SURVEY ON RICE BLAST IN SOME SELECTED AREA OF BANGLADESH AND *IN VITRO* EVALUATION OF SELECTED FUNGICIDES AGAINST *PYRICULARIA ORYZAE*

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JUNE, 2018

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REGISTRATION NO. 12-05022

A Thesis
Submitted to the Faculty of Agriculture
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE

IN

PLANT PATHOLOGY

SEMESTER: JANUARY- JUNE, 2016

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This is to certify that the thesis entitled "**SURVEY ON RICE BLAST IN SOME SELECTED AREA OF BANGLADESH AND *IN VITRO* EVALUATION OF SELECTED FUNGICIDES AGAINST *PYRICULARIA ORYZAE*"** submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfilment of the requirements for the degree of Master of Science in Plant Pathology, embodies the results of a piece of bona fide research work carried out by **Md. Rayhanul Islam**, **Registration No. 12-05022** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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Dated:
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Dedicated to

My

Beloved Parents

ACKNOWLEDGEMENTS

All praises are due to Almighty Allah for best owing mercy upon Author and for imbibing confidence on author to materialize the research work. It is a great gratification to articulate author's gratitude author's respected parents, who entitled much hardship inspiring for prosecuting author's studies, thereby receiving proper education.

The author feel proud to express his cordial gratitude, deep sense of respect and enormous indebtedness to author's research supervisor ***Dr. F. M. Aminuzzaman****, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his educational supervision, incessant encouragement, positive suggestion and unvarying inspiration all through the research work and for taking massive care in preparing this manuscript. His insight and professional skill have made a distinctive contribution to complete this piece of research.*

The authors express his sincere appreciation, insightful sense, respect and immense indebtedness to the respected Co-supervisor ***Dr. M. Salahuddin M. Chowdhury****, Professor, Department of Plant Pathology, SAU. for his constant guidance, unvarying help, timely directions and inspirations throughout the tenure of research work.*

The author is greatly thankful to his respectable teacher ***Dr. Khadija Akhter****, Professor and Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for her valuable teaching, encouragement and co-operation during the entire study period.*

The author would like to expresses cordial thanks to all the respected teachers of SAU and special thanks to Montasir Ahmed, Scientific Officer, BRRI, who helped me very much.

The author would like to expresses cordial thanks to his friends who wished his better life are Farhana Hossain, Lutfunnahar Laila, Tanjina Akter, Arifa Afrin and Liton Chandro Barman helped the author with their valuable suggestions and directions during the preparation of this thesis paper.

The author take an opportunity to express his cordial thanks and sincere gratitude to the staff of the Department of Plant Pathology, SAU for their valuable suggestions and directions to complete this piece of research.

The author expresses his massive thankfulness to all of them who supported and encouraged him to achieve advanced education and regret for his inability for not to mention every one by name.

The Author

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ABSTRACT

Survey was conducted on rice blast in 110 rice field of 12 upazillas under 5 districts of Bangladesh namely Mymensingh, Kishoreganj, Barishal, Naogaon and Cumilla. Among the survey Muktagachha, Mymensingh was found the highest blast disease infected area and Bakerganj, Barishal was found the lowest blast disease infected area in Boro season 2017-2018. *Pyricularia oryzae* was isolated from infected leaf and panicle and identified based on cultural characteristics and conidia morphology. Eight fungicides namely Trooper 75WP, Seltima 100CS, Nativo 75 WP, Amister Top 250 SC, Azonil 56 SC, Autostin 50 WDG, Filia 525 SE, Dithane M-45 were tested for controlling mycelial growth of *Pyricularia oryzae in vitro* in the Plant Pathology laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. Experiment was conducted in Completely Randomized Design (CRD) with four replications. Data on radial mycelial growth was taken at 7, 14 and 21 days after inoculation (DAI). The highest inhibition of mycelial growth was observed when Potato Sucrose Agar (PSA) was amended with Trooper 75 WP (Tricyclazole) followed by Filia and Nativo and lowest inhibition was recorded in Dithane M-45(Mancozeb) amended with PSA media. From these results Trooper (Tricyclazole) was found to be the best effective fungicide and is needed to further field evaluation for confirmation of the result.

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CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa*) is the staple food of Bangladeshi people and it constituted about 90% of the total food grain production (Huda, 2001). It covers about 84.5% of the total cultivable land in Bangladesh (BBS, 2017). The average world yield of rice is 3.84 tons/ha, but the average yield of rice in Bangladesh is only 3.02 t ha^{-1} (FAO, 2017). So the average per hectare production of rice in Bangladesh is extremely lower as compared to other rice growing countries of the world. In Bangladesh, out of 16% annual crop losses occurred due to plant diseases (Fakir, 2004). Rice grain contains on an average 7% protein, 62-65 % starch, 0.7% fat and 1.3% fiber and rice is a main source of vitamin B1 (thiamin), B2 (riboflavin), B3 (niacin) and B5 (pantothenic acid). The Biological value of rice is 63% whereas Biological value of wheat and maize is 49% and 36%, respectively. The domesticated rice comprises two species of food crop in the Poaceae family: *Oryza sativa* and *Oryza glaberrima* (Linscombe, 2006). These plants are native to Tropical and Subtropical Southern Asia and Southeastern Africa, respectively (Linares, 2002). Rice (*Oryza sativa* L.) is the world's most important crop and a primary source of food for more than half of the world's population. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live (Kole, 2006). Globally rice occupies an area of 163 m ha with a production of 719 million hectare of paddy (FAO, 2012).

Rice is a rich source of carbohydrates, proteins and vitamins providing 84 to 310 kilo calories of energy. Nutritionally, hundred grams of rice provides 6.9 g of protein, 1.3 g of dietary fiber, 0.2 g of sugar, 3.7 g of carbohydrates, 0.008 g calcium, 0.23 g of iron, 0.049 mg of riboflavin, 0.10 mg of thiamine, 0.018 mg of pentothenic acid, 1.3 g of nicotinic acid, 0.158 mg of vitamin B6 and also 1.05 mg of manganese, 115 g of phosphorus, 25 mg of magnesium, 115 mg of potassium, 1.09 mg of zinc and 0.01 mg of vitamin C (Swaminathan, 1995).

It is the main source of food for more than half of the world population, especially in South and Southeast Asia and Latin America. In these regions, it represents a high-value commodity crop. Rice is the source of subsistence for more than one third of human population. It is the main staple food in the Asia and the Pacific region, providing almost 39% of calories (Yaduraju and Rao, 2013). Rice is cultivated in 114 countries across the world. The global production of rice has been estimated 697.22 million tons of rice at an average yield of 4.4 tons ha^{-1} is being harvested from 158.43 million ha annually producing 21% of worlds food calorie supply. Asia is the leader in rice production accounting for about 90 per cent of the world's production. Over 75 % of the world supply is consumed by people in Asian countries and thus rice is of immense importance for food security of Asia. In Asian countries more than 80% people had been taking rice in their daily diet as a staple food. The global annual demand for rice is expected to be around 800 million tons by 2025 in view the expected increase in population.

Rice is known to be attacked by many pests and diseases which cause huge annual losses worldwide. Among fungal diseases, rice blast caused by *Pyricularia oryzae* is of significant economic importance. Outbreaks of rice blast is a serious and recurrent problem in all rice growing regions of the world. It is estimated that 60 million people could feed each year by the produce of rice which is destroyed by rice blast (Zeigler *et al*., 1994). Rice crop is subjected to attack by 50 diseases that include 6 bacterial, 21 fungal, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders (Hollier *et al.,* 1993; Webster and Gunnell, 1992; Jabeen *et al*., 2012).

Blast disease has been known since early 1600's and is cited in Chinese and Japanese literature as 'rice fever'. The disease was first recorded in China as seedling blight and the name blast was coined by Metcalf in 1907 (Manibhushan Rao, 1994). Rice blast pathogen is widely distributed and highly destructive under favourble conditions. Temperature, moisture, sunshine, humidity and wind speed play a major role in the infection and development of the disease. Blast disease is caused by the fungus *Pyricularia oryzae* Cavara teleomorph: *Magnaporthe oryzae* B. Couch] (Couch and Kohn, 2009). It causes disease in rice, wheat, rye, barley, pearl millet in which rice and wheat are economically more important. Blast is the most destructive fungal disease affecting global rice production. In temperate flooded and tropical upland rice ecosystems rice blast cause significant yield loses (Shahjahan, 1994).

The fungus is known to occur in 85 countries worldwide. The International Rice Research Institute, Philippines, estimates that in order to feed the growing global population, rice production must be increase by another one-third by the year 2020.

Resistant cultivars and chemical fungicides are important in blast disease control. However, the durability of genetic resistance in improved rice cultivars is often short-lived in the field because the pathogen rapidly evolves to overcome resistance. The key to chemical control of rice blast disease is to overcome fungicide resistant pathogens. However, the frequent use of fungicides on crops may cause hazards to human beings, plant health, beneficial micro-organisms, and develop fungicide resistance into the pathogens and residual toxicity in plant parts.

Objectives

With the facts describe above, the present piece of research work had been under taken to fulfill the following objectives:

i. To determine the incidence and severity of rice blast in the selected rice growing areas of Bangladesh.
ii. To isolate and identify the rice blast pathogen
iii. To evaluate the efficacy of fungicides on mycelial growth, cultural characteristics and sporulation of rice blast pathogen

CHAPTER 2

REVIEW OF LITERATURE

The available literature of work done on blast disease of rice and it's management strategies have been reviewed in this chapter. The review of literature pertaining to this dissertation is presented in the following headings and sub-headings.

2.1. Importance of rice

Rice (*Oryza sativa* L.) is one of the most important cereals of the world and is consumed by 50% of the world population (Luo *et al.*, 1998). There are two species cultivated *Oryza sativa* L (Asian rice) and *Oryza glaberrima Steud* (African rice) (Silue and Notteghem, 1991). *Oryza glaberrima* is traditionally found in diverse West African agro ecosystems but it is largely abandoned in favor of high yielding *Oryza sativa* cultivar that has higher agronomic performance (Seebold *et al.*, 2004).

However, *Oryza sativa* cultivars are often not sufficiently adapted to various abiotic and biotic conditions in Africa. *Oryza glaberrima* has been found to have several useful traits like being moderate to high in their level of resistance to blast (Silue and Notteghem, 1991), rice yellow mottle virus (Attere and Fatokun, 1983; John *et al.*, 1985) and nematodes (Reversat and Destombes, 1995). The variety has also been found to be tolerant to abiotic stresses such as acidity, iron toxicity, drought, and weed competition (Sano *et al.* 1984; Jones *et al.* 1994).

Rice is the most economically important staple food crop in India, China, East-Asia, South East Asia, Africa and Latin America catering to nutritional needs of 70% of the population in these countries (FAO, 1995). It is the main staple food in the Asia and the Pacific region, providing almost 39% of calories (Yaduraju and Rao, 2013). In several developed countries such as North America and European Union (EU) also, rice consumption has increased due to food diversification and immigration (Faure and Mazaud, 1996).

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

Rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5%. During 2012-13 and 2013-14, the world production has increased by 1% (from 472 Million Tonnes to 476 Million Tonnes), trade by 8% (from 38 Million Tonnes to 41 Million Tonnes) and consumption by 3% (from 469 Million Tonnes to 481 Million Tonnes) (Commodity profile for rice - January 2015).

Rice is one of the three most important food crops of the world and the main staple food for nearly a half of the world's population (Von Braun 2007). Its production is concentrated in Asia (90%) in subsistence agriculture farms with the grain destined for local consumption and only 4% exported to international markets (Khush and Kinoshita, 1991). Fifty percent of the production area is located in China and India.

2.2. Significance of blast disease of rice

Magnaporthe grisea (Anamorph *Pyricularia grisea* Sacc. synonym *Pyricularia oryzae* Cav.) causes rice blast disease in rice cultivation areas worldwide (Chin, 1975; Kato, 2001; BPS, 2010). The disease causes yield losses from between 1- 100% in Japan (Kato, 2001), 70% in China, 21-37% in Bali Indonesia (Suprapta and Khalimi, 2012), and 30-50% in South America and Southeast Asia (Baker *et al.*, 1997).

Rice blast is one of the most important diseases of rice, caused by the fungus *Pyricularia oryzae* B. C. Couch (Couch and Kohn 2002). One of the main limitations in production of rice is rice blast disease caused by the fungus *Pyricularia oryzae*. Annual rice losses caused by this fungus during 90's had been estimated at 35% of the worldwide production (Oerke and Dehne,2004). In West Africa, the largest area of African production, this pathogen is the main constraint to production with yield losses ranging from 3-77%. The fungus is able to infect plants at all stages of growth and development in both upland and lowland rice production systems. Lowland rice produced in temperate and subtropical climates of Asia are highly susceptible to the pathogen, while tropical upland areas are susceptible only under irrigation (Nutsugah *et al.*, 2008).

Blast disease caused by *Pyricularia oryzae* Cavara [Synonym *Pyricularia grisea* Sacc., the anamorph of *Magnaporthe grisea* (T.T Hebert) Yaegashi and Udagawa], upsets production statistics of rice in Pakistan (Jia *et al.,* 2000). In Pakistan during the last two decades, rice blast is mostly found in districts of Faisalabad, Toba Tek Singh, Vehari and place like Gaggoo Mandi (Arshad *et al.*, 2008).

The fungus *Pyricularia oryzae* attacks at all stages of the crop and symptoms appear on leaves and nodes (Seebold *et al.*, 2004). The symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base and its rotting (Bonman *et al.*, 1989). Heavy yield losses have been reported in many rice growing countries. For example 75, 50 and 40 percent grain loss may occur in India (Padmanabhan, 1965), Philippines (Ou, 1985) and Nigeria (Awodera and Esuruoso, 1975).

The most usual approaches for the management of rice blast disease include planting of resistant cultivars application of fungicides, and manipulation of planting times, fertilizers and irrigations (Mbodi *et al.*, 1987; Georgopoulos and Ziogas, 1992; Naidu and Reddy, 1989).

Blast is known to attack nearly all above ground parts as well as during all growth stages of plant. Recent reports have shown that the fungus has the capacity to infect plant roots also (Sesma and Osbourn, 2004). The infection of rice blast occur when fungal spores land and attach themselves to leaves using a special adhesive released from the tip of each spore (Hamer *et al.,* 1988). The germinating spore develops an appressorium, a specialized infection cell which generates enormous turgor pressure (up to 8MPa) that ruptures the leaf cuticle, allowing invasion of the underlying leaf tissue (Dean, 1997; Hamer *et al.*, 1988).

The pathogen may infect all the above ground parts of a rice plant at different growth stages: leaf, collar, node, internode, base, or neck, and other parts of the panicle, and sometimes the leaf sheath (Pinnschmidt *et al.*, 1994). The symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base and its rotting (Bonman *et al.*, 1989).

Pyricularia oryzae infects and produces lesions on the following parts of the rice plant: leaf (leaf blast), leaf collar (collar blast) and panicle (panicle blast). In leaf blast initial lesions/spots are white to gray-green with darker borders. Older lesions are white-grey, surrounded with a red-brown margin and are diamond shaped (wide centre and pointed toward either end). Lesion size is commonly 1-1.5 cm long and 0.3-0.5 cm wide. Under favourable conditions, lesions can coalesce and kill the entire leaf. In collar rot, lesions are located at the junction of the leaf blade and leaf sheath and can kill the entire leaf (Padmanabhan, 1974; Bhatt and Singh, 1992; Manibhushanrao, 1994).

Infection to the neck node produces triangular purplish lesions, followed by lesion elongation to both sides of the neck node, symptoms which are very serious for grain development. When young neck nodes are invaded, the panicles become white in colour the so called 'white head' that is sometimes misinterpreted as insect damage. Infected panicles appear white and are partly or completely unfilled. The whitehead symptoms can easily be confused with a stem borer attack which also results in a white and dead panicle. Panicle branches and glumes may also be infected. Spikelets attacked by the fungus change to white in colour from the top and produce many conidia, which become the inoculum source after heading. Panicle blast symptoms include the panicle appearing 11 brown or black. Node infection includes infected nodes appearing black-brown and dry and often occur in a banded pattern. This kind of infection often causes the culm to break, resulting in the death of the rice plant. The pathogen is most common on leaves, causing leaf blast during the vegetative stage of growth, or on neck nodes and panicle branches during the reproductive stage, causing neck blast (Bonman *et al*., 1992).

Leaf blast lesions reduce the net photosynthetic rate of individual leaves to an extent far beyond the visible diseased leaf fraction (Bastiaans, 1991). Neck blast is considered the most destructive phase of the disease and can occur without being preceded by severe leaf blast (Zhu *et al.*, 2005). The neck blast infects the panicle causing failure of the seeds to fill or causing the entire panicle to fall over as it is rotted. Infection of the necks can be very destructive and directly reduces the economic value of the produce. The lesions are often greyish brown discoloration of the branches of the panicle and over time, the branches may break at the lesion. Out of three symptoms, neck blast is more destructive (Srinivas Prasad *et al.*, 2011).

Manandhar *et al.* (1998) reported that *Pyricularia grisea* is one of the most important fungal pathogen of rice because of its widespread occurrence and destructive nature. The fungus can attack any aerial part of the rice plant, including seeds. They also suggested systemic transmission of the fungus from seeds to seedlings.

The fungus *Pyricularia grisea* was able to infect and produce lesions on all organs of the rice plant and when the fungus attacks young leaves, purple spots could be observed changing into spindle shape which has a grey centre and purple to brown border. Brown spots appeared only on older leaves or leaves of resistant cultivars. In young or susceptible leaves, lesions coalesce and cause withering of the leaves, especially at seedling and tillering stage. Infection to the neck results formation of triangular purplish lesions followed by elongation on both sides of neck. When young necks are infected, the panicles become white in colour and later infection caused incomplete grain filling and poor grain quality (Hajimo, 2001).

Ram *et al.* (2007) reported that leaf blast fungus can attack the rice plant at any growth stage and can cause severe leaf necrosis and impede grain filling, resulting in decreased grain number and weight. When the last node is attacked, it causes partial to complete sterility. Rice blast pathogen infect all the above ground parts of rice plants at different growth stages, i.e., leaf, collar, nodes, internodes, base or neck and other parts like panicle and leaf sheath. It starts a typical blast lesion on rice leaf as grey at the center with a dark border and is spindle shaped. The environment with frequent and prolonged dew periods and with cool temperature in day time is most favourable for the spread of the disease (Castilla *et al.*, 2009).

2.3. *Pyricularia oryzae* the causal organism of rice blast

The fungus *Magnaporthe grisea* (Hebbert) Barr (Anamorph: *Pyricularia grisea* (Cooke) Sacc is the causal agent of rice blast disease. The perfect stage of *Pyricularia grisea* was earlier named as *Ceratosphaeria grisea* (Hebert, 1971). Later Yaegashi and Nishihara (1976) suggested the genus *Magnaporthe*. Yaegashi and Udagawa (1978) finally proposed M. grisea as a perfect stage of *Pyricularia grisea*(cooke.) Saccinstead of *Ceratosphaeria grisea*.

The mycelium consists of septate, uninucleate, branched hyphae. However, as the fungus gets older, the hypha become brown. Generally, growth of the pathogen is relatively more on upper surface making the spot more dark on upper side. Conidiophores are simple, septate, basal portion being relatively darker. Conidia are pyriform in shape and hyaline in colour, produced acrogenously, one after another. Conidia is three celled, the middle cell being much wider and darker, and end cell germinates giving out germ tube. Conidia is rarely two celled or four celled. Formation of intercalary or terminal chlamydospores is common, which are globose, thick walled and olive brown.

Commonwealth mycological institute (Hawksworth, 1990) description of the culture: Cultures greyish in colour, conidiophores single or in fascicles, simple or rarely branched, show sympodial growth. Conidia formed singly at the tip of the conidiophore at points arising sympodially and in succession, pyriform to obclavate, narrowed toward tip, rounded at the base, three celled rarely one or two celled, hyaline to pale olive, 19-23 × 7-9 μm, with a distinct protruding basal hilum. Chlamydospores often produced in culture, thick-walled, 5-12 μm diameter.

Fungus produce sexual fruiting bodies called perithecia within 21 days. Perithecia are flask-shaped that carry asci containing ascospores, the products of meiosis. Ascospores are arranged as unordered octads or as larger populations of randomly selected ascospores (Nicholas J. Talbot, 2003).

2.4. Isolation and identification of *Pyricularia oryzae*

Padmanabhan *et al.* (1970) isolated *Pyricularia grisea* from samples of diseased leaves, necks and nodes of the infected rice plant on oat meal agar (OMA) with traces of biotin and thiamine (B and T). Cultures were purified by dilution method and single spore isolates were grown and multiplied on OMA + Biotin and Thiamine at 25^0c.

Palande *et al.* (1977) maintained the blast fungus of rice on oat meal agar medium during their study for the control of rice blast.

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

Kumari and Suryanarayanan (1995) studied 19 isolates of *Pyricularia oryzae* causing blast of rice. These were isolated by standard tissue isolation techniques and maintained throughout the study on oat meal agar.

Greer and Webster (1997) identified *Pyricularia oryzae* by isolating the fungus from panicle neck, nodes and leaf collars of rice on potato dextrose agar.

Kamalakannan *et al.* (2001) isolated *Pyricularia oryzae* causing blast of rice from infected leaves of rice variety, IR 50 by standard method. The pure culture from single spore was maintained on apple agar medium for growth.

Harmon *et al.* (2003) isolated *Pyricularia oryzae* from perennial rye grass in India and cultures were maintained on V8 juice agar and potato dextrose agar.

Uddin *et al.* (2003) maintained the cultures of *Pyricularia grisea* on PDA during their study on detection of *Pyricularia grisea* causing leaf spot of perennial rye grass by Rapid Immuno-Recognition Assay.

Puri *et al.* (2009) isolated *Pyricularia oryzae* from rice leaves showing blast symptoms using standard tissue isolation technique and maintained the culture on oat meal agar and potato dextrose agar.

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

Rice leaves infected with blast were collected by Bonman *et al.* (1987) and isolated by placing each lesion in a moist Petri dish and incubated at 25^0c until sporulation. Conidia from the lesion surface were spread on to water agar and the germinating conidium was isolated and transferred to agar slants.

Correa and Zeigler (1993) collected leaves and panicles infected with rice blast from rice cultivars obtained from germ plasm bank at the Centro Internacional de Agricultura Tropical (CIAT) and the International Rice Research Institute (IRRI). They derived cultures from either mass or single conidial isolates obtained from single lesions. Cultures were maintained on V8 juice agar and multiplied for inoculations on rice-polish agar (Tuite, 1969) at 28^0c under continuous light. They stated that *M. grisea* expressed its virulence spectrum irrespective of geographical location.

Eight samples of rice leaves infected with blast were collected from commercial fields of upland rice cultivars in the state of Goias, Brazil (Silva *et al.*, 2009). Monoconidial isolates were obtained by directly transferring one conidium per lesion on 5% water agar from two to three lesions per leaf. The isolates from panicles in the majority of the cases were obtained from one conidium per panicle. The collected isolates were conserved on sterilized filter paper discs in a freezer at -20 ± 1^0c.

Blast affected leaves of rice cultivars were collected from rice fields in Guilan province of Iran. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed with sterile distilled water and placed on potato dextrose agar in Petri dishes at 25^0c for 2–3 days. Later, Petri dishes were incubated at 25^0c in the dark or artificial fluorescent light on a 12 h light/dark photoperiod for 15–25 days. Monoconidial isolates of the recovered fungi were maintained on half-strength potato dextrose agar slants in test tubes as stock cultures (Motlagh and Javadzadeh, 2010).

Priya Vanaraj *et al* (2013) isolated *Pyricularia oryzae* from blast lesions that surface sterilized with 0.1% mercuric chloride for 1 minute and placed over clean glass slides kept in sterile Petri dishes padded with moist cotton. The Petri dishes were incubated for 48 hours at room temperature (28 ± 2^0c). Single conidia were identified from the sporulating lesions using a stereomicroscope and aseptically transferred to potato dextrose agar (PDA) slants for maintenance. The causal organism was identified as *Pyricularia oryae* based on the spore morphology.

2.6. *In vitro* evaluation of fungicides against *Pyricularia oryzae*

Srivastava (1999) tested some selective fungicides against rice blast (*Pyriculariaoryae*) in Sikkim, India. He conducted trials in Tadong for three consecutive years using the susceptible rice variety, Pusa-33. Bavistin (Carbendazim), Kitazin (Iprobenfos), Cuman L (Ziram), Kavach (Chlorothalonil), Topsin M (Thiophanate-methyl) and Hinosan (Edifenphos).

All these fungicides reduced the disease incidence and increased the grain yield as compared to untreated control. Tricyclazole was the best fungicide in controlling the disease and increasing yield.

Hegade *et al.* (2000) reported that three sprays with Carbendazim (0.1%) or Pyroquilon (0.1%) resulted in the lowest leaf blast incidence.

Hossain and Kulkarni (2001) tested the efficacy of six systemic, four non-systemic fungicides against rice blast *Pyricularia grisea in vitro*. The systemic fungicides, i.e. Carbendazim 50 WP, Difenoconazole 10 WP, Hexaconazole 5 EC, Iprobenfos 48 EC, Propiconazole 25 EC and Tricyclazole 75 WP, were tested at 500 and 1000 ppm. The non-systemic fungicides, i.e. Chlorothalonil 75 WP, Copper oxychloride 50 WP, Mancozeb 75 WP and Iprodione 50 WP, were tested at 1000 and 3000 ppm. Among the systemic fungicides, Iprobenfos, Propiconazole and Carbendazim were the most effective, followed by Hexaconazole and Tricyclazole. Mancozeb was the best non-systemic fungicide in inhibiting the growth of *Pyricularia oryae.*

Tirmali *et al.* (2001) studied the efficacy of fungicides in controlling rice neck blast incidence. All the fungicidal formulations significantly reduced neck blast incidence as compared to the untreated control. Tricyclazole 75 WP @ 0.06% concentration (52.05% disease control) was most effective followed by 0.2 % Epoxiconazole + Carbendazim (46.30%), 0.2% Tebuconazole (42.7%), 0.1% Carpropamid (40.97%), 0.15% Tebuconazole 250EW (40.02%) and 0.05% Carpropamid (36.73%). Maximum grain yield (1179 kg/ha) was obtained with

Tricyclazole 75 WP @ 0.06% followed by 0.2% Epoxiconazole + Carbendazim (979 kg/ha).

Joshi and Mandokhot (2002) conducted a field trail to determine the efficacy of Tricyclazole 75 WP at different concentrations for controlling blast of rice. Among these Tricyclazole 75 WP at three concentrations viz., 0.12, 0.06, and 0.05 were found to be superior.

Sharma and Kapoor (2002) tested the efficacy of Prochloraz, Cyproconazole, Chlorothalonil, Hexaconazole, Propineb and Thiophanate-methyl, along with Tricyclazole and Carbendazim as standard controls, against *Pyriculariaoryae* inciting rice blast. None of the tested fungicides were superior to Tricyclazole. However, Prochloraz was comparable to Carbendazim. Three sprays each of Tricyclazole, Prochloraz, Carbendazim, Hexaconazole, Chlorothalonil and Cyproconazole at the tillering, booting and panicle emergence stages were effective in controlling rice blast.

Vijay (2002) tested the efficacy of Tricyclazole (0.1%), Thiophanate methyl (0.1%), Chlorothalonil (0.25%), Mancozeb (0.25%) and Carbendazim (0.1%) against rice blast caused by *Pyricularia oryzae*. The reduction in disease incidence was highest with Tricyclazole (0.1%) with 17.6 PDI followed by Thiophanate methyl (0.1%) with 19.3PDI.

Prabhu *et al.* (2003) stated that the two applications of Tricyclazole or Benomyl (0.250 kg/ha of a.i.) controlled panicle blast, as indicated by the low values of disease progress curve and relative panicle blast severity and increased grain yield of IAC 202. It is evident, from the AUDPC values of non-treated control that the mean panicle blast severity of four experiments was highest for IAC 202 followed by Caiapo, Araguaia and Rio Paranaiba. Two applications of Tricyclazole (0.250 kg/ha of a.i.) increased grain yield from 847 kg/ha to1,521 kg/ha in IAC 202, and were superior to one application at heading. One application of Tricyclazole at heading increased grain yield from 847 kg/ha to 1,203 kg/ha in IAC 202, and from 994 kg/ha to 1,331 kg/ha in Caiapo. Losses in grain yield of cultivars IAC 202, Caiapó, Rio Paranaíba and Araguaia due to panicle blast were 44.8%, 27.4%, 24.4% and 18.2%, respectively.

Dubey (2005) tested efficacy of different fungicides against blast (*Pyricularia grisea*) of rice. Among 13 fungicides, Carpropamid (0.1%) was the most effective fungicide for blast management, with minimum neck (1.1%) and node infections (1.7%), disease severity (3.8%) and maximum grain yield (4.54 t/ha), followed by Tricyclazole (0.03%) and Thiophanate methyl (0.5%).

Kumbhar (2005) evaluated five new fungicidal formulations viz., Antracol 70 WP (Propineb) (0.5%), Octave 50 WP (Prochloraz) (0.1%), Opus 12.5 SC (Epoxiconazole) (0.2%), Kavach 40 SC (Chlorothalonil) (0.2%) and Kavach 75 WP (Chlorothalonil) (0.1%) against rice blast in the field. All tested new fungicides significantly reduced the neck blast incidence by 7.63 - 26.30% over unsprayed control. Opus 12.5 SC (0.2%) was most effective followed by Octave 50 WP (0.1%), Antracol 70 WP (0.5%), Kavach 40 SC (0.2%) and Kavach 75 WP (0.1%).

Clara *et al.* (2007) tested eight fungicides viz., Carbendazim (0.05%), Thiophanate methyl (0.05%), Mancozeb (0.2%), Ediphenphos (0.05%), Kitazin (0.05%), Propiconazole (0.025%), Hexaconazole (0.005%) and Ridomil (0.15%) against blast disease in field. They found that all the tested fungicides controlled the disease but Carbendazim @ 0.05% was significantly superior in lowering the disease intensity (28.3%) followed by Thiophanate methyl @ 0.05% (29. %) and Mancozeb @ 0.2% (32.8%).

Lukose *et al.* (2007) tested eight fungicides under field conditions against blast disease of pearl millet (*Pyricularia grisea*). Carbendazim @ 0.05 concentrations was significantly superior in controlling the disease followed by Thiophanate methyl and Mancozeb.

Raji and Vimi (2007) tested Isoprothiolane (1.5 ml/lit) for the management of rice blast. All the three doses of the fungicide were effective in reducing the disease and increasing the yield. The lowest dose of Isoprothiolane @ 1.5 ml/lit reduced the leaf blast severity by 45.34% and neck blast severity by 16% and increased the yield by 26.9%.

Mandal and Jha (2008) conducted a field trial for controlling rice blast (*Pyricularia oryzae*) and brown spot (*Helminthosporium oryzae*) with different treatment combinations of fungicides. Among various treatment combinations, Carbendazim + Kasugamycin sprays at 1 ml/lit of water recorded the lowest incidence of both diseases.

Jamal *et al.* (2012) studied that five fungicides viz., Thiophanate-methyl (70%), Carbendazim (50%), Fosetyl-Aluminium (80%), Mancozeb (80%) and Copper oxychloride (50%) were tested by food poisoning method at 10000 ppm, 1000 ppm and 100 ppm by adding at the time of pouring after sterilization of PDA medium. Among tested fungicides, only Mancozeb appeared as highly effective against the M. oryzae, growth of the fungus was completely restricted at 1000 and 10,000 ppm of Mancozeb. All other fungicides failed to inhibit the mycelial growth of the fungus completely, mycelial growth of the test fungus at higher concentration (10,000ppm) of Thiophanate methyl was (20.84 mm), Carbendazim (20.66 mm), Fosetyl-Aluminium (12.80 mm) and Copper oxychloride (22.16 mm).

Naik *et al.*(2012) reported that all the ten fungicides viz., Dithane Z-78 (0.2%), Carbendazim (0.1%), Propiconazole (0.1%), Mancozeb (0.2%), Wettable Sulphur (0.3%), Thiophanate methyl (0.1%), Tricyclazole (0.06%), Benomyl (0.2%), Ediphenphos (0.1%) and Kitazine (0.1%) tested against rice blast proved to be effective in the management of the disease. Out of these, Tricyclazole (0.06%), Kitazine (0.1%) and Ediphenphos (0.1%) were found significantly superior to rest of the fungicides. Significant increase in yield was also recorded in these treatments.

In a trial conducted by Varma and Kumari (2012), the efficacy of five fungicides viz., Isoprothiolane 40% EC (at 1ml/l, 1.5ml/l and 2 ml/l), Carpropamid 27.8% SC (at 0.5 ml/l, 1ml/l and 2ml/l), Carbendazim 50% WP (at 0.75 g/l , 1g/l and 1.5g/l), Tricyclazole 75%WP (at 0.1g/l, 0.6g/l and 1g/l) and Propiconazole 25% EC (at 0.5ml/l, 0.75ml/l and 1ml/l) was evaluated against *Pyricularia grisea*. It was revealed from the results that, Isoprothiolane 40EC (1.5ml/L) significantly reduced the disease intensity (89.7%) which was followed by Carpropamid 27.8SC (1ml/L- 80.5%) and Carbendazim 50 WP (1.5ml/L-73.1%) in comparison to control.

Dehkaei *et al.* (2013), studied the efficacy of a combination fungicide (Tricyclazole + Thiophanate methyl 72.5%WP) against rice leaf, panicle, neck and node blast disease at different doses (400, 500 and 600 g/ha), in comparison with recommended fungicides Tricyclazole (500g/Ha), Carpropamid (400 ml/Ha) and Ediphenphos (1000 ml/Ha) under field conditions. Among all the fungicides under study, Carpropamid and Tricyclazole were significantly superior to rest of the treatments.

Kunova *et al.* (2013), in a study in vitro, found that the mycelial growth of Magnaportheoryzae was inhibited by Azoxystrobin at lower concentration but Tricyclazole was effective at relatively higher concentration. Sporulation was remarkably affected by both the fungicides at their lower concentration.

Bhojyanaik and Jamadar (2014) tested five systemic fungicides viz., Tricyclazole 75EC, Difenoconazole 25EC, Hexaconazole 5E, Propiconazole 25EC, Carbendazim 50WP each at three concentrations (500, 1000 and 1500ppm) against *Pyricularia grisea*. All the trizole group fungicides were effective and statistically at par at the highest concentration. Numerically, Tricyclazole gave maximum inhibition of the mycelial growth (90.06%)

followed by Difenoconazole (88.96%), Hexaconazole (87.93%) and Propiconazole (76.51%). Carbendazim was the least effective fungicide as it recorded only 59.17 per cent inhibition of the mycelial growth.

In a field trial conducted at Gujranwala, Iqbal *et al.* (2014) studied the efficacy of six fungicides such as Copper oxy-chloride (1.25kg/ha); Carbendazim (300g/ha); Chlorothalonil + Metalyxal (1.25kg/ha); Cymiching (1.25 kg/ha); Teboconazole + Floroxystrobin (162.5 g/ha); Teboconazole (1250 ml/ha) and one antibiotic- Kasugamycin (750 g/ha) for controlling blast in transplanted rice in three kharif seasons (2009-2011). The results revealed that Kasugamycin was superior to all the fungicides in all the three seasons.

Kapoor and Katoch (2014) proposed that seed dressing with either Tricyclazole or Pyroquilon followed by a spray of Bavistin (1g/L) at tillering and one spray of Hinosan (1g/L) at heading and one after flowering not only controls the disease but also increases the yield.

On the basis of three years experimentation, Joshi *et al.* (2014) recommended three sprays of Tricyclazole (0.1%) or Isoprothiolane (0.1%) at an interval of 21 days starting from the initiation of blast symptoms for effective management of blast disease of rice.

Singh *et al.* (2014) evaluated seven fungicides, viz., Zineb (0.25%), Tebuconazole (0.1%), Propiconazole (0.1%), Difenoconazole (0.1%), Tricyclazole (0.1%), Azoxystrobin (0.1%) and Azoxystrobin+ Difenoconazole (0.1%) and one antibiotic Kasugamycin (0.1%), against *Pyricularia grisea*.

Maximum disease control was observed in Tebuconazole (83.9 %) followed by Propiconazole (0.1%), Azoxystrobin + Difenoconazole (0.1%), Tricyclazole (0.1%) and Difenoconazole (0.1%) proved significantly superior in controlling the disease with disease severity values of 6.5, 7.0, 7.3, 8.0 and 8.3 per cent, respectively compared with 40.3 per cent in untreated control. Kasugamycin (0.1 %), Azoxystrobin (0.1%) and Zineb (0.25%) proved least effective in controlling the disease.

Fryod *et al.* (1976) reported that the application of Tricyclazole (Beam) a systemic fungicide as seed treatment, foliar spray, soil drenching of nursery bed can give long term effective for blast control.

Tsai *et al.* (1981) reported that beam 75 WP (Tricyclazole), Benzothiazole gave effective control of leaf and neck blast at low volume spraying by motorized mist blower at a flow rate of 0.61/min.

Tripathi *et al.* (1995) reported that Tricyclazole (Beam) gave more effective control than Iprobenfos. Efficacy of seed treatment Fongorene @4g/kg and foliar spray of Bavistin @ 0.05 at various crop stages was also tested against leaf blast. Apparent infection rate found higher ranging from 0.85 to 0.297 at 64 to 71 days 11 after sowing. Foliar spray of Bavistin in nursery tillering and at PI stages was found to be significantly superior in controlling the disease 43.47% and in increasing the grain yield 19.57%.

Saiffula *et al.*, (1998) found that Tricyclazole resulted in resulted in 37.50% reduction in leaf blast and increase the yield up to 307.50% over untreated check.

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 panicle initiation stage, was found to be, the best for blast control (39.20%) and increasing the yield (31.81%).

Garcia Fernandez (2001) found that yield losses caused by *Pyricularia oryzae* use of resistant cultivar and the application of Tricyclazole, Tebuconazole, Isoprothiolane, Kasugamycin, Prochloroz, and Carbendazim + Flusilazole, what maybe manage of leaf blast.

Joshi and Mandokhot (2002) a field experiment was conducted 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 rice yield. Beginning one month after planting, 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 3 concentration of Tricyclazole were significantly superior to the control in reducing disease intensity. There was a liner relationship between disease intensity and yield.

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

Tirmali *et al.* (2004) found that brine solution+ seed dressing with Carbendazim (3g/kg) 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 that there was no phytotoxicity effect at different concentrations of Azoxystrobin. 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.

Khalko and Pan (2009) observed insignificant growth (21.00 mm) in the presence of Difenoconazole 25 EC at 0.32 micro litre/litre, and growth was not observed at higher concentrations. Prochloraz 45 EC and Flusilazole 40 EC totally inhibited the growth of T. harzianum even at 8.0 micro litre / litre. Difenoconazole 25 EC appeared to be partly tolerated by T. harzianum only at lower concentrations.

Varma and Santha Kumari (2012) in vitro study was conducted to evaluate the efficacy of five fungicides against rice blast pathogen, Magnaporthe oryzae, by poisoned food technique. The fungicides were Isoprothiolane 40% EC (Fuji-one) at 1, 1.5 and 2.0 ml, carpropamid 27.8% SC (Protiga) at 0.5, 1.0 and 2.0 ml/l, Carbendazim 50% WP (Bavistin) at 0.75, 1.0 and 1.5 g/l, Tricyclazole 75% WP (Beam) at 0.1, 0.6 and 1.0 g/l and Propiconazole 25% EC (Tilt) at 0.5, 0.75 and 1.0 ml/l. Isoprothiolane at 1.5 ml/l recorded the maximum inhibition of mycelial growth (94.85%), followed by Carpropamid at 1.0 ml/l (91.48%).

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 higher extent than for conidia produced on Azoxystrobin-amended media, even though germination of such conidia was reduced after Azoxystrobin treatment.

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 as compared to the other tested fungicides.

Shiba and Nagata (1981) reported that tricyclazole inhibited mycelial growth, conidial germination and appressorial formation of P. oryzae at concentrations less than 125 ppm. In main field it completely protected the plants from the disease by foliar application at as low as 10-20 ppm.

Carreres *et al.* (1986) evaluated four fungicides (thiabendazole, prochloraz, isoprothiolane and tricyclazole) against blast and found isoprothiolane reduced leaf blast and tricyclazole and isoprothiolane were effective in reducing neck and node infection when the fungicides were applied as fortnightly foliar sprays after disease symptoms appeared (post panicle initiation to flowering).

Singh and Singh (1994) tested the efficacy of new fungicide formulations against neck blast of paddy, viz., tricyclazole, propiconazole, bitertinol, chlorothalonil, carbendazim and blasticidin by spraying twice first at the time of disease initiation on leaves and then at 50% panicle emergence stage. All the test fungicides significantly reduced the disease incidence over check but tricyclazole and propiconazole proved to be the most effective in reducing the disease incidence and increasing grain yield.

Saifulla (1994) tested tricyclazole, blasticidin, carbendazim, cyproconazole, chlorothalonil, bitertanol, propiconazole and hexaconazole against *P. oryzae* in Karnataka using susceptible variety Mandya Vijaya. He reported that maximum disease control and yields were achieved with tricyclazole and blasticidin.

Sood and Kapoor (1997) evaluated seven fungicides against leaf and neck blast of rice caused by *M. grisea*. Fungicides were sprayed at the recommended rates at booting and heading stage. Tricyclazole was found to be most effective in reducing leaf and neck blast by 89.2% and 97.5% and increased yield by 43.3% as compared with untreated control.

Dubey (2000) evaluated twelve fungicides against rice blast. The spray schedule of tricyclazole @ 0.03% and Indifil M-45 (mancozeb) resulted in minimum neck and node infection resulting in higher grain yield.

Santosh *et al.*(2000) reported lowest leaf and neck blast incidence by applying tricyclazole alone or in mixtures.

Tirmali and Patil (2000) tested five fungicide formulations, viz., propineb, capropamid, fluquinconazole, prochlorez, epoxyconazole and tricyclazole. They reported that all the formulations reduced neck blast incidence by 16.27 to 29.23% while epoxyconazole (12.5 SC) being highly effective (29.23%). Tricyclazole (75 WP) showed superiority over all the formulations in reducing neck blast incidence by 50.06% and increasing grain yield by 89.13% followed by epoxyconazole with an yield increase of 41.30% over unsprayed control.

Vijaya (2002) evaluated tricyclazole (0.1%), thiophenate methyl (0.1%), chlorothalonil (0.25%) and carbendazim (0.1%) against rice blast caused by P. grisea and concluded that disease occurrence was lowest with tricyclazole (17.6%) followed by thiophenate methyl (19.3%) and the highest yield was obtained with tricyclazole (5.7 t h^{-1}) followed by thiophenate methyl (5.6 t h^{-1}).

Coresti and Guidietta (2003) compared the efficacy of azoxystrobin and trifloxystrobin to tricyclazole and tricyclazole + propiconazole applied at the beginning of stem elongation and at late booting. They concluded that two applications of azoxystrobin (250 g ha^{-1}) and triflixystrobin (125 g ha-^{1}) were more effective than tricyclazole (225 g ha^{-1}). However the difference between two treatments of strobilurins and one treatment of tricyclazole at 450 g ha-^{1} was not significant. They reported that both strobilurins and tricyclazole were highly effective against leaf blast and neck blast with 90-100% and 75-90% reduction in disease occurrence respectively.

Prajapati *et al.* (2004) tested cyproconazole 50 EC (0.1%), tricyclazole 75 WP (0.045%), carbendazim 50 WG (0.05%), bitertenol 25 WP (0.025%) and propiconzole 25 EC (0.025%) against carbendazim 50 WP (0.05%) as standard control using a highly susceptible rice cultivar Pankhali 203. They concluded that tricyclazole was significantly superior in reducing the leaf blast and neck blast by 62.9 and 64.1% respectively.

Ali Anwar and Bhat (2005) studied efficacy of isoprothiolane 40 EC (1ml/kg), tricyclazole 75 WP (0.6 g/l), edifenphos 50 EC (1ml/kg), hexaconazole 5 EC (0.5ml/kg) and mancozeb 75 WP (3g/kg) as seed treatment against rice blast using variety K 39. They stated that isoprothiolane 40 EC (1ml/kg) and tricyclazole 75 WP (0.6 g/l) were superior as seed treatment with maximum protection against leaf blast up to 35 days, while the leaf blast symptoms were observed in edifenphos 50 EC treated plots at 30 days. Mancozeb 75 WP was the least effective of all the fungicides tested.

Dubey (2005) studied the efficacy of caprapomid (0.1%), tricyclazole (0.03%), thiophenate methyl (0.05%), carbendazim (0.05%), chlorothalonil (0.15%), validamycin (0.05%) and copper oxychloride (0.2%) against rice blast in the main field using susceptible rice cultivar Birsa Dhan 202. He concluded that caprapomid was the most effective fungicide for blast management with the minimum neck (1.1%) and node infections (1.7%), PDI (3.8%) and maximum grain yield (4.54 t ha -1) followed by tricyclazole and thiophenate methyl.

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

Muralidharan (2005) reported that tricyclazole and carbendazim were the most effective fungicides and application of these fungicides increased yield by 41 and 87% in 2000 and 2001 with greater reduction in the neck blast incidence (28-35% reduction).

Ghazanfar *et al.* (2009) tested nine fungicides, i.e, tetrachloropthalide, tebuconazole + trifloxystrobin, sulphur, copper Sulphate, difenconazole25%, propiconazole + tricyclazole, propiconazole + difenconazole and propiconazole. They reported that all the fungicides were found effective in management of rice blast disease but tetrachloropthalide, tebuconazole + trifloxystrobin and difenconazole proved to be effective and reduced the disease percentage by 11.4, 12.5 and 12.85, respectively.

Narayana Swamy *et al.* (2009) reported that application of Nativo (trifloxystrobin + tebuconazole) @ 0.4 g/l was found most effective in controlling leaf blast to an extent of 84% compared to control followed by Filia (tricyclazole + propiconazole) and Gain (tricyclazole).

Singh *et al.* (2011) tested fernoxyl + isoprothiolane (RIL013SF 35SC), isoprothiolane, metominostrobin and tricyclazole against neck blast incidence. They found that out of the four fungicides, fernoxyl + isoprothiolane (RIL 013SF 35SC) @ 2.0 ml/l was found most promising fungicide in reducing the disease incidence (12.8%) with higher grain yield 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}. The metominostrobin found least effective against neck blast with disease incidence of 27.3% and grain yield 2550 kg ha^{-1} compared to control (29.7% and 2780 kg ha^{-1}).

CHAPTER 3

MATERIALS AND METHODS

The various aspect of present investigation on blast disease of rice (*Oryza sativa L.*) incited by *Pyricularia oryzae* were conducted in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 during 2016-2018.

The materials used and methods or techniques adopted during the course of present investigations are describe in this chapter.

3.1. Experimental site

Survey was conducted in different districts of Bangladesh namely Mymensingh, Kishoreganj, Barishal, Naogaon and Cumilla. Isolation of *Pyricularia oryzae,* determination of cultural characteristics and *in vitro* study on efficacy of fungicides were tested in the Plant Pathology laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka -1207.

3.2. Survey on incidence and severity of rice blast and collection of diseased sample

The survey on rice blast disease was conducted in farmers' fields of Bangladesh during Boro (November to May; irrigated ecosystem) season. Disease incidence and severity was recorded during survey of farmers' field.

Disease incidence was recorded on the basis of the percent diseased panicle present in the field. Disease severity was recorded as 0 to 9 scale (Table 1) developed by International Rice Research Institute (IRRI, 2014)

Table 1. Scale for panicle blast disease based on symptom

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

The diseased leaves and panicle of rice showing typical symptom of rice blast were collected from surveyed area. Samples were sun dried and put into brown paper envelop and were brought to the laboratory for further studies.

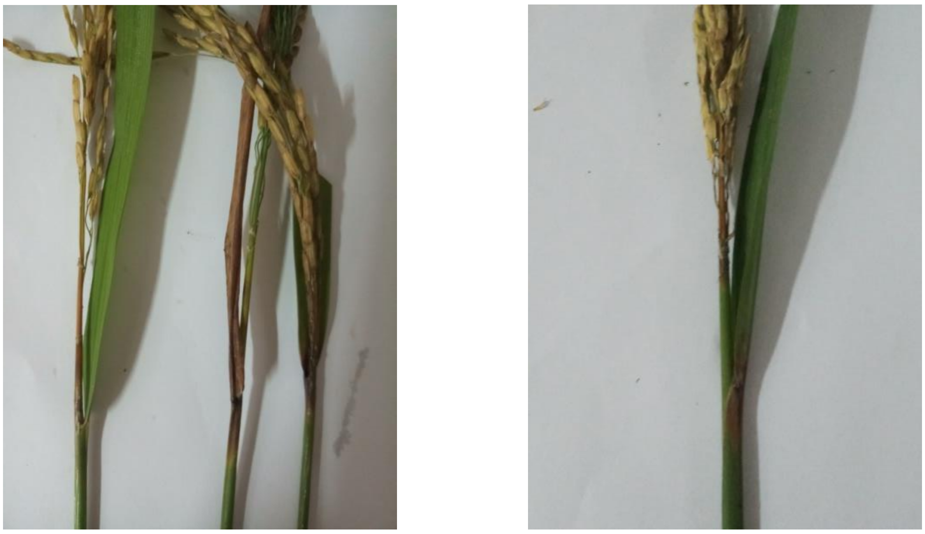

Figure 1. Blast infected sample collected from different districts of Bangladesh in Boro season 2017-18

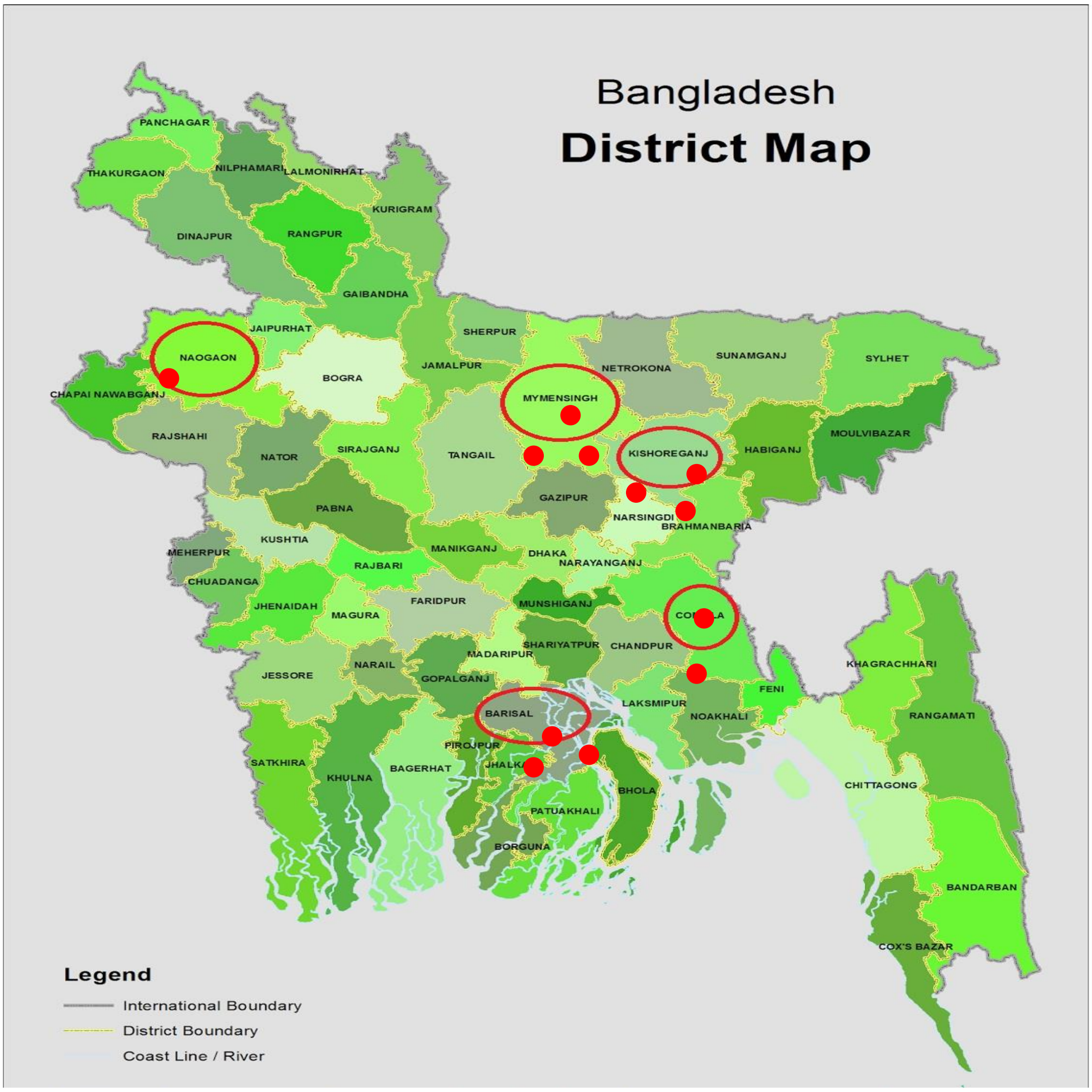

Figure 2. Rice blast survey and sampling area (●) of different districts of Bangladesh (http://maps-of-bangladesh.blogspot.com/)

3.3. Culture medium used and preparation

Water agar (WA) and Potato Sucrose Agar (PSA) medium was used for isolation, purification of the causal organism from the infected rice blast samples.

3.3.1. Water agar (WA) medium preparation

Thirty gram of agar was put it in a 2-liter capacity flask. 1000 ml distilled water was mixed with agar in the flask. Then the flask was plugged with cotton and autoclaved for 15 min at 15 psi. (Hayashi *et al*, 2009).

3.3.2. Potato sucrose agar (PSA) medium preparation

Two hundred gram of potato was boiled in 500 ml distilled water for one hour. After boiling, potato juice was filtrated through cheese cloth or nylon mesh. 20 g Sucrose and 20 gm Agar was poured into the potato juice and the volume was adjusted to 1000 ml by adding distilled water. Finally, the flask was plugged with cotton and autoclaved for 15 min at 15 psi (Okunowo, 2010).

3. 4. Isolation of *Pyricularia oryzae*

The necrotic patches of diseased leaves were cut into small pieces. These pieces were surface sterilized by dipping in mercuric chloride solution (1:1000) for one minute and were washed by sterilized water for several times. The cut pieces were inoculated in sterilized Petri dish containing potato dextrose agar medium (Riker, 1936) under aseptic condition and kept in incubator at 25 ± 1^0c for development of fungal growth. The fungus cultures were also maintained in culture tube to avoid contamination.

3.5. Purification of *Pyricularia oryzae*

Isolation techniques were used for getting pure culture of the fungus was on sterilized PDA plates.

The marginal mycelial growth that developed subsequently was picked-up aseptically for sub-culturing. The sub culturing was done at an interval 15 days and preserved at low temperature (5 ± 1^{0}C) in refrigerator.

3.6. Purification and maintenance of pure culture

When pure growth of the fungus was achieved, 5 mm culture discs of the fungal mycelium were cut with the help of sterilized cork borer and transferred aseptically in oat meal agar slants and allowed to grow. The pure culture slants were sealed with paraffin wax and stored in a refrigerator for further use.

3.7. Identification of the causal organism

The pathogen isolated from the diseased specimen and established in pure form was identified on the basis of colony and morphological characters.

3.8. Pathogenicity Study

A pot culture technique was used to prove the pathogenicity of the test organism. The blast susceptible BRRI dhan 28 seeds were sown in sterilized earthen pots containing sterilized soil + Farm Yard Manure (FYM) (1:1). The seedlings with vigorous growth were selected for artificial inoculation. Then the pathogen was inoculated by spraying over the seedlings. After spraying the seedlings were covered with polythine paper. The observations on the development of symptoms were recorded daily for a period of 15 days from the day of inoculation.

3.9. Mycelial growth and morphological characterization

Mycelial growth of *Pyricularia oryzae* on PDA was recorded at 7 Days after Inoculation (DAI), 14 DAI and 21DAI. Data on spore morphology viz. shape, size and colour of conidia were also determined.

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

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

3.10. *In vitro* efficacy of different fungicides against *Pyricularia oryzae*

Poisoned Food Technique (Nene and Thapliyal, 1993) was applied in present assay for determination of fungicides efficacy. Each fungicide with a control were tested against the *Pyricularia oryzae*. PSA was used as basal medium and distributed in 100 ml aliquots in each 250 ml Erlenmeyer flasks, which were 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°C. 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 sterile cork borer. Each disc was transferred aseptically to the center of each Petri plate, already poured with poisoned medium. The PSA plates without fungicide (with water only) were also inoculated and maintained as control. The plates were incubated at 27 ± 1°C for 7 days. Four replications per treatment were maintained. The observations on colony growth were recorded until Petri plate in control treatment was fully covered with mycelial growth.

3. 11. Percent growth inhibition

Percent inhibition of growth of test fungus was calculated by the following formula (Horsfall, 1956).

$$X = \frac{Y}{Z} \times 100$$

Where,

X = Percent inhibition

Y = Growth of fungus in control (mm)

Z = Growth of fungus in treatment (mm)

3.12. Treatments used in this study

Eight fungicides and a control (No fungicide) were used as treatment in *in vitro* experiment. The name and concentration of different treatments were enlisted in Table 2.

Table 2. Details of fungicides tested in this study

Treatments	Trade name of Fungicides	Dose (ppm)	Active ingredient	Company name
T_1	Amister Top 250 SC	100	Azoxystrobin + Difenoconazole	Syngenta Bangladesh
T_2	Autostin 50 WDG	100	Carbendazim	Auto Crop Care
T_3	Azonil 56 SC	100	Azoxystrobin + Chlorothalonil	Haychem Bangladesh Ltd.
T_4	Dithane M-45	100	Mancozeb	Bayer CropScience Ltd.
T_5	Filia 525 SE	100	Tricyclazole + Propiconazole	Syngenta Bangladesh
T_6	Nativo 75 WP	100	Trifloxystrobin + Tebuconazole	Bayer CropScience Ltd.
T_7	Seltima 100 CS	100	Pyraclostrobin	BASF
T_8	Trooper 75 WP	100	Tricyclazole	Auto Crop Care Ltd.
T_9	Control (Only water)	-	-	-

Figure 3. Fungicides used in this study(A. seltima 100 CS, B. Amister Top 250 SC, C. Filia 525 SE, D. Azonil 56 SC, E. Dithane M-45, F. Trooper 75 WP, G. Autostin 50 WDG, H. Nativo 75 WP)

3.13. *In vitro* experimental design

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

3.14. Statistical analysis of data

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

CHAPTER 4

RESULTS AND DISCUSSIONS

Blast of rice, caused by *Pyricularia oryzae* is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favorable conditions. During the last few years, incidence of leaf and neck blast was observed regularly during both the seasons in some pockets of the northern and southern region of Bangladesh. This changing pattern of occurrence of blast in Bangladesh may be due to changes in climatic conditions and adoption of new susceptible varieties for cultivation by the farmers. Occurrence the disease in both the seasons created interest to conduct this study.

In lieu of this, the present study was taken to initiate the work on survey, isolation, pure culture and *in vitro* evaluation of fungicides against rice blast causal organism *Pyricularia oryzae*. The results of the experiments conducted on these lines are presented in this chapter.

4.1. Survey on rice blast disease

In Boro season 2017-18, a survey was done in different districts of Bangladesh. The blast disease incidence and severity were recorded during the survey is shown in Table 3.

Table 3. Survey on blast disease in different districts of Bangladesh in Boro season 2017-18

Name of districts	Name of Upazillas	Varieties	No. of field visited	Blast disease	
				Incidence (%)	Severity Score
Mymensingh	Gafargaon	BRRI dhan28,BRRI dhan81	7	30	5
	Bhaluka	BRRI dhan28, BRRI dhan63	10	20	3
	Muktagacha	BRRI dhan29,BRRI dhan81	15	60	5
Kishoreganj	Hossainpur	BRRI dhan28	8	20	7
	Mithamain	BRRI dhan29	10	20	5
	Pakundia	BRRI dhan28, BRRI dhan63	6	30	5
Barishal	Mehendigaj	BRRI dhan74, BRRI dhan89	5	10	3
	Babuganj	BRRI dhan74	10	5	3
	Bakerganj	BRRI dhan89	12	5	1
Naogaon	Manda	Zira dhan, BRRI dhan63	8	20	5
Cumilla	Chandina	BRRI dhan28, BRRIdhan29	10	50	5
	Burichang	BRRI dhan28, BRRI dhan29	9	30	3
Total 5	12	7	110		

From the survey, it is exposed that the highest incidence of blast disease was recorded from Muktagachha (60%) and severity score was 5. The highest severity score of blast disease was observed in Hossainpur, Kishoreganj (7) but percent incident was observed only 20%.

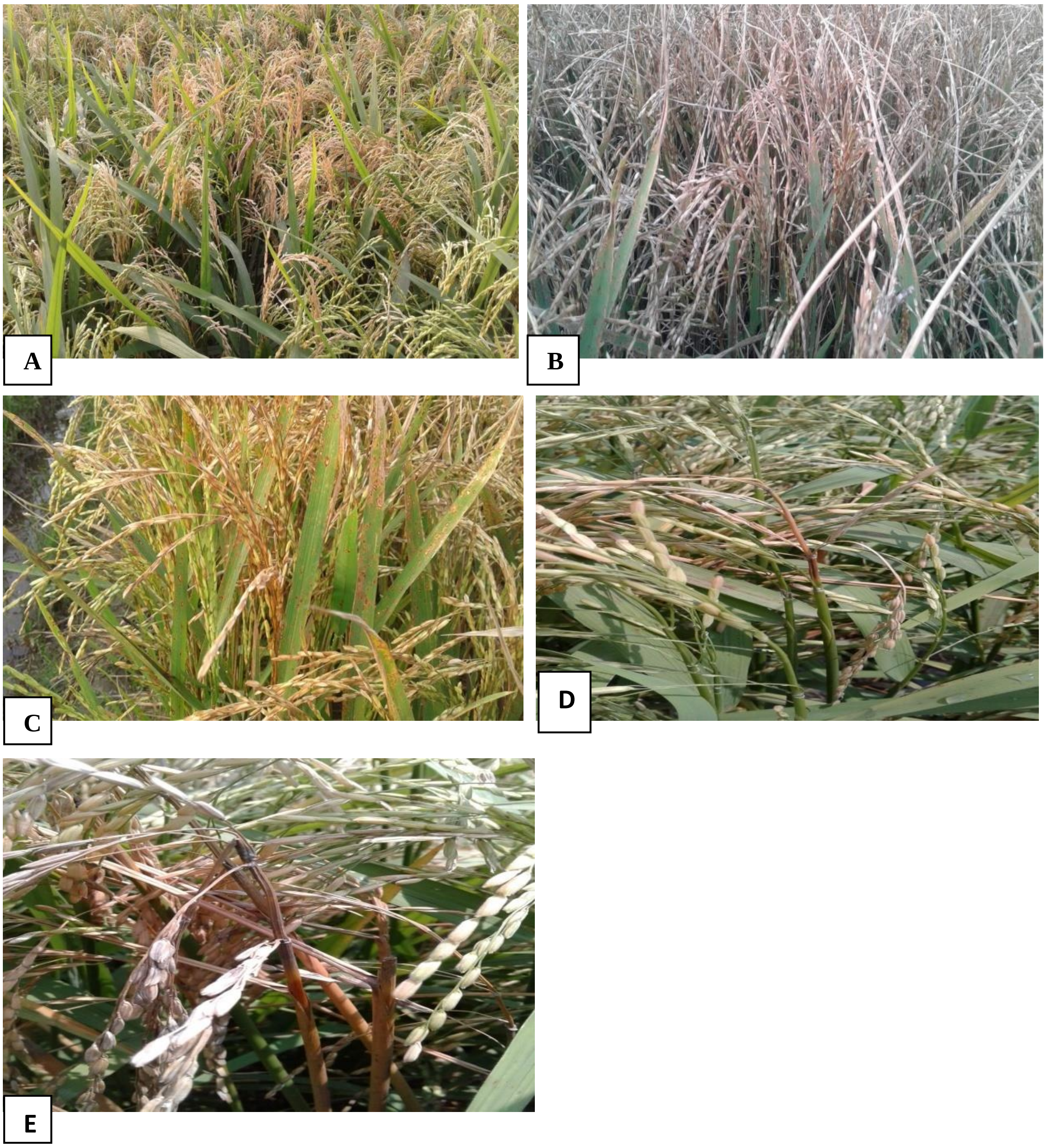

Figure 4. Field view of infected rice in Mymensingh and Kishoreganj (A,B and C); Field view of infected neck seen in Barishal, Naogaon and Cumilla region (D and E)

4.2. Isolation and pure culture of *Pyricularia oryzae*

Collected infected leaf and panicle samples were cut in 3-5 cm sections. These sections were surface sterilized by dipping in 10% Clorox (The Clorox Company, USA) for one minute and were washed by sterilized water for several times and then the cut sections were placed on moist filter paper (Whatman: 9.0cm) in a sterile petri dish (Figure 5). Plates were incubated for 24 hours at room temperature (24^0C). After 24 hours the infected parts were examined under stereo microscope (Motic SMZ-168). Conidial masses (Figure 6) were picked by using very fine tip needle and spread on 3% water agar plate. After then the agar plate was put on the stage of stereo microscope inside out. A single conidia was focused on the agar plate. Single conidia on plate were picked by using needle and then transferred it to another agar plate. That plate was incubated at room temperature for 2 or 3 days and finally the mycelium was transferred to potato sucrose medium plates (Hayashi, 2009).

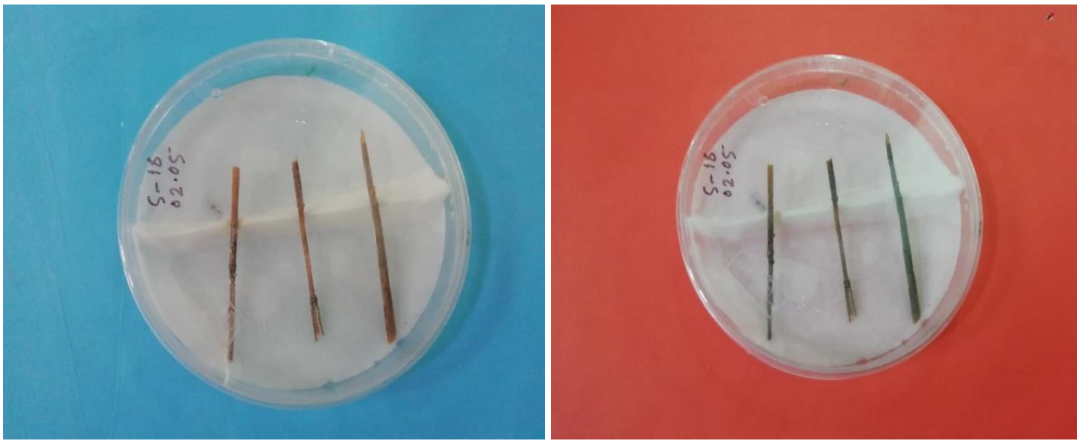

Figure 5. Infected neck segment of panicle in moist chamber

Figure 6. Conidial mass seen under stereomicroscope (×50)

4.3. Confirmation of *Pyricularia oryzae*

Conidial masses were picked by using very fine tip needle and placed in glass slide. Then the slide was observed under compound microscope with cover slip. Typical pyriform conidia was found (Figure 7)

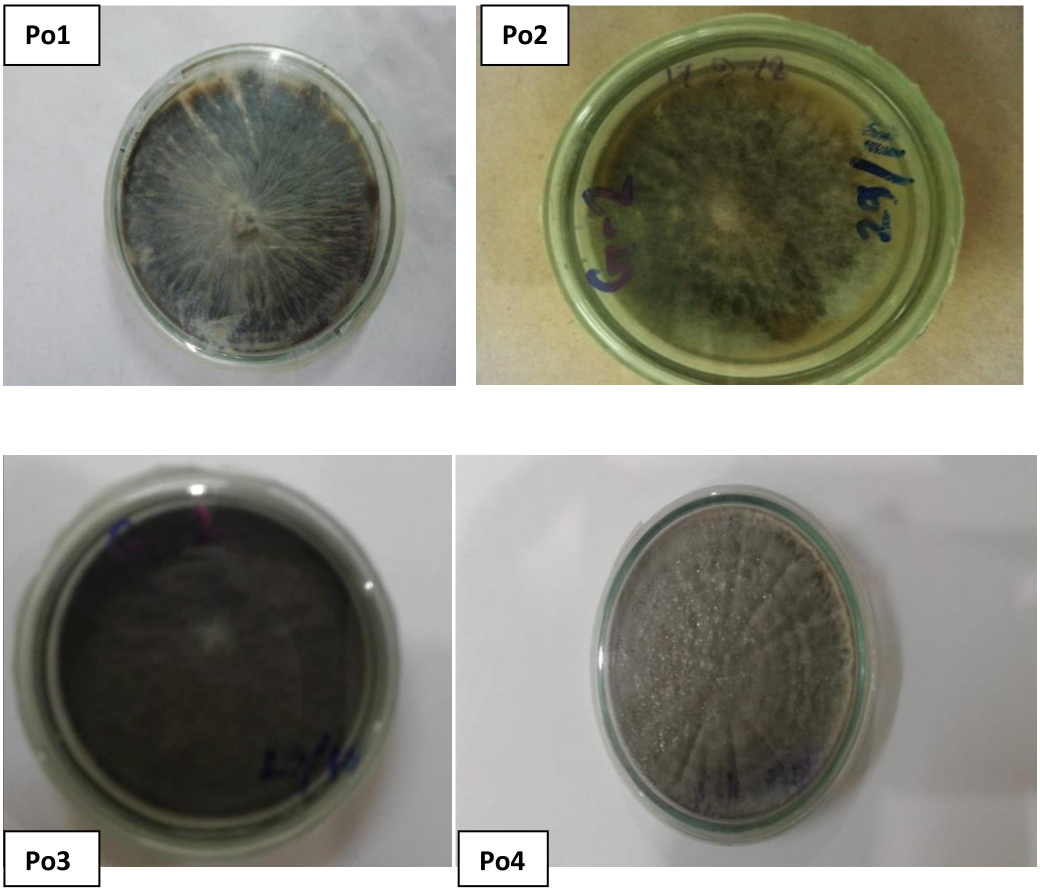

Figure 7. Pure culture of four isolates of *Pyricularia oryzae* on potato sucrose agar (PSA)

Figure 8. Mycelial growth of four isolates of *Pyricularia oryzae* on oat meal agar

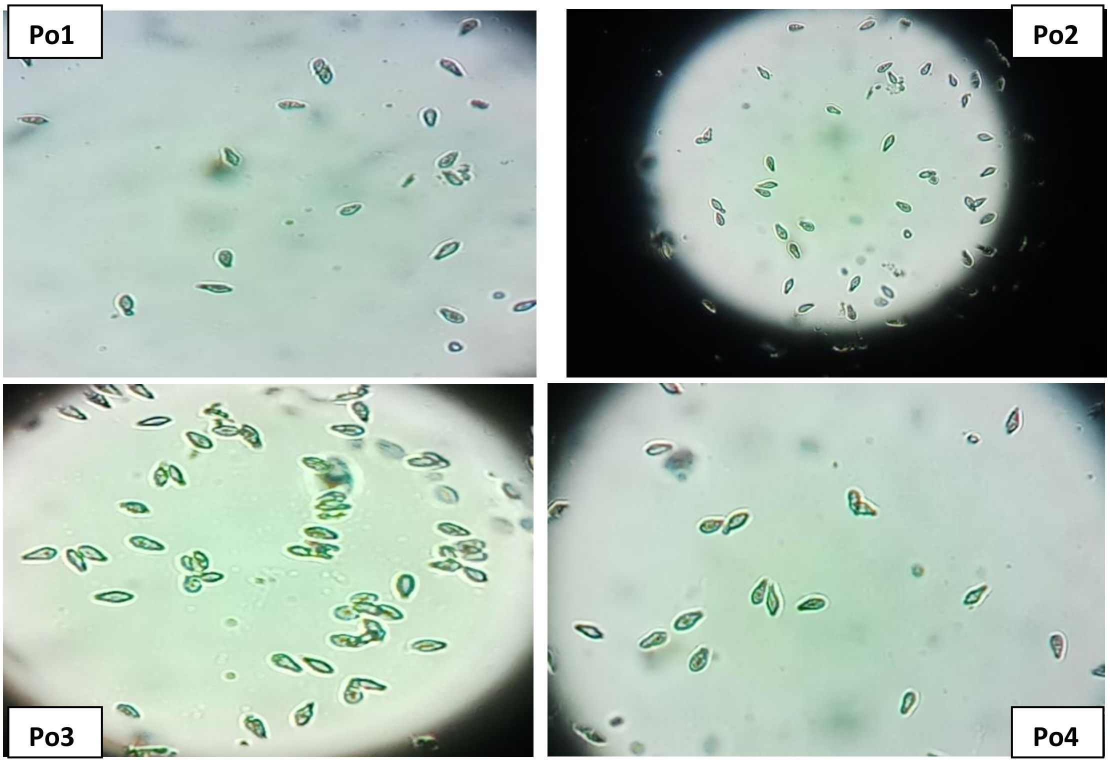

Figure 9. Conidia of four isolates of *Pyricularia oryzae* on oat meal agar observed under compound microscope (×200)

From the Table 4, the higest mycelial growth (79.50 mm) was observed in case of the isolates *Po*4 and the lowest mycelial growth (68.33 mm) was observed in case of the isolates *Po*1. The mycelial growth of isolates *Po*3 and *Po*4 nearly similar. *Po*3 showed the highest sporulation (4) and *Po*1 and *Po*4 showed the lowest (2).

Table 4. Mycelial growth and sporulation of four isolates of *Pyricularia oryzae* on oat meal agar

Isolates of *P. oryzae*	**Mycelial growth (mm)**	**Sporulation Index**	
		Index	**Sporulation Type**
Po 1	68.33c	2	Fair
Po 2	74.83b	3	Good
*Po*3	77.67a	4	Excellent
Po 4	79.50a	2	Fair
CV(%)	1.88		

4.4. *In vitro* mycelial growth at 7 days after inoculation

Eight fungicides belonging to different groups were tested *in vitro* for their efficacy against *Pyricularia oryzae*, by employing poisoned food technique and using Potato Sucrose Agar (PSA) as basal medium. The data obtained on the effect of various fungicides *in vitro* on the vegetative growth and inhibitions of the pathogen.

In vitro Mycelia growth at different treatment found significantly different (Table 5 and Appendix I). Maximum growth inhibition of *Pyricularia oryzae* was achieved with Trooper (80.29%), Filia (76.19%) and Nativo (71.15%), which was significantly different and superior to rest of the treatments (Figure 10). The next fungicides in order of inhibition merit were Seltima and Azonil with 64.65% and 63.94% inhibition of the test fungus as compared to control. Trooper (80.29%) was found to be the best effective fungicide. Dithane M-45 (27.40%) was found to be the least effective fungicide against *Pyricularia oryzae*.

Joshi and Mandokhot (2002) conducted a field trial to determine the efficacy of Tricyclazole 75 WP at different concentrations for controlling blast of rice. Among these, Tricyclazole 75 WP was found to be superior at three concentrations (0.12, 0.06 and 0.05%).

Vijay (2002) tested the efficacy of Tricyclazole (0.1%), Thiophanate methyl (0.1%), Chlorothalonil (0.25%), Mancozeb (0.25%) and Carbendazim (0.1%) against rice blast caused by *Pyricularia oryzae*.

The reduction in disease incidence was the highest with Tricyclazole (17.6 %) followed by Propiconazole (19.35%). The application of Tricyclazole resulted in least blast severity (18.31%) with significantly higher grain yield (45.66 q/ha).

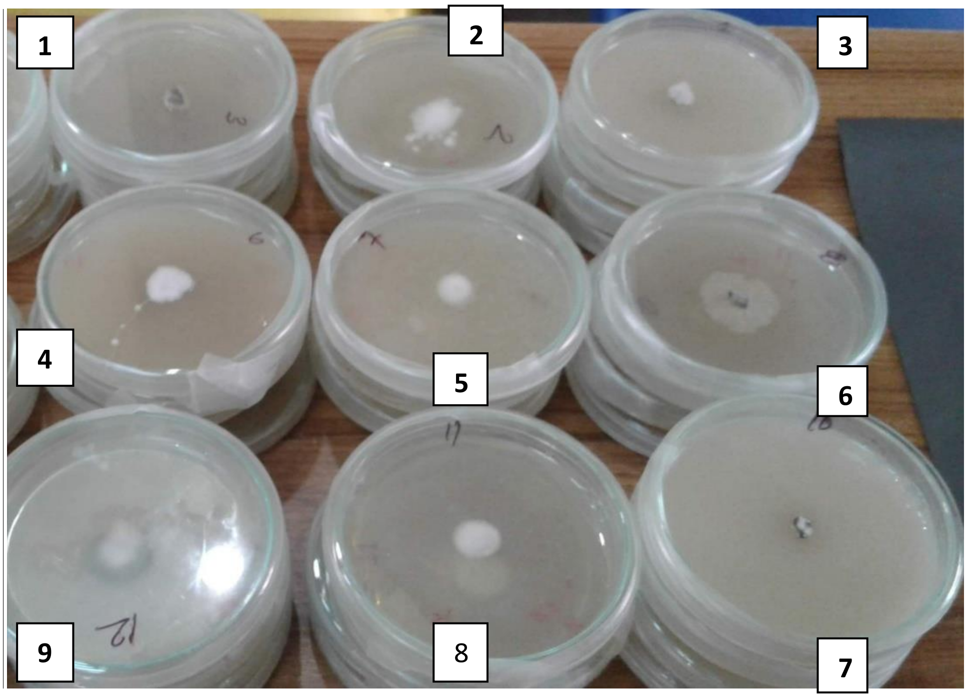

Figure 10. *In vitro* mycelial growth of *Pyricularia oryzae* at 7 days after inoculation

Table 5. Effect of fungicides on mycelial growth and percent inhibition *in vitro* at 7 days after inoculation (DAI)

Treatments	Mycelial growth (mm) at 7 DAI	% inhibition of mycelia growth over control
T_1= Trooper 75 WP	10.25 f	80.29
T_2= Filia 525 SE	12.38 ef	76.19
T_3= Nativo 75 WP	15.00 e	71.15
T_4= Amister top 250 SC	24.75 c	52.40
T_5= Azonil 56 SC	18.75 d	63.94
T_6= Dithane M-45	37.75 b	37.75
T_7 = Autostin 50 WDG	25.62 c	50.73
T_8= Seltima 100 CS	18.38 d	64.65
T_9=Control	52.00 a	-
±SE	1.63	-
CV(%)	9.64	-

4.5. *In vitro* mycelial growth at 14 days after inoculation

In vitro Mycelial growth of *Pyricularia oryzae* on different combinations at 14 DAI was found significantly different (Table 6 and Appendix II). The maximum inhibition of *Pyricularia oryzae* was achieved with Trooper (76.72%), Filia (73.48%) and Nativo (67.89%), which was significantly different and superior to rest of the treatments (Figure 11). The next fungicides in order of merit were Seltima and Azonil with 60.78% and 62.10% inhibition of the test fungus as compared to control. Trooper (76.72%) was found to be the best effective fungicide. Dithane M-45(29.10%) was found to be the least effective fungicide. These results are in agreement with those reported by Hossain and Kulkarni (2001) who found Tricyclazole and Propiconazole are to be effective fungicides followed by Tebuconazole and Carbendazim. Dubey (2005) found Tricyclazole (0.1%) to be the most effective fungicide followed by Tebuconazole (0.02%) and Carbendazim (0.2%) in control of rice blast. Similar results were also recorded by Bhojyanaik *et al*. (2014) who tested five systemic fungicides viz., Tricyclazole 75EC, Difenoconazole 25EC, Hexaconazole 5E, Propiconazole 25EC, Carbendazim 50WP each at 500 ppm, 1000 ppm, 1500 ppm against *Pyricularia oryzae*. Among these tricyclazole 75EC (1500 ppm), gave maximum inhibition of the mycelial growth (90.06%) of the pathogen followed by Difenoconazole 25EC (1500 ppm) (88.96%), and Propiconazole 25EC (1500 ppm) (76.51%) and were found to be on par with each other as well as significantly superior over Carbendazim 50WP (1500 ppm) (59.17%) which was found to be the least efficient in inhibiting mycelial growth of the pathogen.

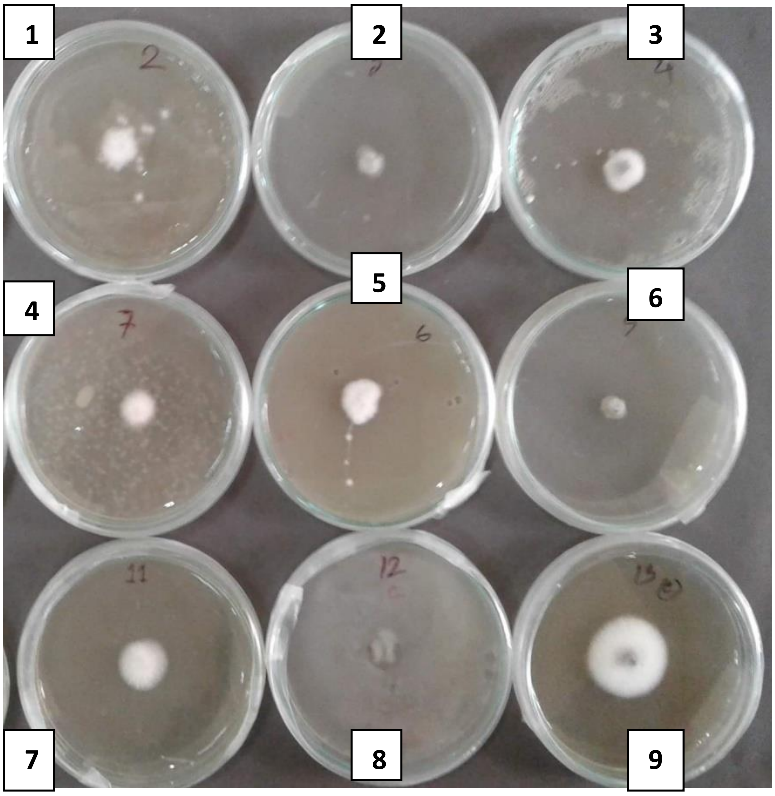

Figure 11. ***In vitro*** **mycelial growth of** ***Pyricularia oryzae*** **at 14 days after inoculation**

Table 6. Effect of fungicides on mycelial growth and percent inhibition *in vitro* at 14 days after inoculation (DAI)

Treatments	**Mycelial growth (mm) at 14 DAI**	**% inhibition of mycelia growth over control**
T_1= Trooper 75WP	13.5 h	76.72
T_2= Filia 525SE	15.38 gh	73.48
T_3= Nativo 75 wp	18.62 fg	67.89
T_4= Amister Top 250SC	28.38 d	51.07
T_5= Azonil 56SC	21.98 ef	62.10
T_6= Dithane M-45	41.12 b	29.10
T_7 = Autostin 50WDG	35.5 c	38.79
T_8= Seltima 100CS	22.75 e	60.78
T_9=Control	058 a	-
±SE	1.86	-
CV(%)	9.29	-

4.6. *In vitro* mycelial growth at 21 days after inoculation

In vitro Mycelial growth of *Pyricularia oryzae* on different combinations at 21 DAI was found significantly different (Table 7 and Appendix III). The maximum inhibition of *Pyricularia oryzae* was achieved with Trooper (71.46%), Filia (71.26%) and Nativo (64.57%), which was significantly different and superior to rest of the treatments (Figure 12). The next fungicides in order of merit were Seltima and Azonil with 59.06% and 58.08% inhibition of the test fungus as compared to control. Trooper (71.46%) was found to be the best effective fungicide. Dithane M-45 (28.94%) was found to be the least effective fungicide. The results are in concurrence with those of Joshi *et al.* (2014) who reported three sprays of Tricyclazole (0.1%) or Isoprothiolane (0.1%) are recommended at an interval of 21 days starting from the initiation of blast symptoms for effective management of blast disease of rice. Similar results were also recorded by Bhojyanaik *et al.* (2014) who tested five systemic fungicides viz., Tricyclazole 75EC, Difenoconazole 25EC, Hexaconazole 5E, Propiconazole 25EC, Carbendazim 50WP each at 500 ppm, 1000 ppm, 1500 ppm against *P. oryzae.* Among these tricyclazole 75EC (1500 ppm), gave maximum inhibition of the mycelial growth (90.06%) of the pathogen followed by Difenoconazole 25EC (1500 ppm) (88.96%), Hexaconazole 5E (1500 ppm) (87.93%) and Propiconazole 25EC (1500 ppm) (76.51%) and were found to be on par with each other as well as significantly superior over Carbendazim 50WP (1500 ppm) (59.17%) which was found to be the least efficient in inhibiting mycelial growth of the pathogen.

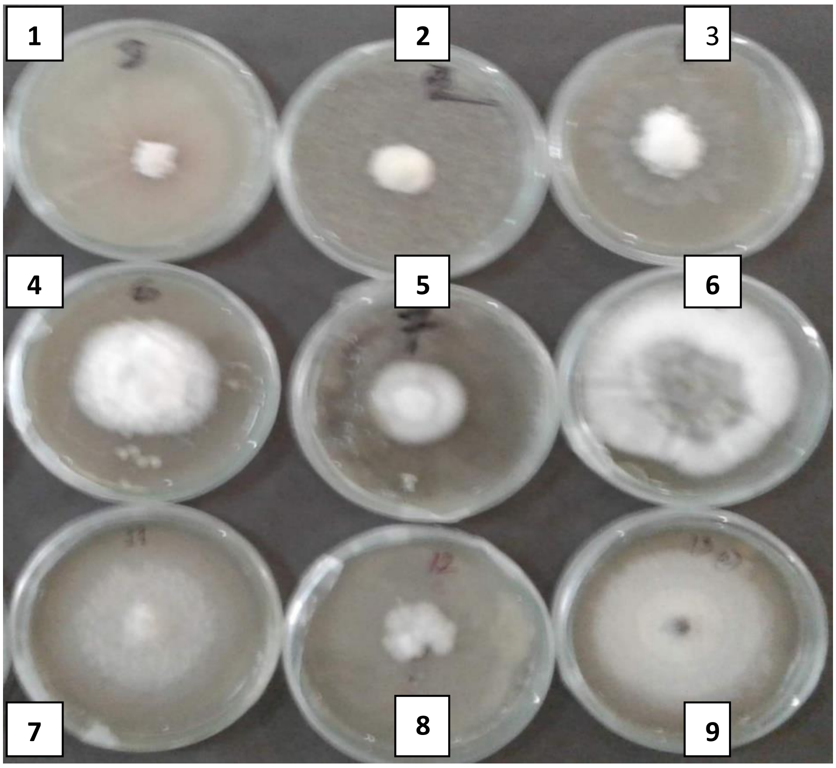

Figure 12. ***In vitro*** **mycelial growth of** ***Pyricularia oryzae*** **at 21 days after inoculation**

Table 7. Mycelial growth and percent inhibition by different treatment on *in vitro* at 21 days after inoculation (DAI)

Treatments	**Mycelial growth (mm) at 21 DAI**	**% inhibition of mycelia growth over control**
T_1= Trooper 75WP	18.12f	71.46
T_2= Filia 525SE	18.25 ef	71.26
T_3= Nativo 75 wp	22.50 de	64.57
T_4= Amister top 250SC	32.75 c	48.43
T_5= Azonil 56SC	26.0d	59.06
T_6= Dithane M-45	45.12 b	28.94
T_7 = Autostin 50WDG	41.12 b	35.24
T_8= Seltima 100CS	26.62 d	58.08
T_9=Control	63.50 a	-
±SE	2.11	-
CV(%)	9.14	-

CHAPTER 5

SUMMARY AND CONCLUSION

Rice (*Oryza sativa L.*) is the most important staple food grain crop of the world which constitutes the principle food for about 60 per cent of the world's population. It contributes 43 percent of total food grain production and 46 per cent of total cereal production in India. Rice based production system provides the income and employment for more than 50 million households. One third of Asia's rice production is consumed in China and one fifth in India. Rice is known to be attacked by many pests and diseases which cause huge losses annually worldwide. Among fungal diseases of rice, rice blast caused by *Pyricularia oryzae* is of significant economic importance. The blast disease of rice (*Oryza sativa* L.) caused by *Pyricularia oryzae* was noticed in severe form at different districts of Bangladesh during boro season, 2018. Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world. It is estimated that each year enough of rice is destroyed by rice blast alone to feed 60 million people. The survey on rice blast disease was conducted in farmers' fields of Bangladesh during Boro (November to May; irrigated ecosystem) season. From the survey it is exposed that highest severity score of blast disease is observed in Hossainpur, Kishoreganj (7) but percent incident is only 20%. The highest incidence of blast disease is recorded from Muktagachha (60%) and severity was 5. Eight fungicides and a control (No fungicide) were used as treatment *in vitro* experiment. Poisoned Food Technique was applied in present assay for determination of fungicides efficacy. Each fungicide with a control were tested against the *Pyricularia oryzae*. The pathogenic fungus was isolated on Potato Sucrose agar (PSA) medium from infected leaves of rice. On the basis of typical symptoms and microscopic observations, was identified as *Pyricularia oryzae*. Efficacy of fungicides was assessed by poisoned food technique. It was revealed that, all the fungicides tested caused significant inhibition of pathogen but Trooper (80.29%), Filia (76.19%) and Nativo (71.15%) were the most effective fungicide followed by Seltima (64.65%), Azonil (63.94%), Amister Top (52.40%), Autostin (50.73%) and Dithane M-45 (27.40%) were the least effective fungicide. From the above result it can be concluded that in all cases Trooper (71.46%) was found to be the best effective fungicide. Dithane M-45 (28.94%) was found to be the least effective fungicide. From above results, Trooper (Tricyclazole) was found the best effective fungicide in *in vitro* test against the test fungus. Thus it is suggested that a field trial should be performed for testing the field performance of Trooper 75 WP, which can be effective for reduce the blast disease of rice in field condition.

CHAPTER 6

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CHAPTER 7

APPENDICES

Appendix-I: ANOVA for Mycelial length at 7 DAI

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	8	5762.1250	720.2656	135.93	0.0000
Error	27	143.0625	5.2986		
Total	35	5905.1875			

Appendix-II: ANOVA for Mycelial length at 14 DAI

Source	DF	Sum of Square	Mean Square	FValue	Pr(> F)
Treatment	8	6595.5800	824.4475	118.72	0.0000
Error	27	187.5075	6.9447		
Total	35	6783.0875			

Appendix-III: ANOVA for Mycelial length at 21 DAI

Source	DF	Sum of Square	Mean Square	F Value	Pr(>F)
Treatment	8	7124.2500	890.5312	99.87	0.0000
Error	27	240.7500	8.9167		
Total	35	7365.0000			