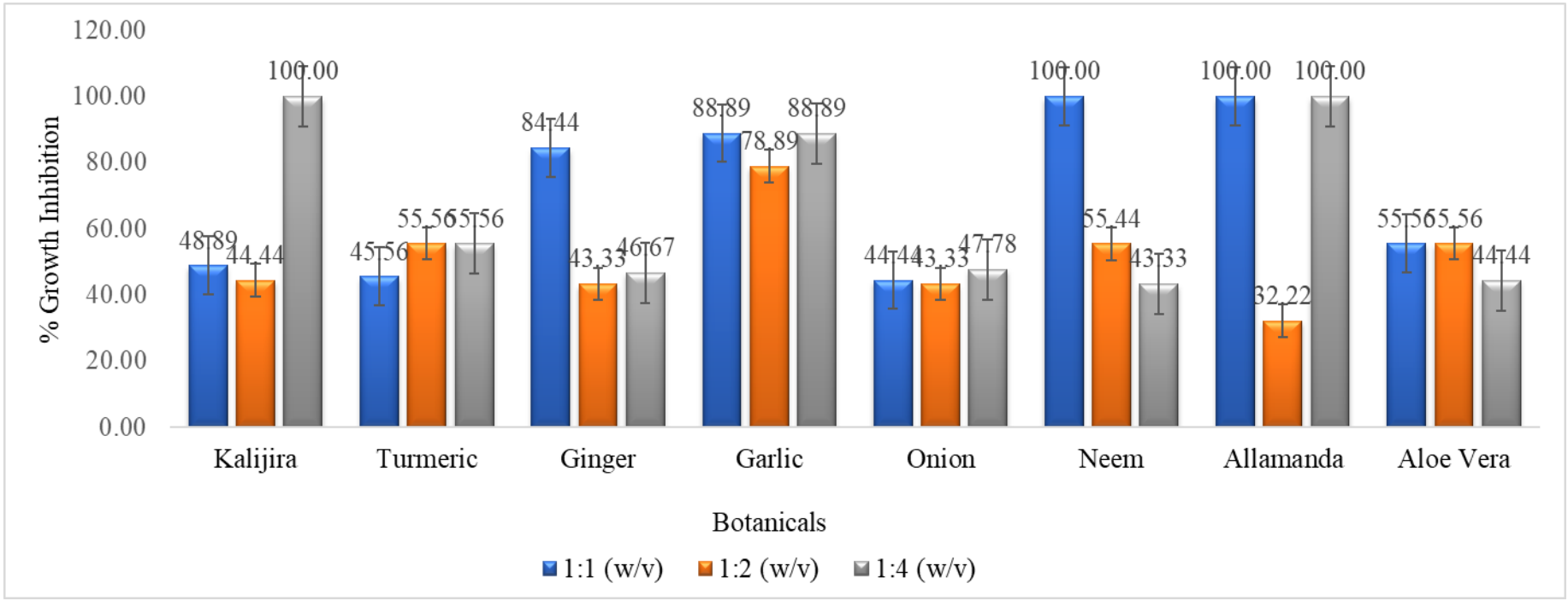


Figure 17. Percent inhibition of radial mycelial growth of *Magnaporthae oryzae in-vitro* against different concentration of botanical extracts with water at 7 DAI

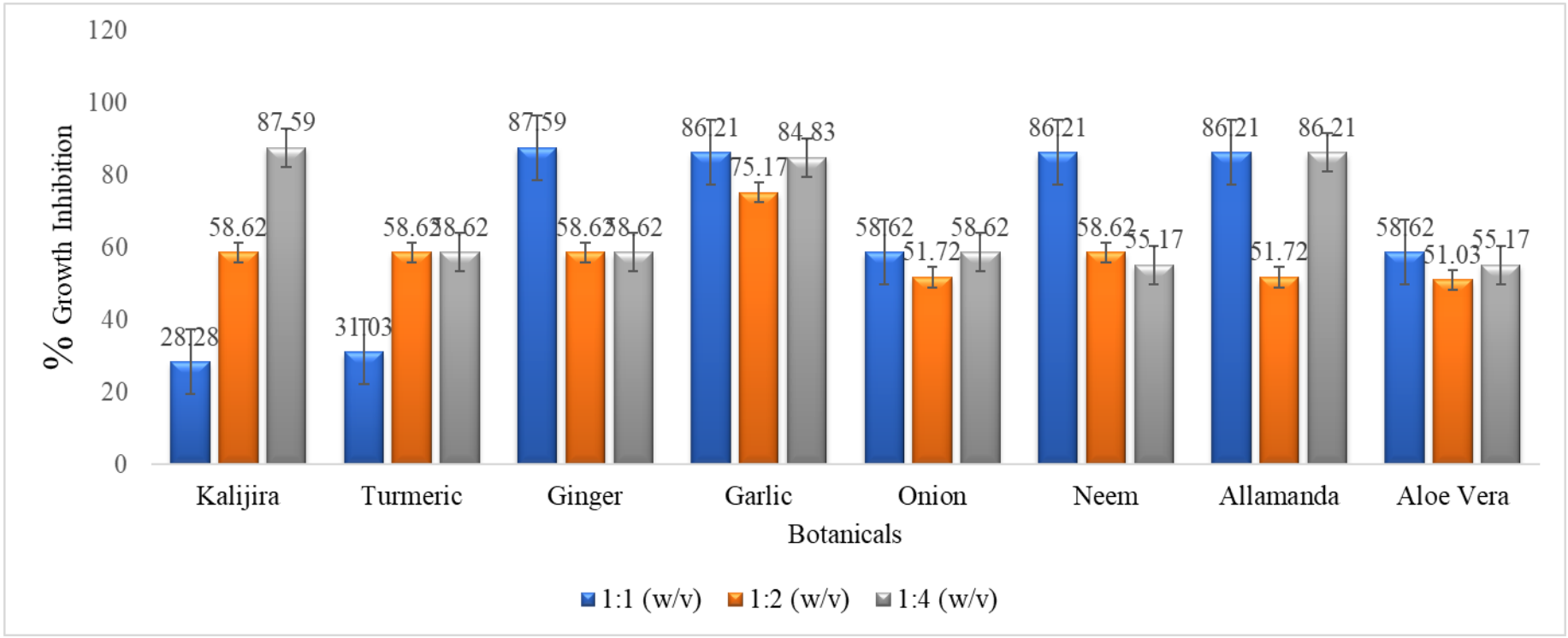


Figure 18. Percent inhibition of radial mycelial growth of *Magnaporthe oryzae in-vitro* against different concentrations of botanical extracts with water at 14 DAI

4.22 *In vitro* efficiency of bio-agent against *Magnaporthe oryzae*

Different bio-agents were tested in vitro with water to estimate their efficiency against *Magnaporthe oryzae*. The data obtained as an effect of different bio-agents on the mycelial growth and inhibitions of the pathogen at 6 and 15 DAI and significant variation was recorded in terms of mycelial growth as bio-agents *Trichoderma* sp. and *Phasiliomyce lilacinus* (Table 21 and 22).

4.23. Effect of different bio-control agents on mycelial growth of *Magnaporthe oryzae*

Two different bio-control agents such as *Trichoderma harzianum* and *Purpureocillium lilacinus* were tested under laboratory conditions against rice blast causing fungus, *Magnaporthe oryzae*. The inoculum of each bio-control agent was obtained from the Plant Pathology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. Four cm disc of test fungus and bio-control agent were placed at opposite sides in Petri dishes in five different designs containing sterilized PDA medium. There were 3 replications of each bio-control agent, and *Magnaporthe oryzae* were incubated at 30^0C. Petri dishes containing the test fungus and bio-control agents separately incubated at 30^oC served as control. Colony diameter of both the bio-control agent and the test fungus were recorded after 48 hours by giving a straight line in the center of both colonies with a permanent marker. The mechanism of antagonism was observed when the colonies of both fungi met. The following interactions between the test fungus and antagonist were noted (Yaqub and Shahzad, 2005).

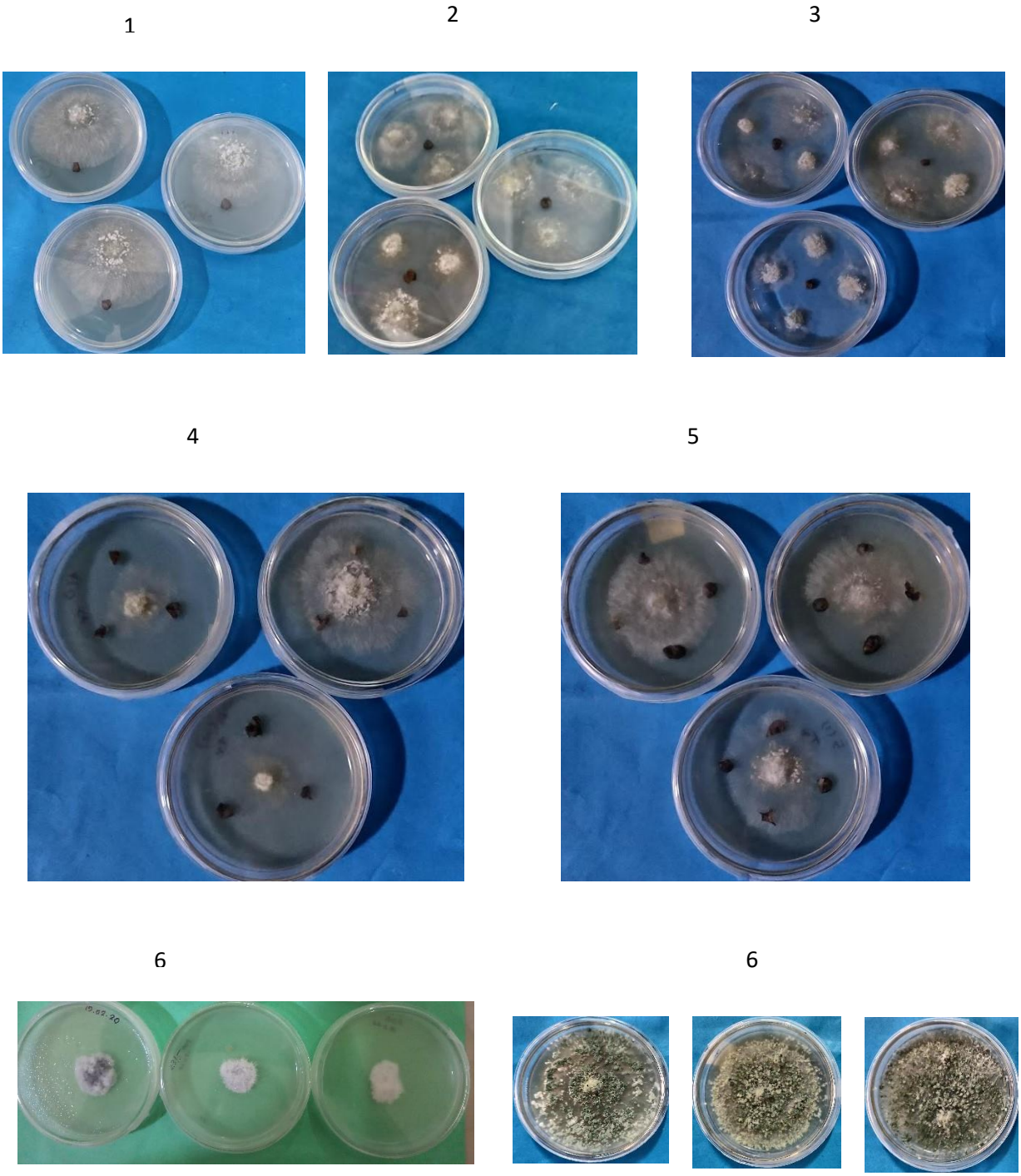


Figure 19. Growth of *Magnaporthe oryzae* and bio-agent *Trichoderma harzianum* in both dual culture technique and control condition in PDA at 6 days after inoculation.

4.24. In *vitro* radial mycelial radial growth of *Magnaporthe oryzae* with of bio-agent (*Trichoderma harzianum*) in PDA media

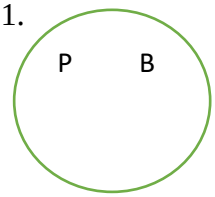
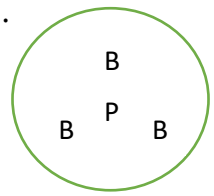
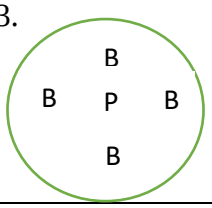
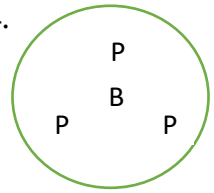
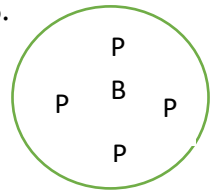
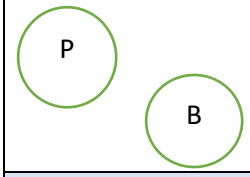
The trial was performed following five different designs of the dual culture method to understand how the *Magnaporthe oryzae* deals with *Trichoderma harzianum.* In the first design, one disc of *Magnaporthe oryzae* was placed against one disc of *Trichoderma harzianum* using a dual culture technique. In the second design, three-disc of *Trichoderma harzianum* were mounted on the periphery of the Petri dish, circling one of the disks of *M. Oryzae* at the middle of the Petri dish adopting the methodology of dual culture. In the third design, four disks of *Trichoderma harzianum* were mounted on the periphery of the Petri dish, circling one of the disks of *M. Oryzae*, in the center of the Petri dish, is following a dual culture approach. In the fourth design, three-disc of *M. Oryzae* were set on the periphery of the Petri dish surrounding one disk of *Trichoderma harzianum* at the middle of the Petri dish adopting the methodology of dual culture. In the fifth design, four-disc of *M. Oryzae* were set on the periphery of the Petri dish surrounding one disk of *Trichoderma harzianum* at the middle of the Petri dish adopting the methodology of dual culture. Two separate Petri dishes were set as control conditions for the tested *Magnaporthe oryzae* fungus and the bio-agent *Trichoderma harzianum* (Figure 19).

In the third design, four disks of *Trichoderma harzianum* were mounted on the periphery of the Petri dish, circling one of the disks of *M. Oryzae*, in the center of the Petri dish, is following a dual culture approach successfully inhibited the growth of the fungus and percent infection of *M. oryzae* (0.00 mm). In contrast, the bio-agent growth was 16.67 mm followed by second design three discs of *Trichoderma harzianum* were mounted on the periphery of the Petri dish circling one of the disks of *M. Oryzae* at the middle of the Petri dish inhibited the growth of the fungus (0.00 mm), whereas the bio-

agent growth was 22.33 mm at 6 DAI (Table 21). In the first, fourth, and fifth design, the radial mycelial growth of *M. oryzae* was 0.00 mm, whereas the radial mycelial growth of bio-agent *Trichoderma harzianum* was 41.67 mm, 36.67, and 41.67 mm. The control condition radial, mycelial growth of test fungus, and bio-agent were 12.33 mm and 41.67 mm, respectively. Table 21 shows no significant difference found in the first, fourth, fifth, and control design.

In the in vitro condition third design, four disks of *Trichoderma harzianum* were mounted on the periphery of the Petri dish, circling one of the disks of *M. Oryza*e center of the Petridis following dual culture technique gave most satisfactory result.

Table 21 ***In vitro*** **radial mycelial radial growth of** ***Magnaporthe oryzae*** **with of bio-agent (*Trichoderma harzianum*) in PDA media**

Treatment design	6 DAI		6 DAI	
	Radial mycelial growth of pathogen (mm)	Inhibition of growth (%)	Radial mycelial growth of bio-agent (mm)	Inhibition of growth (%)
1. 	0.00 b	100.00	41.67 a	0.00
2. 	0.00 b	100.00	22.33 b	46.41
3. 	0.00 b	100.00	16.67 b	60.00
4. 	0.00 b	100.00	36.67 a	12.00
5. 	0.00 b	100.00	41.67 a	0.00
6. Control 	12.33 a	--	41.67 a	--
$LSD_{(0.01)}$	0.590	--	7.412	--
Level of significance	0.01	--	0.01	--
CV(%)	11.47	--	8.89	--

DAI= Days After Inoculation

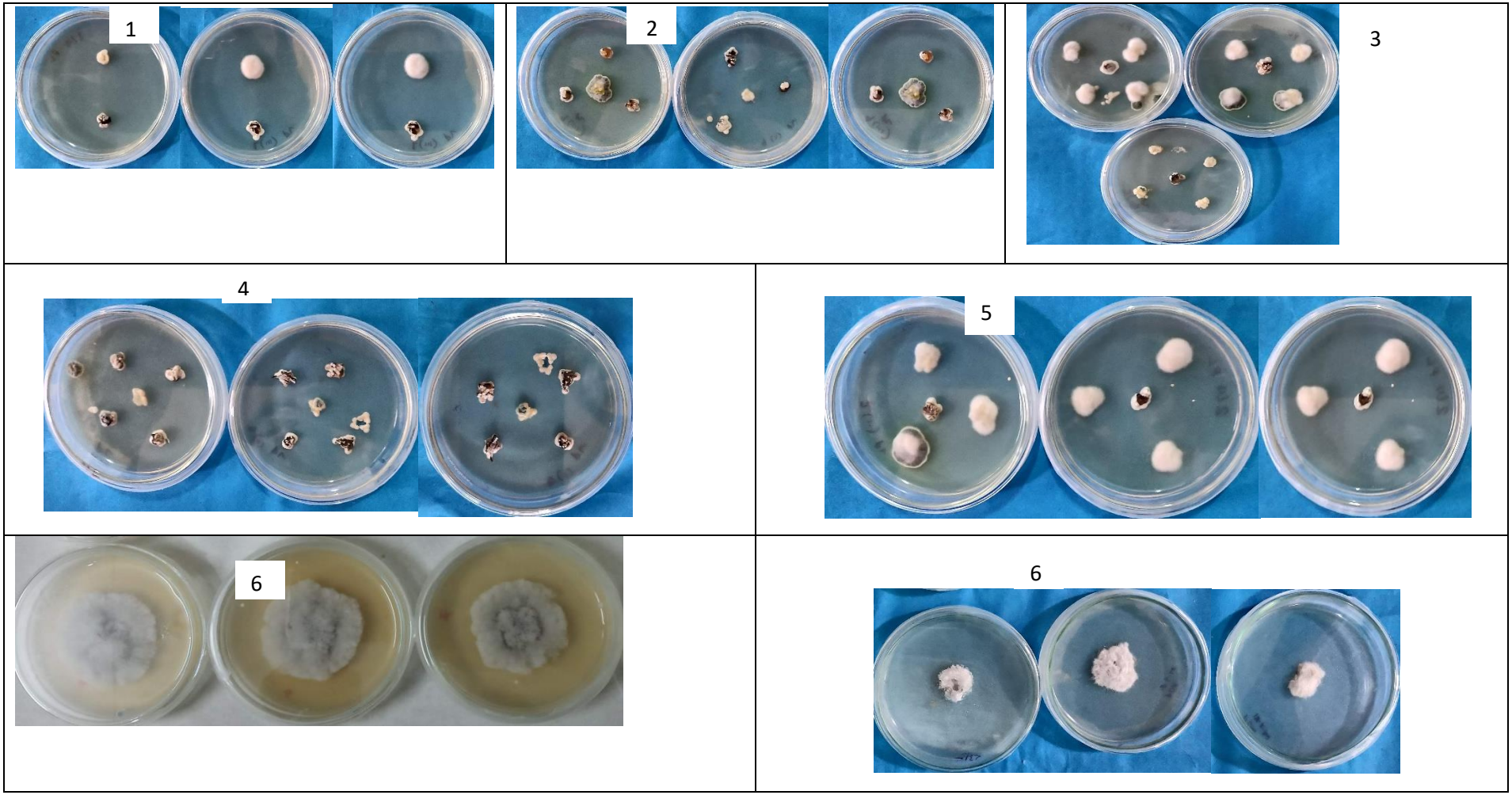


Figure 20. Growth of *Magnaporthe oryzae* and bio-agent *Purpureocillum lilacinum* in both dual culture technique and in control condition in PDA at 6 days after inoculation.

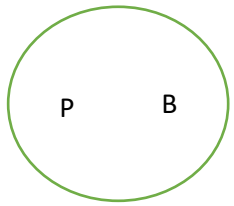
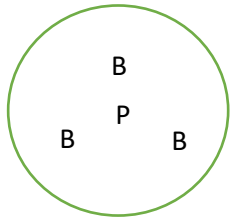
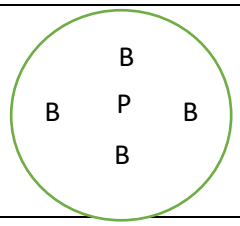
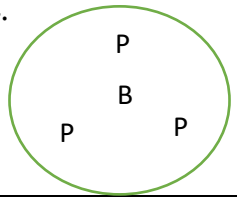
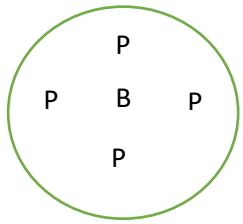
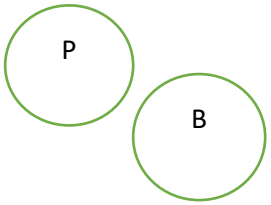
4.25. *In vitro* radial mycelial growth of *Magnaporthe oryzae* with of bio-agent (*Purpureocillium lilacinum*) in PDA media

The trial was performed following five different designs of the dual culture method to understand how the *Magnaporthe oryzae* deals with *Purpureocillium lilacinum.* In the first design, one disc of *M. oryzae* was placed against one disc of *P. lilacinms* using a dual culture technique. In the second design, three-disc of *P. lilacinm* were mounted on the periphery of the Petri dish, circling one of the disks of *M. oryzae* at the middle of the Petri dish adopting the methodology of dual culture. In the third design, four discs of *P. lilacinum* were mounted on the periphery of the Petri dish, circling one of the disks of *M. oryzae*, in the center of the Petri dish, following the following: a dual culture. In the fourth design, three discs of *M. oryzae* were set on the periphery of the Petri dish surrounding one disk of *P. lilacinus* at the middle of the Petri dish adopting the methodology of dual culture. In the fifth design, four discs of *M. oryzae* were set on the periphery of the Petri dish surrounding one disk of *P. lilacinum* at the middle of the Petri dish adopting the methodology of dual culture. Two separate Petri dishes were set as control conditions for the tested *M. oryzae* fungus and the bio-agent *P. lilacinum* (Figure 20).

In the second design, three discs of *P. lilacinum* were mounted on the periphery of the Petri dish, circling one of the disks of *M. oryzae* at the middle of the Petri dish adopting the methodology of dual culture inhibited the radial mycelial growth of pathogen *M. oryzae* (0.00 mm). In contrast, the growth of bio-agent was (10.00 mm) followed by first and third design 10.67 mm and 0.00 mm at 6 DAI in PDA (Table 22). Finally, in the fourth and fifth design radial, the mycelial growth of *M. oryzae* was 4.67 mm and 0.00 mm, whereas the radial mycelial growth of bio-agent *P. lilacinum* was 0.00 mm and 0.00 mm respectively.

In the control condition, the radial mycelial growth of test fungus and bio-agent was 10.67 mm and 11.67 mm, respectively. In the In-vitro condition second design, three disks of *P. lilacinum* were mounted on the periphery of the Petri dish circling one of *M. oryzae*, in the centre of the Petridis following dual culture technique gave most satisfactory result.

Table 22. *In vitro* radial mycelial radial growth of *Magnaporthe oryzae* with of bio-agent (*Purpureocillium lilacinum*) in PDA media

Treatment design	6 DAI		6 DAI	
	Radial mycelial growth (mm)	Inhibition of growth (%)	Radial mycelial growth (mm)	Inhibition of growth (%)
1. 	0.00 c	100.00	10.67 ab	8.57
2. 	0.00 c	100.00	10.00 b	14.31
3. 	0.00 c	100.00	10.67 ab	8.57
4. 	4.67 b	56.23	0.00 c	100.00
5. 	0.00 c	100.00	0.00 c	100.00
6. Control 	10.67 a	--	11.67 a	--
$LSD_{(0.01)}$	0.831	--	1.019	--
Level of significance	0.01	--	0.01	--
CV(%)	13.04	--	5.70	--

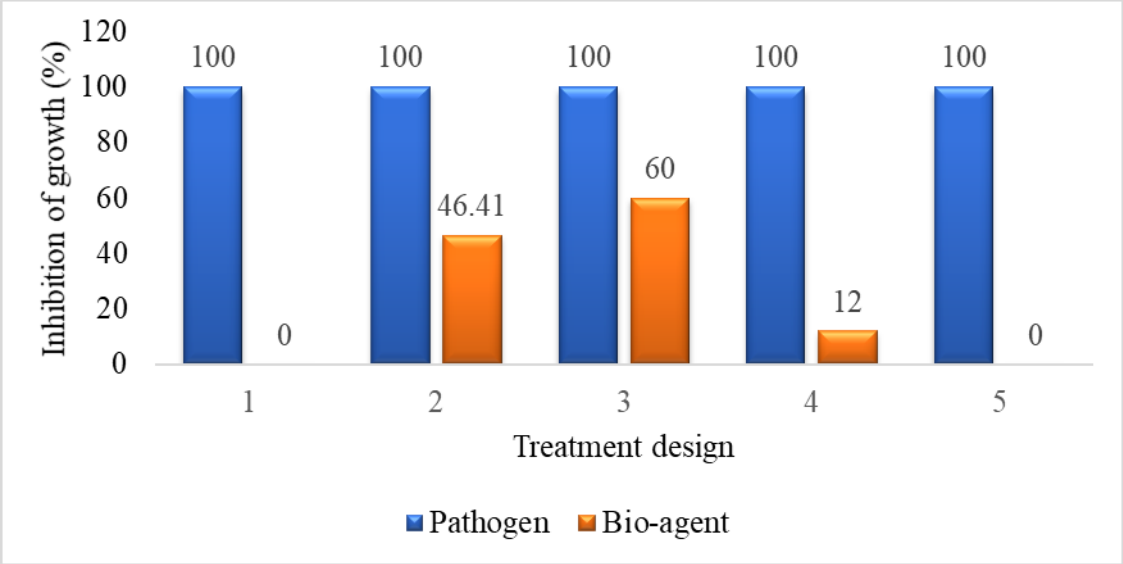


Figure 21. Percent inhibition of growth of *Magnaporthe oryzae* with bio-agent (*Trichoderma harzianum*) in PDA media (1= First design, 2= Two design, 3 = Third design, 4= Fourth design, 5= Fifth design)

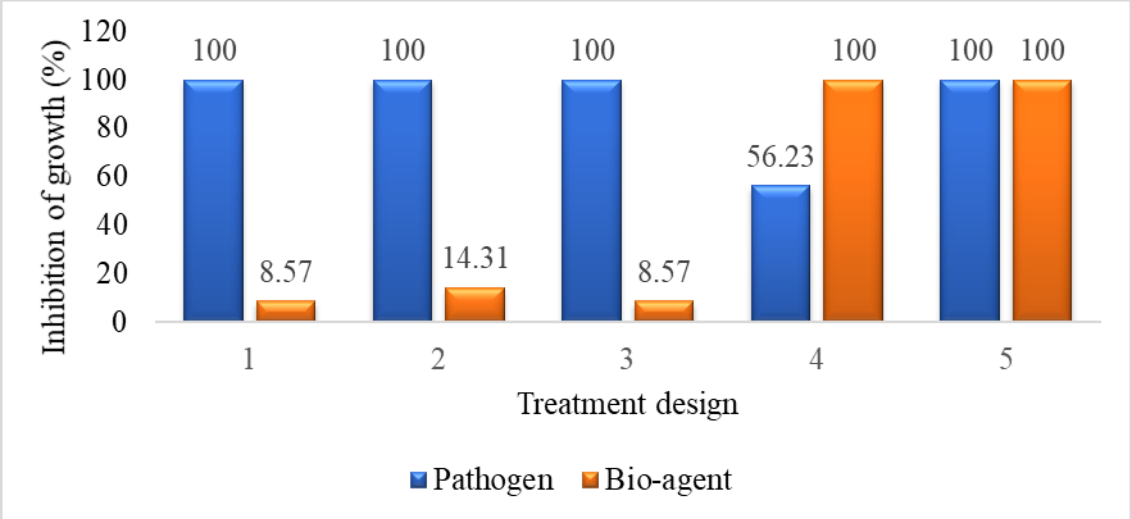


Figure 22. Percent inhibition of growth of *Magnaporthe oryzae* with bio-agent (*Purpureocillium lilacinum*) in PDA media (1= First design, 2= Two design, 3 = Third design, 4= Fourth design, 5= Fifth design)

The present study findings corroborate the findings of Gouramanis (1995), who found that antagonistic like *T. harzianum* inhibited *M. oryzae* mycelial and conidial growth by 70-88 percent. In addition, Watanable (1985) tested Trichoderma species against 24 airborne plant pathogens, including *M. oryzae*, and discovered that *T. hamatun, T.*

harzianum, T. koningii, T. pseudokoningii, and T. viride had the high antagonistic ability. He also found that *T. harzianum* and *T. viride* isolates had vigorous antimicrobial activity against *M. oryzae,* whereas *T. polysporum* was a weaker antagonist. According to Srinon *et al*. (2006), most antagonistic fungi effectively inhibited plate pathogen spore formation. The growth inhibition (GI) of spore development was significantly enhanced in some of the antagonistic fungi used. This occurred due to antagonistic fungi such as *Trichoderma sp.* and *Penicillium sp*. producing enzymes. Elad (2000) also reported that a T39 isolate of *Trichoderma harzianum* could be used as a model for demonstrating biocontrol in commercial settings and the process involved. Wasimfiroz *et al.,* (2018) evaluated the efficacy of four bio-agents on inhibition of growth of the pathogen. The maximum inhibition of growth of the pathogen was observed in *Trichoderma viride* with 64.44 percent, followed by *Trichoderma harzianum* with inhibition of 59.04 percent followed by *Pseudomonas fluorescens* (52.48 %). On the other hand, the least inhibition of growth of the pathogen was observed in *Bacillus subtilis,* with an inhibition of 47.48 percent. Similar results were obtained by Kumar *et al.* (2017), who reported *T. viride* is the best bio-control (24.53%) which was followed by *T. harzianum* (23.08%), while Rao and Kumar (2020) found different results where maximum growth inhibition was observed with *P. fluorescens* (63.7%) followed by *Trichoderma koningii* (52.2%) and lowest *Trichoderma viride* (47.1%) in mycelial growth inhibition of *Pyricularia grisea*. Hajanu *et al.* (2012) evaluated six bio-control agents *viz*., *Trichoderma harzianum, Trichoderma polysporum, Trichoderma pseudokoningii, Gliocladium virens, Paecilomyces variotii* and *Paecilomyces lilacinus* were used where maximum mycelial inhibition of *M. oryzae* was provided by *P. lilacinus* followed by *Trichoderma* spp. Fungal bio-control agents like *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp.,

etc have proved to be effective for controlling numerous destructive plant pathogens (Larena *et al.,* 2002; Elad, 2000; Srinon *et al*., 2006; Dawar *et al*., 1993; Yaqub & Shahzad, 2005). Dar and Murtaza (2021) found that *Trichoderma harzianum* was the best bioagent for controlling the radial mycelial growth against *Magnaporthe grisea.* That found the same result was 100 percent radial mycelial growth inhibited by fungal bio-agent *Trichoderma harzianum* against blast pathogen of rice (Rana and Paul, 2019). Similar findings were reported by (Kulmitra *et al*. 2017; Kumar & Ashraf, 2019; Mouria *et al.,* 2018). Chakraborty *et al.* (2021) stated that Commercial formulations of biocontrol agents and bioactive natural products could be cost-effective and sustainable, but their availability is minimal. Jambhulkar *et al.* (2018) investigated that the Combined application of *T. harzianum* Th3 and *P. fluorescens* RRb11 synergistically reduced the severity of RB by 69.5% in comparison to the untreated control, displaying a synergy factor (SF) of 1.29. The combined *T. harzianum* Th3 and P. fluorescens RRb 11 enhanced several rice plant growth and yield parameters. A similar result that the *Trichoderma* isolates obtained from screening effectively inhibited *M. grisea* in rice (Ali & Nadarajah, 2014; De Sousa *et al.,* 2021). Nazifa *et al.* (2021) showed that the design where four 5.0 mm mycelial discs of *T. harzianum* THR 4 were set on the periphery of the Petri dish surrounding one disc of MoO on the center of the Petri dish in dual culture gave the most satisfactory result with 92% growth inhibition of MoO in compare to control plate of MoO.

Table 23. Screening of rice genotypes against *M. oryzae*

Sl. No.	Rice genotype	Reaction
1.	BADC BRRI Dhan 28	S
2.	BRRI Dhan 34	S
3.	BRRI Dhan 71	S
4.	Chinigura	S
5.	Jeera Vog	R
6.	Badsha Vog	MR
7.	Chini Atop - 2	S
8.	Begun Bichi	S
9.	Katari Vog	S
10.	BADC BRRI Dhan 29	S
11.	NeelSagar Seed Jholok Hybrid Dhan	S
12.	LalTeer Tia Hybrid Dhan	S
13.	Kalijeera	S
14.	Tej Gold BAYER Hybrid	MR
15.	BINA 7	S
16.	BRRI dhan 34 (Atap)	S
17.	BRRI dhan33	R

4.26 Screening of rice genotypes against *M. oryzae*

Seventeen (17) rice genotypes were collected and evaluated in the Uniform Blast Nursery (UBN), BRRI to screen resistant rice genotypes against *Magnoporthe oryzae*. Among the seventeen rice genotypes, BADC BRRI Dhan 28, BRRI Dhan 71, Chinigura, Chini Atop-2, Begun Bichi, Katari Vog, BADC BRRI Dhan 29, LalTeer Tia Hybrid Dhan, Kalijeera, and BRRI dhan 34 (Atap) were found susceptible against *Magnoporthe oryzae.* Badsha Vog and Tej Gold BAYER Hybrid were found moderately resistant against *M. oryzae.* Jeera Vog and BRRI dhan 33 showed resistant reaction against *M. oryzae* (Table 23). The present study also confirmed the findings of Sabin *et. al.* (2016) who reported that Sabitri was the resistant genotypes with the lowest percentage of incidence and severity during observation whereas Taichung-176 and Sankharika showed the highest percentage of incidence and severity of the disease. In another study, Challagulla *et al.* (2015) evaluated 13 rice genotypes from Australia using three distinct inoculation methods (spot, filter paper and standard methods) at

seedling, vegetative and reproductive stages. Among the 13 rice genotypes screened, AAT9 expressed a highly resistant response, and AAT4, AAT6, AAT10, AAT11, AAT13, AAT17 and AAT18 expressed resistance at various stages. Ghazanfar *et al.* (2009) discovered One line, IR70181-1-1-1 resistant to *M. oryzae*. Nine-course type lines showed a somewhat resistant reaction, however, none of the fine type lines did. Seventy-seven course (35) and fine (42) rice lines were found to be moderately sensitive. Twenty-four fine rice lines had susceptible to the extremely susceptible response. However, resistance to the rice blast pathogen is more widespread in the course compared to fine germplasm lines of rice. Ghimire *et al.* (2019) found that the Sabitri genotype provides adequate resistance to rice blast disease in rice grown in the Baitadi district's hill region under Direct Seeded Rice (DSR) conditions.

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

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Consequently, four experiments were conducted to evaluate the morphological and pathogenic variation of *Magnaporthe oryzae* and the management of rice blast through fungicides, botanicals, and bio-agents. Firstly, the survey was conducted in different regions of Bangladesh on the blast incidence and severity diseases of rice, and subsequently, blast infected leaves, stems and panicles were collected from the farmer's BRRI dhan28 rice field. In vitro evaluation of fungicides, botanicals, and bio-agents was carried out against *Magnaporthe oryzae* at the Mycology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, from April to August 2016 to 2018.

5.1 Summary

The survey experiment was conducted in the BRRI dhan28 rice field from January to February 2016. Survey data revealed that rice blast disease prevalence was 100% for all the surveyed areas. However, in the case of disease incidence, the highest disease incidence (60%) was found in Bogura district, whereas the lowest (10%) was observed in Dhaka, Sunamgonj, and Moulvi Bazar districts. In addition, the highest severity score (5) was recorded in Kishoregonj district with a 25% disease incidence, while the lowest (2) was found in Dhaka, Cox's Bazar, and Sunamganj districts, respectively, with the disease incidence of 10%, 30%, and 10%, respectively.

The isolates of *M. oryzae* were identified based on morphological and cultural characteristics. Under compound microscope with coverslip typical two septate, three celled pyriform conidia were observed. It was revealed that mycelial radial growth of

M. oryzae varied for Water Agar (WA), Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Rice Flour Yeast Agar (RFYA), Oat Meal Agar (OMA) and Potato-Rice straw Dextrose agar (PR_SDA) culture media. The highest mycelial radial growth of *M. oryzae* (29.67 mm) was recorded for OMA, whereas the lowest mycelial radial growth of *M. oryzae* (15.00 mm) was observed in PR_SDA culture media.

The average radial mycelial growth of collected 26 isolates of *M. oryzae* was measured for 7 and 15 days after being cultured in PDA media. In 7 days after cultured, the longest radial mycelial growth (51.50 mm) was recorded from the isolate collected from Sepaipara union under Boda Upazila of Panchagar district, while the shortest growth (32.00 mm) was from the isolate of Kolmakanda union under Kolmakanda Upazila of Netrokona district. At 15 days after culture, the longest radial mycelial growth (85.83 mm) was found from the isolates collected from Rasulpur union under Kaharol Upazila of Dinajpur, the shortest radial mycelial growth (60.33 mm) of Birunia union under Bhaluka Upazila of Mymensingh District.

Radial mycelial growth of collected total 15 isolates of *M. oryzae* was measured for 7 and 15 days after cultured in PDA plates and in 7 days after cultured, the longest radial mycelial (46.00 mm) was recorded from the isolate of KHAL01 and KHMP03, while the shortest radial mycelial growth (35.00 mm) from the isolate of KMSL09. At 15 days after culture, the longest radial mycelial growth (82.50 mm) was found from the isolates of KHMP03, KHML04, and KHDL05, whereas the shortest (61.00 mm) was from MPBS13.

The radial mycelial growth of the collected 15 isolates of *M. oryzae* was measured for 7 and 15 days after being cultured in OMA plates. In 7 days after cultured, the longest

radial mycelial growth (45.00 mm) was recorded from the isolate of KHAL01, while the shortest radial mycelial growth (20.00 mm) was observed from the isolate of KHML04 and KISL07. At 15 days after culture, the longest radial mycelial growth (86.00 mm) was found from the isolates of MTKS14, whereas the shortest growth (47.50 mm) from KISL07.

In vitro mycelial growth and % inoculation of *Magnaporthe oryzae* at 5 days after inoculation revealed that among the 12 fungicides, maximum growth inhibition (100%) of *M. oryzae* was found in Folicular 250 EC (Tebuconazole) (10%), Seltima (100 CS) (Pyraclostrobin (10%), Filia (525 SE) (Propiconazole (12.5%) plus Tricyclazole (40%) and Difar (300 EC) (Difenoconazole (15%) plus Propiconazole (15%), whereas the minimum inhibition (3.00%) was recorded in Autostin (50 WDG). In vitro mycelial growth of *M. oryzae* at 10 DAI at different fungicidal treatments, the maximum growth inhibition (100%) of *M. oryzae* was found in Folicular 250 EC (Tebuconazole) (10%), Seltima (100 CS) (Pyraclostrobin (10%), Filia (525 SE) (Propiconazole (12.5%) plus Tricyclazole (40%) and Difar (300 EC) (Difenoconazole (15%) plus Propiconazole (15%) and the lowest inhibition (7.14%) was recorded in Autostin (50 WDG). *In vitro* mycelial growth and percent inoculation of *Magnaporthe oryzae* at 15 days after inoculation revealed that among the 12 fungicides, maximum growth inhibition (100%) of *M. oryzae* was found in Folicular 250 EC (Tebuconazole) (10%), Seltima (100 CS) (Pyraclostrobin (10%), Filia (525 SE) (Propiconazole (12.5%) plus Tricyclazole (40%) and Difar (300 EC) (Difenoconazole (15%) plus Propiconazole (15%), whereas the lowest (0.00%) inhibition was recorded in Autostin (50 WDG).

In vitro mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 100% botanical with ethanol extract revealed that neem, Alamanda, and Aloe vera were

performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after inoculation, which is 0.00 mm radial mycelial growth and 100% growth inhibition and the maximum radial mycelial growth was found in turmeric (42.00 mm) compared to control (49.67mm) at 7 days after inoculation. Among the eight botanicals mixed with ethanol in 1:1 (w/v) at 14 DAI, the highest mycelial radial growth was found in Kalijira (56 mm) compared to control.

In vitro mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 50% botanical with ethanol extract revealed that neem, Alamanda and Aloe vera were performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after inoculation, which is 0.00 mm radial mycelial growth and 100% growth inhibition. The maximum radial mycelial growth was found in Kalijira (38.00 mm) compared to control (49.67 mm) at 7 days after inoculation. Among the eight botanicals mixed with ethanol in 1:2 (w/v) at 14 DAI, the highest mycelial radial growth was also found in Kalijira (56.00 mm) compared to control (60.00 mm). The minimum mycelial radial growth was also found in Aloe vera (4.00 mm), which was 93.33% of percent growth inhibition.

In vitro mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 25% botanical with ethanol extracts showed that neem, Alamanda, and Aloe vera performed best significantly inhibited radial mycelial growth of *M. oryzae* at 14 days after inoculation, which is zero mm radial mycelial growth and 100% growth inhibition. The maximum radial mycelial growth was found in Kalijira (38.00 mm) compared to control (49.67mm) at 7 days after inoculation. Among the eight botanicals mixed with ethanol in 1:4 (w/v) at 14 DAI, the highest mycelial radial growth was also found in Kalijira (50.00 mm) compared to control (60.00mm). The minimum mycelial

radial growth was also found in Aloe vera (4.00 mm), which was 93.33% of percent growth inhibition.

In vitro mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 100% botanical with water extract revealed that neem and Alamanda were performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after inoculation, which is zero mm radial mycelial growth and 100% growth inhibition. The maximum radial mycelial growth was found in Onion (25.00 mm) compared to control (45.00 mm) at 7 days after inoculation. Among the eight botanicals mixed with water in 1:1 (w/v) at 14 DAI, the highest mycelial radial growth was found in Kalijira (52 mm) compared to control (72.50 mm). No significant difference was found among Kalijira (52.00 mm) and Turmeric (50.00 mm), compared to control at 14 DAI of test fungus.

In vitro mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 50% botanical with water extract revealed that Garlic was performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after inoculation, which was 9.50 mm radial mycelial growth and 78.89% growth inhibition. The maximum radial mycelial growth was found in Alamanda (30.50 mm) compared to control (45.00mm) at 7 days after inoculation. Among the eight botanicals mixed with water in 1:2 (w/v) at 14 DAI, the highest mycelial radial growth was also found in Aloe vera (35.50 mm) compared to control (72.50 mm). The minimum mycelial radial growth was also found in Garlic (18.00 mm) which is 75.17% of percent growth inhibition.

In vitro mycelial growth of *Magnaporthe oryzae* at 7 and 14 days after inoculation at 25% botanical with water extract showed that Kalijira and Alamanda were performed best and significantly inhibited radial mycelial growth of *M. oryzae* at 7 days after

inoculation, which is zero mm radial mycelial growth and 100% growth inhibition. The maximum radial mycelial growth was found in neem and Aloe vera (25.50 mm) and (25.00) compared to control (45.00 mm) at 7 days after inoculation. Among the eight botanicals mixed with water in 1:4 (w/v) at 14 DAI, the highest mycelial radial growth was also found in Aloe vera (32.50 mm) compared to control (72.50mm). The minimum mycelial radial growth was also found in Kalijira (9.00 mm), 87.59% growth inhibition.

Radial mycelial growth of *Magnaporthe oryzae* against bio-agent (*Trichoderma harzianum*) in PDA media trial was performed following five different designs of dual culture method to understand how the *M. oryzae* deals with *Trichoderma harzianum* and is following a dual culture approach successfully inhibited the growth of the fungus and percent infection of *M. oryzae* (0.00 mm). In contrast, the bio-agent growth was (16.67 mm) followed by second design three-disc of *Trichoderma harzianum* were mounted on the periphery of the Petri dish circling one of the disks of *M. oryzae* at the middle of the Petri dish inhibited the growth of the fungus (0.00 mm) whereas the bio-agent growth was (22.33 mm) at 6 DAI. In the first, fourth, and fifth design, the radial mycelial growth of *M. oryzae* was 0.00 mm, whereas the radial mycelial growth of bio-agent *Trichoderma harzianum* was 41.67 mm, 36.67, and 41.67 mm. In the control condition radial, the mycelial growth of test fungus and bio-agent was 12.33 mm and 41.67 mm, respectively, whereas no significant difference was found in the design of first, fourth, fifth, and control.

5.2 Conclusion

In this study, four experiments were carried out from April to August season of 2016 to 2018 at different regions of Bangladesh and Shere-e-Bangla Agricultural University to

evaluate the morphological and pathogenic variation of *Magnaporthe oryzae* and in vitro management of rice blast pathogen *Magnaporthe oryzae* through fungicides, botanicals, and bio-agents.

Under the above facts and findings, we may conclude that

1) Rice blast caused by *M. oryzae* is widely distributed in the growing regions of Bangladesh. Among the different rice-growing areas, the disease incidence and severity varied from 10-60% and 10-25%, respectively.

2) Variabilities exist among the isolates *M. oryzae* associated with rice blast disease in Bangladesh. The existence of physiological races of the pathogen in Bangladesh might be the reason for the diversified severity of the disease in different growing regions in the country.

3) Results of a pathogenicity test of 26 isolates of *M. oryzae were* pathogenic, causing rice blast of Boro rice. However, the isolates were sharply varied in terms of the degree of pathogenicity.

4) The highest mycelial radial growth of *M. oryzae* (29.67 mm) was recorded for OMA, whereas the lowest (15.00 mm) in PR_SDA culture media. The redial mycelial growth varied location-specific, and it was 32.00 mm to 51.50 mm at 7 DAI and 60.33 mm to 85.83 mm at 15 DAI.

5) Based on findings of *in vitro* evaluation of fungicides Folicular 250 EC (Tebuconazole) (10%), Seltima (100 CS) (Pyraclostrobin (10%) (100%), Filia (525 SE) (Propiconazole (12.5%) plus Tricyclazole (40%) (100%) and Difar (300 EC) (Difenoconazole (15%) plus Propiconazole (15%) (100%) were found to be most effective against *M. oryzae.*

6) Based on results of the *in vitro* evaluation of botanicals with ethanol, Neem (*Azadirachta indica*), Allamanda (*Allamanda cathertica* L.) Aloe vera (*Aloe vera*) in all concentrations were superior to the rest of the botanical treatment against *M. oryzae. In-vitro* evaluation of botanicals with water at 7 DAI Kalijira, Neem (*Azadirachta indica*) and Allamanda (*Allamanda cathertica* L.) are found to be most effective against *M. oryzae,* and at 14 DAI Kalijira, 1:4 (w/v) (87.14%) Ginger 1:1 (w/v) (87.14%) are found to be most effective against *M. oryzae.*

7) Based on the results of the *in vitro* evaluation of bioagent *Trichoderma harzianum,* the third design, four disks of were mounted on the periphery of the Petri dish, circling one of the disks of *M. oryza*e center of the Petridis following dual culture technique gave most satisfactory result. *In vitro* evaluation of bioagent *P. lilacinus* second design, three disks of *P. lilacinus* were mounted on the periphery of the Petri dish circling one of *M. oryzae,* in the center of the Petridis following dual culture technique gavethe most satisfactory result.

8) In screening test among seventeen rice genotypes, Jeera Vog and IRRI dhan33 were found resistant reaction against *M. oryzae.*

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