Study on Blast Disease of Rice and It's Management strategies

A thesis

Submitted to the Uttar Banga Krishi Viswavidyalaya

in partial fulfillment of the requirements for the Degree of

Master of Science (Agriculture)

In

PLANT PATHOLOGY

By

Suman Dutta

Registration No.: A-2015-31-M

DEPARTMENT OF PLANT PATHOLOGY

Faculty of Agriculture

UTTAR BANGA KRISHI VISWAVIDYALAYA

Pundibari, Cooch Behar, West Bengal-736165

2017

Dedicated

To

My beloved PARENT

A strong and gentle soul of my life who taught mebeing patient in hard times and believe in hard work and that so much could be done with little

My Grand parents

For being my first teachers and without whom I will be nothing today

My family

For the emotional strength which you are all giving till today

Farmers 'society and all my well wishers

UTTAR BANGA KRISHI VISWAVIDYALAYA

DEPARTMENT OF PLANT PATHOLOGY

FACULTY OF AGRICULTURE

Pundibari, Cooch Behar, West Bengal -736165, India

From:

Dr. Sekhar Bandyopadhyay

Assistant Professor (Stage 3)

Phone no: 91+9434685676

E mail: sekhar29@gmail.com

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled ***"Study on Blast disease of rice and it's management strategies"****submitted by* ***MR. SUMAN DUTTA*** *in partial fulfillment of the requirements for the degree of Master of Science in Agriculture (Plant Pathology) of Uttar Banga Krishi Viswavidyalaya, is a faithful record of bona-fide research work carried out under my personal supervision and guidance. Results of the thesis have not been submitted for any other Degree or Diploma. Assistance and help received during the course of investigation has been duly acknowledged.*

Place: Pundibari, Cooch Behar **(Dr. Sekhar Bandyopadhyay)**

Dated: ……..……., 2017 **Chairman of the**

Advisory committee

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)

We, the undersigned, having been satisfied with the performance of Mr. Suman Dutta (A-2015-31-M), in viva-voce on Final Evaluation of thesis, conducted today, the ……..…. 2015, recommend that the thesis be accepted for the award of the degree of Master of Science(Agriculture) in Plant Pathology of Uttar Banga Krishi Viswavidyalaya.

Sl.No	**Name**	**Signature**
1	Dr. Sekhar Bandyopadhyay Assistant Professor (Stage 3) & Chairman, Advisory Committee, Department of Plant Pathology	_____
2	(External Examiner)	_____
3	Dr. P.M. Bhattacharya Member, Advisory Committee, Department of Plant Pathology	_____
4	Dr. Surojit Khalko Member, Advisory Committee, Department of Plant Pathology	_____
5	Dr. Biplab Mitra Member, Advisory Committee, Department of Agronomy	_____
6	Dr. Ayon Roy Head, Department of Plant Pathology	_____
7	Dean, Post Graduate Studies	_____

ACKNOWLEDGEMENT

This work would not have been completed without the providence of the Almighty God whose help and grace was always eminent.

It is a moment of great pleasure to put in record my heartfelt gratitude and indebtedness to honourable guide and chairman of my Advisory Committee ***Dr. Sekhar Bandyopadhyay****, Assistant Professor (Stage 3), Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal for his able magnificent guidance, inspiration, constructive criticism and encouragement during the course of investigation and preparation of the thesis.*

I am highly thank full to ***Dr. P. M. Bhattacharya****, Associate Professor, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal and Member of my Advisory Committee for his keen interest, help and co-operation during the course of my investigation.*

I am highly obliged to the members of by Advisory Committee namely, ***Dr. S. Khalko,*** *Assistant Professor (Stage 2), Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal,* ***Dr. B. Mitra****, Assistant Professor, Department of Agronomy, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal for their generous help, valuable suggestions and necessary help provided during the course of present investigation.*

I take this opportunity to express my gratitude to ***Dr. Ayon Roy****, Associate Professor & Head, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya for his generous help and co-operation during my entire M. Sc. programme.*

I would like to convey my thanks to ***Dr. Apurba Kumar Chowdhury****, Professor, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya for his generous support, encouragement and technical help during the course of my investigation.*

I am grateful to convey my deep sense of regards to ***Dr. A. Debnath, Miss. S. Das*** *and* ***Dr. S. Hembram****, Assistant Professor in Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal for their valuable and kind co-operation during the course of study.*

Sincere thanks are extended to ***Rabi Da, Aminul Da, Ajit Da, Sukumar Da, Somnath Da*** *and special thanks to* ***Soumendu Da & Amar Da****, and all the non-teaching staffs of the Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari and Library staffs of the University.*

A Special word of thanks to ***Bijan Da, Barun Da, Dolpriya Di, Abhishek Da, Sunu Da, Ranjana Di, Swarnavo Da, Asha Di*** *for their valuable suggestions, co-operation and encouragement during my study.*

I express my heartfelt thanks to my friends like ***Shankar, Bivas, Prantik, Tanmoy, Abhijit, Susmita, Soumen, Samaresh, Rakesh*** *and all friends help and constant enlivening company and good wish that greatly expedited my thesis work and the course of study.*

I give heartfelt thanks to my junior ***Pulak, Aparajita, Sini*** *and* ***Asaha*** *for their help and co-operation during my study period.*

Words are not enough to express my affection and heartfelt thanks to my adorable parents ***Mr. Ajit kr. Dutta*** *and* ***Mrs. Jharna Dutta****, my grand father late* ***Kalabaron Dutta****, my grand mother* ***Dasibala Dutta****, my beloved sister* ***Deepshika & Riya*** *and my beloved brother* ***Ananta, Anirban & Raju****, and my Uncle* ***Mr. Shemal Dutta****, without whose inspiration, support and affection, I would have never achieved this level, who forever existing smile on my face have embraced all sorts of pains, grief and worries that they came across. I felt as the "God" himself has incarnated in the form of my beloved parent.*

I would like to record my special thanks the department of Plant Pathology for providing all facilities during the course of my study.

In thanking all those people, I must emphasize that I am fully responsible for any shortcoming that remains.

Place: Pundibari, Cooch Behar ----------

Date: (Suman Dutta)

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS AND ABBREVIATIONS

%	per cent
C	Celsius
µm	micrometer
cm	centimeter
g	gram
h	hours
ha	hectars
i.e.,	that is
l	liter
µl	micro liter
°C	degree celcius
mg	milligram
ml	milli liter
mm	milli miter
t/ha	Tons per hectare
CRD	Completely Randomized Design
RBD	Randomized Block Design
et al.	and others
No.	Number
RH	Relative Humidity
Viz.,	Namely
PDI	Per cent Disease Index
PDS	Per cent Disease severity
@	At the rate of
SE (m)	Standard Error of mean
DAT	Days After Transplanting
DAI	Days After Inoculation
AUGPC	Area Under Growth Progress Curve
BOD	Biological Oxygen Demand
PDB	Potato Dextrose Browth
cfu	Colony forming unit
AUCTPC	Area Under Canopy Temperature Progress Curve
AUCTDPC	Area Under Canopy Temperature Depression Progress Curve
AUSDC	Area Under Spad Decline Curve
CTD	Canopy temperature depression
HCL	Hydrochloride acid
AUDPC	Area Under Disease Progress Curve

CHAPTER- 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the grain with the second highest worldwide production after maize (Boumas, 1985). Rice grain contains on an average 7% protein, 62-65 % starch, 0.7% fat & 1.3% fibre and rice is a main source of vitamin B1 (thiamin), B2 (riboflavin), B3 (niacin) & B5 (pantothenic acid).The Biological value of rice is 63% whereas Biological value of wheat & maize is 49% & 36%. The domesticated rice comprises two species of food crop in the Poaceae ("true grass") 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 MT of paddy (FAO, 2012). Rice (*Oryza sativa* L.) 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, especially poor. It is the main staple food in the Asia and the Pacific region, providing almost 39 % of calories (Yaduraju, 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 per cent of the world supply is consumed by people in Asian countries and thus rice is of immense importance to food security of Asia. In Asian countries more than 80% people taken 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 occupies a pivotal place in Indian agriculture and is the staple food for more than 65 per cent of the Indian population, accounting for more than 43 per cent of total food grain production and 55 per cent of cereals production in the country. In India it is a means of livelihood for millions of rural households. The present annual rice production in India is about 104.40 million tons from around 42.4 million ha area and a productivity of 2.46 tons ha^{1}

(Anonymous, 2013). The productivity of rice should be increased to about 3.25 t ha^{-1} to meet the projected demand since the scope for increasing area under rice cultivation is limited.

Rice [*Oryza sativa*] is a major staple food and a mainstay for the rural population and their food security. It is widely cultivated in India, China, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Japan, Philippines and Brazil. China is the leading rice producer followed by India, Indonesia and Bangladesh in 2013-14. India was the largest exporter of rice in 2013-14 followed by Thailand, Vietnam and USA. Developing countries account for 95% of the total production, with China and India alone responsible for nearly half of the world output. Rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5%.

India is one of the world's largest producers of white rice and brown rice, accounting for 20% of all world rice production. Rice is India's pre-eminent crop, and is the staple food of the people of the eastern and southern parts of the country. The regions cultivating this crop in India are distinguished as the western coastal strip, the eastern coastal strip, covering all the primary deltas, Assam plains and surrounding low hills, foothills and Terai region along the Himalayas and states like West Bengal, Bihar, Eastern Uttar Pradesh, Eastern Madhya Pradesh, Northern Andhra Pradesh and Orissa.India's rice production has increased at compound annual growth rate (CAGR) of 3.32 per cent during 2002-03 and 2013-14. Area under rice cultivation has not increased substantially during 2002-03 and 2013-14. Rice production during 2014 was 88.02 Million Tons with a cultivable area of 101.92 M ha (Department of Agriculture, 2015). In Andhra Pradesh, the crop is grown to an extent of 3.62 Million hectares with a production of 11.4 MT and productivity of 3173 Kg ha^{-1} (IPNI, 2013).In West Bengal rice is grown approximately 5.9 M ha with a production of 15-16 million tons.

The International Rice Research Institute, Philippines, estimates that in order to feed the growing global population, rice production must increase by another one-third by the year 2020.
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 *Magnaporthe oryzae* is of significant economic importance.

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 (Zeigler, Leong, & Teng, 1994).

Rice crop is subjected to attack by 50 diseases 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). The major rice diseases that often cause great economic losses are rice blast (*Magnaporthe grisea*), sheath blight (*Rhizoctonia solani*), Bacterial blight (*Xanthomonas oryzae*) and Tungro virus disease especially in South and South East Asia (Ling, 1980).

Rice blast and brown spot were the major diseases noticed during pre independent India and before introduction of high yielding varieties. After introduction of HYV, along with them, BLB, tungro and sheath blight have become major diseases. Recently diseases like sheath rot, false smut, stem rot and grain discolouration which were minor and occurring sporadically are emerging and causing considerable yield loss. This is primarily due to climate change, crop intensification and changes in practice. Out of the total yield loss due to diseases in rice, 35% is by blast, 25% by sheath blight, 20% by BLB, 10% by tungro and remaining 10% by other diseases. In the present study, the author will concentrate on blast and sheath blight of rice since both are most prevalent in West Bengal particularly during kharif season.

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; Scardaci *et al.*, 1997).

Serious yield losses due to epiphytotic of blast diseases have been recorded in different regions in India, such as Tanjore delta, Nellore, Hyderabad, Bombay, parts of Orissa, Kashmir & Kerala. In India first recorded outbreak of blast in 1918 in Tanjore district of Tamil Nadu was reported by MacRae who estimated the loss as 69% (Thomas & Krishnaswamy, 1948). In 1952, the crop was completely wiped out in Deras Farm in Orissa. In 1955-56 season the early rice was severely damaged by blast.

Keeping these facts in view the present investigation was carried out to "Studies on Blast Disease of rice and it's management strategies.**"** with the following objectives:

Objectives:

(1) Isolation and establishment of Koch's Postulate for leaf blast pathogen of rice.

(2) Morphological and cultural characterization of the pathogen.

(3) Chemical management strategies against the pathogen (*Pyricularia grisea*).

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), rice gall midges, insects (Alam, 1988) 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, 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 Toenniessen 1991). Fifty percent of the production area is located in China and India.

2.2 Importance of blast disease of rice:

Magnaporthe grisea (Anamorph *Pyricularia grisea* Sacc. synonym *Pyricularia oryzae* Cav.) causes rice blast disease in rice cultivation areas worldwide (BPS, 2010; Chin, 1975; Kato, 2001). 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; Scardaci *et al*., 1997).

Rice blast is one of the most important diseases of rice, caused by the fungus *Magnaporthe oryzae* B. C. Couch (Couch and Kohn 2002). One of the main limitations in production is rice blast disease caused by the fungus *Magnaporthe 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 (Georgopoulos and Ziogas, 1992; Moletti, 1988; Mbodi *et al.*, 1987; 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 & 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, Howard, Chumley, & Valent, 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).

2.3 Nature and disease symptoms:

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).

Magnaporthe oryzae infects and produces lesions on the following parts of the rice plant: leaf (leaf blast), leaf collar (collar blast), culm (culm nodes), panicle neck node (neck rot) 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, 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 *P. 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 *P. 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).

2.4 Occurrence & distribution:

Rice blast disease is distributed in about 85 countries in all continents where the rice plant is cultivated, in both low land and upland conditions. Rice blast is present wherever rice is cultivated, but the disease occurs with highly variable intensities depending on climate and cropping system. Environments with frequent and prolonged dew periods and with cool temperature in daytime are more favorable to blast (Chiba *et al*., 1996; Liu *et al*., 2004).

In Pakistan during the last two decades, rice blast is mostly found in districts of Faisalabad, Toba Tek Singh, Vehari and places like Gaggoo Mandi (Arshad *et al*., 2008). Rice blast has been recorded in the Northern Territory (Stahl 1955; Heaton 1964), Brazil (Prabhu and Morais,1986), Queensland, Australia (Perrot 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 serious and recurrent problem in all rice growing regions of the world. 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 (Supriya Devi

& G D Sharma, 2010). It is estimated that each year enough of rice is destroyed by rice blast alone to feed 60 million people (Zeigler, Leong, & Teng, 1994).

It was first reported as 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 by Astolifi (1828) and in India it was first reported in 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 found in approximately 85 countries throughout the world (Kapoor Pooja and Abhishek Katoch, 2014**).**

The disease is also a major problem in Penna river belts and Godavari in Andhra Pradesh (http://www.rkmp.co.in). 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 as well as in submerged or deep water rice. Rice blast is the most serious disease found in the extensive rice areas of Latin America, Africa, and Southeast Asia and is a worldwide problem in rice production. Rice blast disease is a significant constraint to global food security and agricultural trade (Leong, 2004).

In the West African sub-region, 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 a number of 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 an important disease in Ghana by Clerk (1974) and Oduro (2000). Various reports by Twumasi (1996, 1998), Twumasi and Adu-Tutu (1995), Nutsugah (1997 a, b) and Nutsugah and Twumasi (2001) have identified the blast disease of rice as a serious threat to rice production in Ghana.

2.5 About *Magnaporthe grisea*:

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* (Habert, 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.) Sacc instead 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 (CMI, 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).

SYSTEMATIC POSITION:

Kingdom: **Fungi**

Phylum: **Ascomycota**

Class: **Sordariomycetes**

Order: **Magnaporthales**

Family: **Magnaporthaceae**

Genus: ***Pyricularia*** **(Anamorph)**

Magnaporthe (Teleomorph)

Species: ***grisea***

2.6 Isolation of *P. grisea:*

Padmanabhan *et al.* (1970) isolated to *P. 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 + B & T at 25^0C.

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 *et al*. (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 25ºC for 2–3 days. Later, Petri dishes were incubated at 25ºC 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) Blast lesions were surface sterilized with 0.1% mercuric chloride for 1 minute and placed over clean glass slides kept in sterile Petri dishes padded with moist cotton. The Petri dishes were incubated for 48 hours at room temperature (28±2°C). Single conidia were identified from the sporulating lesions using a stereomicroscope and aseptically transferred to potato dextrose agar (PDA) slants for maintenance. The causal organism was identified as *Pyricularia oryae* based on the spore morphology.

2.7 Sporulation of the pathogen:

Culturing of different isolates of *Pyricularia oryzae* was studied by Priya Vanaraj *et al.* (2013) and reported that colonies of *P. oryzae* appeared as white on oat meal, rice polish and malt extract agar, 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. The spores of rice isolates from Erode and Gopichettipalayam were significantly smaller in length and width.

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

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

Mycelium in cultures was first hyaline in colour, then changed to olivaceous, 1 – 5.2 µm in width, septate and branched. The spore measurements were 15 – 22 µm × 4 – 7 µm (Average, 17.4 µm × 5.2 µm) (Mijan Hossain, 2000).

Ram *et al.* (2012) found isolates of the fungus from different hosts differed in their response in media for mycelial growth and sporulation. Radial mycelial growth and days of sporulation of *P. grisea* were studied by culturing three fungal isolates from rice, finger millet and *Panicum* sp. on six different media: prune agar (PA), oat meal agar (OMA), potato dextrose agar (PDA), finger millet leaf decoction agar, finger millet polish agar (FPA) and finger millet meal agar. The highest RMG was found in the isolates from finger millet and the lowest in the isolates from rice. The shortest days of sporulation (1 week) was found in the isolate from rice and the longest (>2 weeks) in the isolate from finger millet. Among the different media used, PA and OMA were found to be the best for mycelial growth and sporulation of the isolates both from rice and finger millet. The shape, color and compactness of the fungal colonies varied with the media and isolates used. Cross inoculation studies showed that the fungus isolates from rice were able to infect all the plant species while isolates from finger millet were only able to infect three plant species (*E. coracana*, *Setaria* sp. and *E. indica*).

2.8 Morphological characters of the pathogen:

Nishikado (1917) described morphology of *P. grisea* spores which measured 16 – 33 × 5 – 9 µm. Usually 22 – 27 × 7 – 8 µm with a small basal appendage, other dimensions were, basal appendage 1.2 – 1.8 (1.6) µm in width, basal cell 4.8 – 11.5 (7.8µm), middle cell 1.8 – 11.5 (6.6 µm), apical cell 6 – 14 (7 µm) in length.

Tochinai and Shimamura (1932) classified 39 isolates into nine forms on the basis of cultural characteristics. On steamed rice straw, the conidia of the isolates belonging to four forms were short, the mean value ranged from 19.3 to 22.8µm. The conidia of other five forms were long, the mean value ranged from 26.8 to 29.9µm. All isolates from the affected spikes or glumes of rice plants were of the long conidium type, while most isolates from the nodes were of the short conidium type. This suggests considerable difference in the length of conidia among the isolates of *Pyricularia* on rice.

Aoki (1955) measured 16 isolates in potato dextrose agar culture and showed that, the average length of the isolate ranged from 21.2 to 28.4µm, and the average width from 7.3 to

9.0µm. Ono and Nakazato (1958) observed that, the size of conidia of *P. grisea* varied with the culture media also. Mijan Hossain (2000) observed mycelium in cultures was first hyaline in colour, then changed to olivaceous, 1 – 5.2 µm in width, septate and branched. The spore measurements were 15 – 22 µm × 4 – 7 µm (Average, 17.4 µm × 5.2µm).

Linear growth of the colonies of the *Pyricularia* isolated from rice was measured on standard medium agar, oat meal agar, french bean agar and decoction agar made out of the leaf material of rice. He also determined the weight of mycelial mat produced by the isolates in the standard medium, Richards's medium, Browns medium and decoctions of leaf material of rice. The isolates produced good growth on the decoctions of their host material (Ramakrishnan, 1948). Veeraraghavan and Padmanabhan (1965) reported that the dimensions of conidia produced by *P. oryzae* ranged from 17.6 to 24.0 µm in length and 8.0 to 9.6 µm in width.

2.9 Cultural characterization of the pathogen

2.9.1 Growth of the pathogen in different media:

Ravindramalviya (2014) used four culture media for the study of mycelial growth of *P. grisea* under *in vitro.* Among them 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, Czapek-Dox medium was not found effective for both vegetative growth and sporulation of the test pathogen.

Mahdieh S (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 light was provided either continuously or at intervals. A combination of 16/8 hr light/darkness intervals and adding rice materials to culture media could induce *P. oryzae* for abetter sporulation.

Sun *et al.* (1989) studied the effects of 17 media on 41 isolates of *P. oryzae.* They found that, corn meal and rice straw agar media were most conducive for sporulation. Arunkumar and Singh (1995) studied *Pyricularia grisea* (*M. grisea*) from rice on different solid culture media. They found that, maximum colony diameter of rice isolate occurred on malt extract agar and Leonin agar.

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

Culturing of different isolates of *Pyricularia oryzae* was studied by Priya Vanaraj *et al.* (2013) and reported that colonies of *P. oryzae* appeared as white on oat meal, rice polish and malt extract agar, 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. The spores of rice isolates from Erode and Gopichettipalayam were significantly smaller in length and width.

Du Xinfa *et al.* (1995) stated that *Pyricularia* isolates from hosts including rice and common weeds in rice fields sporulated abundantly on sterilized barley or sorghum grains.

2.9.2 Effect of different temperatures:

Influence of temperatures viz; 20^0C, 25^0C, 30^0C and 35^0C on mycelial radial growth of *Pyricularia grisea* was studied. Maximum mycelial radial growth was recorded at 25^0C followed by 20^0C. However, lesser growth was observed at temperatures of 30^0C and 35^0C (Ravindramalviya, 2014).

Pal (2014) observed that 25^0C is the best temperature for mycelial growth and sporulation of the rice blast pathogen.

The blast outbreak is unpredictable, however, low temperature (about 22-25^0C) and long dew appearance are considered as two important factors recognized to induce blast epidemic and environmental conditions have an effect on the incidence of rice blast (Singh, 1988; Chaudhary and Vishwadhar, 1988; Manibhushanrao *et al*., 1989; Kim and Kim, 1991; Vijaya, 2003; Fukuda *et al.,* 2004; Monma *et al.,* 2004; Iwadate *et al.*, 2004).

The optimum temperature for the mycelial growth of *P. grisea* is said to be 25 to 30^0C (Awoderu *et al.,* 1991; Okeke *et al.* 1992; Arunkumar and Singh, 1995) while minimum temperature for the growth of the species is 8 – 9^0C and thermal death point is 51–52^0C (Nishikado, 1927; Yang *et al.,* 2011). Choi *et al*. (1987) recorded that the optimum temperature

for conidial germination of *Pyricularia oryzae* on a glass slide was 26-30^{0}C, at which temperature at least 4 hours of leaf wetness was required.

The temperature and incidence of paddy blast was negatively correlated i.e. -0.88, -0.80, -0.95, -0.84 respectively. This indicated that the disease incidence increases with the decrease of temperature (Shafaullah *et al*., 2011). The pathogen from rice grows luxuriantly on oat-meal, potato dextrose, ragi-meal agar medium at pH of 6.9 and temperature 30°C (Sirkant Kulkarni and Govindu, 1976).

Perezsendin *et al.* (1982) recorded 30°C as the optimum temperature for sporulation of *M. grisea* from rice. Sporulation of *M. oryzae* and disease progress was favored by high relative humidity (>89%), optimal temperature (25-28°C) and a minimum of 4 hours of leaf wetness (Teng, 1994).

Kim (1994) and Teng (1994**)** reported that conidiophores and first conidia were produced 4 to 6 h after dew formation and released shortly thereafter under optimal conditions. Sporulation of *P. grisea* from rice is favored by relative humidity ≥89%, optimal temperature of 25-28°C and a minimum of 4 hours of leaf wetness (Ou, 1985., Kim, 1994 and Teng, 1994).

Huang *et al.* (1980) concluded the blast varied from place to place in favourable temperature of 22-26^{0}C. Murlidharan and Venkatrao (1980) studied for forecasting epidemic outbreak in the plains with the help of trap method at Nellore. They noted that minimum temperature below 20^{0}C and R.H. 90% and above generally prevail for many days during the month of December, January and February when the blast become severe. It was further noticed that the use of high level of nitrogen and seed rate resulted in increasing the blast infection.

Awoderu *et al.*, (1991) observed that the minimum, optimum and maximum temperature for growth and conidial production of *P. grisea* were 10^{0}C, 25^{0}C and37^{0}C, respectively. Okeke *et al.*, (1992) noted that the growth of *P. grisea* was optimum at 28^{0}C, moderate at 23^{0}C and minimum at 15^{0}C and growth was inhibited at a temperature of 37^{0}C.

Tripathi *et al.*, (1997) reported maximum leaf blast severity in the second fortnight of October followed by first night of November when the maximum and minimum temperature varied between 31.5 to 18.10C- 16.60C and relative humidity 90-95%, respectively.

Kapoor *et al.*, (2004) studied the disease severity of leaf blast during 1997,1998 and 1999 in Kangra district, Himachal Pradesh and found that high humidity >80%, prevalence of low

temperature (16-19^{0}C) and maximum temperature (<28^{0}C)for 6-8 days or cloudy weather and 5-6 rainy days in a week were predisposing factor for disease development.

Behera *et al.,* (2008) observed maximum and minimum temperature had a negative correlation to the spore count of *Pyricularia oryzae.* Maximum temperature, minimum temperature, afternoon relative humidity and rainfall exhibited a negative correlation to the percentage of spores causing grain discolouration whereas maximum temperature had significant impact on spore count for grain discolouration. The most favourable weather condition for was at maximum temperature (30-32^{0}C)and minimum temperature (16.8-21.9^{0}C).

Chakrabatri and Wilcoxson (1970) observed that light stimulates sporulation of *P. oryzae*. They found that there was a distinct interaction between temperature and light. It was noticed that ultra violate light is most favourable for conidia production *P. oryzae*.

2.9.3 Growth of the pathogen in different carbon sources:

Pal (2014) evaluated five carbon sources against the *P. grisea* in different period of incubation for the assessment of mycelial growth and sporulation. Among the tested carbon sources, maltose gave optimum mycelial growth followed by dextrose and glucose and minimum mycelial growth was observed in Sucrose and fructose. None of carbon sources induced the sporulation of *P. grisea.*

Carbon compounds like maltose, sucrose, glucose, inulin and mannitol as well as organic acids such as succinic acid were the best carbon sources whereas, lactose and galactose were not suitable for *Pyricularia grisea* (Otani, 1953).

Otsuka *et al.* (1957) classified *P. grisea* based on their biochemical characters. Sucrose, glucose, maltose, fructose, lactose and xylose were the most suitable carbon sources for all the 47 isolates tested. Starch, dextrin, mannose, arabinose, galactose, rhamnose, raffinose and dulcitol was utilized by all the isolates, but not inositol or organic acids. Some strains of *P. grisea* utilized sorbitol, inulin and also mannitol.

Awoderu *et al.* (1991) reported that all carbon sources tested enhanced growth and production of conidia *P. oryzae* as compared untreated check. Mijan Hossain (2000) reported that starch supported the maximum dry mycelial weight followed by maltose and next best was cellulose. Least growth of *P. grisea* was observed in carbon tetrachloride.

2.9.4 Growth of the pathogen in different nitrogen sources:

Pal (2014) studied that maximum mycelial growth of *P. grisea* was reported in barium nitrate followed by Ammonium nitrate. It was noted that minimum mycelial growth was observed in Sodium nitrate and Potassium nitrate over control. None of nitrogen sources induced the sporulation of *P. grisea*.

According to Otani (1952), among the nitrogenous compounds KNO_3, $NaNO_3$, glycine, L – alanine, asparatic acid and asparagine markedly accelerated the growth, while $NaNO_2$, cystine, taurine and creatine inhibited the growth of the isolates of *P. grisea* . Apparao (1956) studied that asparagine, peptone, $NaNO_3$ and KNO_3 supported good growth of *P. grisea.* Nitrate could be utilized by the pathogen and obtained the growth**.**

Otsuka *et al*. (1957) concluded that among the several N compounds tested against 47 fungal isolates, KNO_3, $NaNO_3$, L – asparatic acid, L – asparagine, L – arginine, L – alanine, L – serine, L - histidine, L – glycine were the most suitable for the growth of the pathogen while oxyproline, L – cystine and L – phenylalanine were not utilized.

Veeraju and Prasad (1972) proved that performed inhibitor level changes differently in response to inoculation with virulent races of *P. grisea* and different level nitrogen fertilizer increased the synthesis of inhibitor but a higher level reduce the synthesis.

According to Mijan Hossain (2000), asparagine was the best nitrogen source for *P. grisea* which was significantly superior from other nitrogen sources tried in the studies. Minimum growth was observed in ammonium sulphate under study.

2.9.5 Growth of the pathogen at different pH level:

Ravindramalviya (2014) studied that the mycelial radial growth of *P. grisea* was significantly high at pH 6 followed by pH 7.0. The minimum mycelial radial growth was recorded at the alkaline pH of 8. However, the acidic range of 5.0 also did not favour the mycelial radial growth.

Pal (2014) studied that the mycelial growth of *P. grisea* was maximum at pH 7 followed by pH 6. The least mycelial growth was recorded at the pH 9. However, low pH 4 had totally inhibited the growth of fungus and pH 6 and pH 7 was found suitable for both growth and sporulation.

Sy *et al.*, (1977) proposed the effect of the pH on the mycelial growth, formation of conidia and conidial germination of *P. oryzae.* They found that, increase in weight occurred at all pH levels used except 2.35 – 2.95. Mycelial growth was maximum at pH 4 – 6, formation of conidia was maximum at pH 4.60 – 6.45, whereas germination was best at 4.60 – 5.45. The optimum pH range for the growth of *P. oryzae* was 5.5 – 10.5 and for conidial formation was 5.5 – 7.0 (Awoderu *et al.*, 1991).

Arun Kumar and Singh (1995) observed differential response of *P. grisea* isolates from rice, finger millet and pearl millet to pH. They found that, pH 6.5 was the best for the growth of rice and pearl millet isolates and pH 7.0 for finger millet isolates.

Mijan Hossain (2000) studied that, mycelial growth of *P. grisea* increased with increase in pH from 3.5 to 6.5. The pathogen showed the maximum mycelial growth at pH6.5 and least growth was observed at pH 3.5.

2.10 *In vitro* fungicidal testing against *Pyricularia grisea*:

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.* (2002) conducted an experiment to evaluate 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 higher extent than for conidia produced on Azoxystrobin-amended media, even though germination of such conidia was reduced after Azoxystrobin treatment.

The minimum inhibitory concentration of tricyclazole (Beam) and pyroquilon to inhibit the growth of *P. oryzae* were found to be 500 and 700 ppm respectively (El-Kazzaz *et al.*, 1990).

The efficiency of seven fungicides was evaluated by Arun kumar and Singh (1995) on the growth of *Magnaporthe grisea* isolated from rice, finger millet, pearl millet and observed that, rice and finger millet isolates were most sensitive to carbendazim (Bavistin) followed by thiophanate methyl (Topsin-M), edifenphos (Hinosan) iprobenfos (Kitazin), mancozeb (Dithane M-45), copper oxychoride (Blitox 50) and pyroquilon (Fongonrene).

Sood and Kapoor (1997) evaluated seven fungicides and found that tricyclazole 75% WP was the most effective and reduced the leaf and neck blast by 89.2% and 94.5% respectively. The efficacy of six systemic fungicides *i.e*., carbendazim, difenoconazole, hexaconazole, iprobenfos, propiconazole and tricyclazole at 500 and 1000 ppm concentration and 4 non-systemic fungicides chlorothalonil, copper oxychloride, mancozeb and iprodione at 1000 and 3000 ppm concentration (Hossain and Kulkarni, 2001). 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 *P. grisea.*

Gohel *et al*. (2008) reported that tricyclazole, mancozeb, carbendazim, iprobenfos, propiconazole and edifenphos were found highly fungitoxic with cent per cent growth inhibition of *Pyricularia oryzae.* Gohel *et al*. (2009) tested nineteen fungicides against *P. oryzae in vitro*. Among these 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 highly fungitoxic with 90.0 % growth inhibition.

The efficacy of fungicides *viz*., thiophanate-methyl, carbendazim, fosetyl-aluminium, 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 Yamini varma *et al.* (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 data of the results showed that for isoprothiolane at 1.5ml/l

concentration showed maximum inhibition of mycelial growth (94.85%) followed by carpropamid at 1ml/l concentration (91.48%).

Chander 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 per cent growth inhibition at 10 ppm while Folicur, Amistar top and Score exhibited 100 per cent growth inhibition at 25 ppm. Merger and Baan exhibited 50 per cent growth inhibition at 10 and 25 ppm respectively. However, Kasu-B registered the least (60 per cent) growth inhibition of fungal growth at 100 ppm. These studies revealed that Tilt followed by Amistar top, Score and Folicur are 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 as well as there was 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} where as 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.* (2014) 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* (2×10^8 cfu/ml) were evaluated against *Pyricularia oryzae* by dual culture technique under laboratory conditions. The maximum inhibition (100%) was observed by Carbendazim 50% WP 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 be inhibit the mycelial growth of *Pyricularia oryzae* significantly (59.72 %) (Kishan Lal *et al.*, 2015).

2.11 Management of rice blast disease under field condition:

Chemical management is more effective for management of blast disease of rice caused by *Pyricularia oryzae* (Peterson, 1990; Saifulla and Seshadri, 1992; Sood and Kapoor, 1997; Vijaya, 2002; Tripathi and Jain, 2005; Swamy *et al.*, 2009; Perini *et al.*, 2011; Dey *et al*., 2013). Varier *et al.*, (1993) reported that seed treatment with Tricyclazole at 4 g/kg seed which proved effective after 40 days of sowing.

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 found to be a highly effective.

Ravindra malviya (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 shows better result.

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

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

Dubey (1995) conducted field trials of eight fungicides for managing *Pyricularia oryzae*, Topsin M + Indofil M-45 proved to be most effective against leaf blast disease of rice. Minami and Ando (1994) reported that probenazole induces a resistant reaction in rice plants against infection by rice blast fungus. Gouramanis (1995) found that fungicides Carbendazim, Pyroquilon, Thiophanate methyl and Chlobenthiazone reduced the leaf blast disease of rice. On the other hand Tricyclazole was effective in reducing the neck blast.

Enyinnia (1996) evaluated two systemic fungicides Benomyl and Tricylazole on Faro / 29, a rice cultivar, at full booting stage and reported good control of natural infection of rice leaf blast. Filippi and Prabhu (1997) reported that pyroquilon fungicide (4.0 g a.i. per Kg of seed) was effective in controlling leaf and panicle blast. Sood and Kapoor (1997) evaluated 7 fungicides against leaf and neck blast of rice caused by *Magnaporthe grisea.* The fungicides were sprayed at the recommended rates at booting and heading stage. Tricylazole was the most effective, reducing leaf and neck blast by 89.2% and 97.5% and increasing the yield 43.3% as compared with the untreated control.

Tirmali and Patil (2000) conducted field experiment on susceptible rice cultivar E. K. 70 and 5 new fungicide formulations viz. 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 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 efficicacy 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 at heading stage of crop and found that all these new fungicides resulted in significantly reduced neck blast. Chaudhary (1986) reported that Edifenphos addition of either Sandovit (0.1%) or Tispre (0.1%) was effective in reducing foliage infection by *Pyricularia oryzae*. Reddy and Satyanarayana (1988) recorded that Edifenphos and carbendazim gave good control of *Pyricularia oryzae*.

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 the 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 significant increase in yield in Tricyclazole sprayed plots (7783.33 kg/ha.) followed by Ediphenphos (6941.66 kg/ha.), Kitazine (6850.00 kg/ha.) with B:C ratio 1:2.64, 1:2.39, 1:2.31, respectively (Ganesh *et al.*, 2012).

Varma and Santhakumari (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. 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 (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 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 concentrations 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 to be 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.

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. 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 (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%).

The efficacy of azoxystrobin and trifloxystrobin to tricyclazole and tricyclazole + propiconazole was compared when applied at the begining of the stem elongation and at 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 highly effective against leaf blast and neck blast and reduced incidence and disease severity by 90-100%, respectively. There was no significant improvement in efficacy of tricyclazole (225 g ha^{-1}) in combination with propiconazole (125 g ha^{-1}) when compared to tricyclazole alone (Cortesi and Giuditta, 2003).

The response of four upland rice cultivars to foliar fungicide application in relation to 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 per cent 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 minimum neck (1.1%) and node infections (1.7%), disease severity (3.8%) and the maximum grain yield (4.54 t/ha) 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 per cent over the control. A maximum increase of 60.99 per cent in grain yield was achieved with tricyclazole 75

WP followed by epoxiconazole 12.5 SC which recorded an increase of 34.85 per cent over the control (Kumbhar, 2005).

Groth (2006) applied azoxystrobin as 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 compared to unsprayed plots with high blast pressure. 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 compared with 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 percentage 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 untreated control. 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}. Metominostrobin found least effective against neck blast with disease incidence of 27.3% and grain yield 2550 kg ha^{-1} compared with control (29.7% and 2780 kg ha^{-1}).

Debashis 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 the three weeks in reducing the disease more in 3rd 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 controling 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 disease with leaf blast severity of 8.8% and neck blast incidence of 4.7%. This treatment also recorded 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 leaf blast disease severity to 11.9% and neck blast incidence to 6.2% with concomitant grain yield of 24.8 q ha^{-1}.

2.12 Relationship between AUDPC, AUCTPC, AUCTDC and AUSDC

Ashajyothi (2016) found that there is a positive correlation between AUCTPC and AUDPC (r=0.78, p=0.0476) i.e., the increase in canopy temperature directly associated with increase in the disease severity. The AUCTDC (r=0.79, p=0.0476) and AUSDC (r=0.26, p=0.2744) were found to be negatively correlated with the AUDPC in sheath blight infected rice plants. She told that a decline in canopy temperature resulted in decreased disease severity. Thereby the chlorophyll content as well as canopy temperature can be correlated with disease severity. Wakiyama (2002) found that rice plants with high chlorophyll content are having low canopy temperature than the plant with low chlorophyll content.

CHAPTER 3

MATERIALS AND METHODS

The present investigation entitled "Studies on blast disease of rice and it's management strategies." was carried out during 2015-17 at Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar.The field experiment was conducted at experimental area of this university and the laboratory work done in Research Laboratory, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar. The details of the materials used and methodologies adopted during the course of present study are described below:

3.1 Experimental site

The experiment was conducted at the instructional farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar located at 26°24'02.2"N latitude, 89°23'21.7"E longitude and at an elevation of 43 meters above mean sea level. It was carried out for one year , *i.e.*, *kharif* 2016-17.

3.2 Climatic condition

The experimental site was bestowed with sub-tropical per humid climate. The annual precipitation varied from 2800-3000 mm of which 70-90% used to be received during monsoon months (June – September). Temperature began to rise from February – March and reached its peak during April – May. The relative humidity remained very high almost throughout the year except during winter months.

3.3 Experimental soil

The soil, on which the experiment was carried out, was sandy loam in texture (61-63 % sand, 20% silt and 16-18% clay) acidic, with a pH of 5.51.with good drainage facility.

3.4 Meteorological data:

The meteorological data (Maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity, rainfall and total rainy days) for the *kharif* season, 2016 were obtained from the Department of Agronomy, GKMS project, UBKV, Pundibari, Cooch Behar.

Table 1:Weekly metrological data during kharif season 2016-17

Date	Rainfall (mm)	No of rainy days	Temperature (^{0}C)		Humidity (%)	
			Max.	Min.	Max.	Min.
1-7 June	8.31	2	35.19	23.89	79.57	71.86
8-14 June	18.10	4	32.87	23.83	85.71	77.14
15-21 June	44.69	7	30.89	24.41	98.29	85.86
22-28 June	54.63	4	31.64	21.40	92.43	85.14
29 Jun-5 July	13.17	7	31.61	25.46	91.57	81.00
6-12 July	15.76	7	33.54	25.71	86.14	79.14
13-19 July	34.17	7	31.81	25.96	97.86	90.29
20-26 July	54.09	7	29.30	24.79	98.00	91.29
27 July-2 Aug	5.83	5	33.11	25.13	91.43	79.43
3-9 Aug	17.50	2	33.93	26.19	86.71	78.57
10-16 Aug	3.21	3	33.97	26.30	85.57	78.57
17-23 Aug	1.71	2	34.23	26.07	82.43	77.14
24-30 Aug	33.86	3	34.54	25.83	85.86	78.86
31 Aug-6 Sep	15.19	6	32.67	25.20	92.00	87.00
7-13 Sep	11.26	5	31.90	25.26	92.57	87.29
14-20 Sep	6.49	6	33.19	25.13	89.29	84.57
21-27 Sep	39.50	5	30.43	23.37	91.57	85.71
28 Sep-4 Oct	0.55	3	34.20	25.82	86.00	82.42
5-11 Oct	13.07	6	31.80	24.50	94.28	85.71
12-18 Oct	7.64	2	31.42	21.04	83.00	74.57

Date	Rainfall (mm)	No of rainy days	Temperature (^{0}C)		Humidity (%)	
			Max.	Min.	Max.	Min.
19-25 Oct	0.00	0	33.48	19.55	72.42	65.57
26 Oct-1 Nov	0.00	0	33.42	19.11	75.57	66.42
2-8 Nov	0.00	0	32.57	17.85	75.57	66.28
9-15 Nov	0.00	0	31.77	18.48	76.42	71.28
16-22 Nov	0.00	0	30.02	14.97	76.00	72.28
23-29 Nov	0.00	0	30.10	14.80	72.57	68.14
30 Nov-6 Dec	0.00	0	29.14	14.8	72.00	66.42
7-13 Dec	0.00	0	28.74	11.0	78.71	69.14
14-20 Dec	0.00	0	28.05	11.67	82.14	69.85
21-27 Dec	0.00	0	27.94	12.84	89.57	77.85

Source : GKMS project, UBKV, Pundibari, Cooch Behar

3.5 Cleaning and sterilization of glassware

For all the laboratory studies, Borosil glassware was used. The glassware were first cleaned with a detergent, followed by thorough cleaning with tap water. The cleaned glassware were placed in potassium dichromate solution, for 24 hours and finally rinsed with distilled water for 3-4 times. Then they were air dried and sterilized in hot air oven at180°C for one hour.

Media and water used in the study were sterilized at 15 lb psi (121.6°C) for 20 minutes in an autoclave. Work benches were sterilized by ethyl alcohol. Cork borer, scalpel and inoculation loop were sterilized by flame.

3.6 Equipments used

The following equipments and materials were used in present investigation:

1. BOD incubator for incubation
2. Autoclave for media sterilization
3. Hot air oven for glassware sterilization
5. Inoculation needle, forceps, needles, blades,
6. Laminar air flow for isolation and purification

7. Thermometer
8. Electronic weighing balance
9. Refrigerator
10. Spirit lamp

3.7 Isolation of pathogen (*Pyricularia grisea):*

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 and Riker, 1936) amended with streptomycin sulphate under aseptic condition and kept in BOD incubator at 25 ± 1^0C for development of fungal growth. The fungus cultures were also maintained in culture tube to avoid contamination.

3.8 Purification of pathogen (*Pyricularia grisea*):

Fungus isolation techniques were used for getting pure culture of the fungus was transferred 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($5\pm1^{\circ}$C) in refrigerator.

3.9 Koch's Postulate test for rice blast pathogen:

First the diseased sample was collected from the experimental field and then the pathogen was isolated *in vitro* in PDA. Again, disease free healthy leaves were collected from the field. After that the leaves were arranged in a tray over moist blotting paper and cut end of the leaves covered by absorbent cotton. Suspension of the pathogen was made by adding sterilized distilled water on the grown pathogen culture. After that the pathogen suspension was inoculated in the healthy leaf with the help of a syringe and incubated it under room temperature for 3 days. After 3 days symptoms were developed on leaves. Then again the pathogen was re-isolated from the newly developed symptoms in PDA media and the growth of the fungus on the medium was observed.

3.10 Spore (conidia) Morphology:

For sporulation of the fungus, rice grain media was used. First rice grain was sterilized in conical flask in an autoclave at 15lb psi (121.6°C) for 20 minutes. Then the pathogen was inoculated in the sterilized rice grain and it was kept in a BOD at 25±1°C. After 55-60 days spore was formed. This spore suspension was again inoculated in fresh rice leaves following the procedure mentioned earlier. Those leaves developed similar type of blast lesion and those lesions were moist chambered in BOD at 25±1°C for 3 days. The conidia both from leaf sample and from media were observed under a microscope. Photograph of the spore (both in media and leaf sample) were taken with a binocular microscope fitted with Moticam 3.0 MP camera. 50 spores both from media and leaf sample were measured (length and width) in µm with the help of image analyzing software and also septation pattern was observed from both leaf sample and media.

3.11 Experiment detail *in vitro:*

Design : CRD

Replication : 03

Maintenance of pure culture: Potato Dextrose Agar Medium (PDA, Riker and Riker, 1936)

Culture used : 15 days old culture used

Observation : Mycelial growth and sporulation of *Pyricularia grisea* at 2 Days after Inoculation (DAI), 4DAI & 6DAI.

3.12 Cultural studies of the pathogen

3.12.1 Effect of different cultural media on the growth of the pathogen

Cultural characteristics of *Pyricularia grisea* was studied in five different culture media namely Potato Dextrose Agar (PDA), Oat meal agar, Malt extract agar, Rice polish agar & White rice agar. The composition of the used media was given as under:

Potato Dextrose Agar (PDA)

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

Oat Meal Agar

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

Malt Extract Agar

Composition	Quantities (g / litter)
Malt Extract	30
Peptone	06
Agar-agar	15
Chloramphenicol	0.05

Rice Polish Agar

Composition	Quantities (g / litter)
Rice polish	20
Agar-agar	20
Chloramphenicol	0.05

White Rice Extract Agar

Composition	Quantities (g / litter)
White rice extract	20
Agar-agar	20
Chloramphenicol	0.05

For measuring the radial growth rate, 5 days old culture of *Pyricularia grisea* was inoculated in the petri plate at the center of 90 mm petri plate. Each treatment was replicated thrice. Inoculum was in the form of 5 mm mycelial discs taken from margin of colonies grown on PDA plates. The plates were incubated in a BOD incubator in 25±1^{0}C temperature and the radial growth was measured (in mm) 2, 4 & 6 days after incubation. Colony characteristics (Growth type, growth pattern, colony colour, etc.) were observed by visual observation of the growth pattern of *Pyricularia grisea* after 144 hrs (6 days) of inoculation. Area Under Growth Progress Curve (AUGPC) was calculated following the formula:

$$AUGPC = \sum_{i=1}^{n-1} [\{(X_{(i+1)} + X_i)/2\} (T_{(i+1)} - T_i)]$$

Where, X_i is the growth of the pathogen on ith date, T_i is the day on which observation was recorded and n is the number of scoring days.

3.12.2Effect of different temperature levels on growth of the pathogen:

The effect of different temperatures on the growth and sporulation of *P. grisea* was studied at six temperature levels *viz*., 10, 15, 20, 25, 30 and 35°C. 20 ml of potato dextrose agar medium was poured into a 90 mm petri plate. When the media is solidified then a 5 mm mycelial disc cut from actively growing cultures was inoculated at the centre of the plate and incubated at different temperature levels. Each treatment was replicated thrice. The mycelial growth &sporulation was recorded at 2, 4 & 6 days after inoculation at 25±1°C in a BOD incubator. Area Under Growth Progress Curve (AUGPC) was calculated following the formula:

$$AUGPC = \sum_{i=1}^{n-1} [\{(X_{(i+1)} + X_i)/2\} (T_{(i+1)} - T_i)]$$

Where, X_i is the growth of the pathogen on ith date, T_i is the day on which observation was recorded and n is the number of scoring days.

3.12.3 Effect of difference carbon sources on growth of the pathogen:

Potato dextrose agar medium was used as basal medium to study the influence of carbon compounds on growth and sporulation. Various carbon compounds, viz. glucose, dextrose, sucrose, fructose and maltose were tested and the concentration of each carbon source equivalent to carbon present in the amount of dextrose @ 20gm/l in the basal medium was added. Then 5 mm disc of actively growing culture was cut and put in the middle of the plate in each treatment.3 replications were kept. The mycelial diameter was recorded after incubation (25±1°C) at 2, 4, & 6 days after inoculation for *P .grisea*. Area Under Growth Progress Curve (AUGPC) was calculated following the formula:

$$AUGPC = \sum_{i=1}^{n-1} [\{(X_{(i+1)} + X_i)/2\} (T_{(i+1)} - T_i)]$$

Where, X_i is the growth of the pathogen on ith date, T_i is the day on which observation was recorded and n is the number of scoring days.

3.12.4 Effect of different nitrogen sources on growth of the pathogen:

For nitrogen requirement study, Potato dextrose agar medium was taken as a basal medium. Different nitrogen compound viz. Sodium nitrate, Calcium nitrate, Potassium nitrate and Ammonium nitrate @ 2 gm/l were added in potato dextrose agar medium. Then 5 mm disc of actively growing culture was cut and put in the middle of the plate in each treatment.3 replications were kept. Mycelial diameter (in mm) & sporulation was recorded at 2, 4, & 6 days after incubation at 25±1°C in a BOD incubator for the test pathogen. Area Under Growth Progress Curve (AUGPC) was calculated following the formula:

$$AUGPC = \sum_{i=1}^{n-1} [\{(X_{(i+1)} + X_i)/2\} (T_{(i+1)} - T_i)]$$

Where, X_i is the growth of the pathogen on ith date, T_i is the day on which observation was recorded and n is the number of scoring days.

3.12.5 Effect of different hydrogen ion concentration (pH) on growth of the pathogen:

Potato dextrose agar medium was adjusted by Phillips digital pH meter to various pH levels 5, 6, 7, 8, 9 and 10 with 0.1 N hydrochloride acid (HCl) on 0.1 sodium hydroxide (NaOH) and buffer with citrate phosphate of corresponding pH. Mycelial culture (5mm disc) was seeded in the adjusted pH medium and mycelial growth& sporulation was recorded at after incubation in 25±1°C in a BOD incubator at 2, 4, & 6 days after inoculation for *P. grisea*. Area Under Growth Progress Curve (AUGPC) was calculated following the formula:

$$AUGPC = \sum_{i=1}^{n-1} [\{(X_{(i+1)} + X_i)/2\} (T_{(i+1)} - T_i)]$$

Where, X_i is the growth of the pathogen on ith date, T_i is the day on which observation was recorded and n is the number of scoring days.

3.13 Bioassay of different fungicides in various concentrations against *Pyricularia grisea:*

Seven different fungicide molecules or combination of molecules in different concentrations as mentioned below were used for *in vitro* evaluation of fungitoxicity towards *P. oryzae* by using poisoned food technique.

Growth in all concentrations was taken and % inhibition over control as well as ED 90 values for all the treatments were found out.

Sl. No.	Treatments	Dose (ppm)
T1	Azoxystrobin 23% SC	50,100,200,300,400 & 500
T2	Tricyclazole 75% WP	50,100,200,300,400 & 500
T3	Azoxystrobin 23% EC+Difenoconazole 25% EC	50,100,200,300,400 & 500
T4	Tebuconazole 50% EC+Trifloxystrobin 25% WG	50,100,200,300,400 & 500
T5	Tebuconazole 25% EC	50,100,200,300,400 & 500
T6	Difenoconazole 25% EC	50,100,200,300,400 & 500
T7	Tricyclazole 75% WP+Mancozeb 75% WP	50,100,200,300,400 & 500
T8	Control	No chemical

Design: Completely Randomized Design (C.R.D.)

1 ml of fungicides were taken with the help of a sterilized glass rod and mixed in 100 ml of sterilized distilled water in case of liquid fungicide and 1 g of fungicide were mixed in 100ml of water in case of dust form fungicide. From this stock solution, 0.5 ml,1 ml,2 ml,3 ml,4 ml & 5 ml fungicide solution was mixed with 99.5 ml, 99 ml, 98 ml,97 ml, 96 ml & 95 ml of sterilized non-solidified potato dextrose agar medium in a conical flask each, respectively and shaking of all the conical flasks was done. 20 ml of amended medium was poured in a 90 mm sterilized petri dish and allowed it to solidify. Mycelial discs of 5 mm diameter from 15 days old culture was placed at the center of the Petri plate and then incubated at 25 ± 1^0C for 7 days. Control was maintained without fungicide. Three replications were maintained for each treatment.

3.14 Effect of new generation fungicides for the control of leaf blast of Rice *in vivo*

3.14.1 Experiment detail *in vivo:*

Field experiment	: UBKV farm, Pundibari, Cooch Behar
Experimental variety	: Swarnamasuri (MTU 7029)
Season	: Kharif 2016
Date of seed sown in nursery	:26/06/2016
Area of nursery	: 56 sq.m
Date of swoing in main field	: 21/07/16
Age of seedling	: 24 days
Spacing	: 20 cm (Row to Row) × 15 cm (Plant to Plant)
Replication	: 3
Treatments	: 8
Plot size	: 5m × 3m
Seed treatments	: Tricyclazole 75% WP @3gm/2kg of seeds
Experimental Design Used	: RBD (Randomized Block Design)
Fertilizer Application	: 80 : 40 : 40 (N : P : K) [Basal dose- ¼ N : Full P : ¾ K] [1st Top dressing- ½ N] [2nd Top dressing – ¼ N & ¼ K]
Weeding	: 30 & 60 Days After Transplanting (both were manually)

Irrigation - 40 Days After Transplanting (DAT)

Fungicide spray -1st spray 60 DAT

2nd spray 70 DAT

3rd spray 80 DAT

Disease data -1st reading was taken before first spray and then at 10 days interval for 3 times

Artificial inoculation of the pathogen in the field was done at 60 days after transplanting by the pathogen grown in PDB medium (10^5cfu).

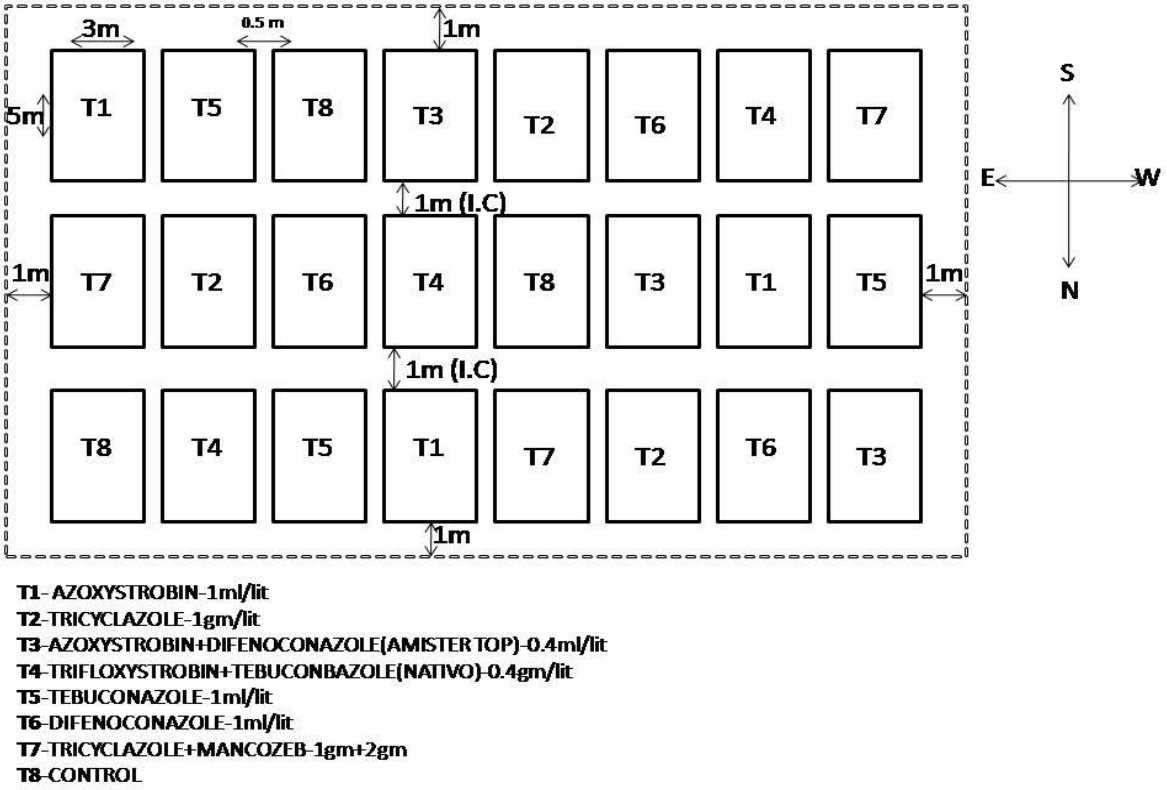


Fig 1: Lay out of the experimental field

Different treatment combinations are as follows:

Treatments	Chemical	Dose
T1	Azoxystrobin 23% SC	1.0 ml/L
T2	Tricyclazole 75% WP	1.0 gm/L
T3	Azoxystrobin 23% EC+Difenoconazole 25% EC	0.4 ml/L
T4	Tebuconazole 50% EC+Trifloxystrobin 25% WG	0.4 gm/L

T5	Tebuconazole 25% EC	1.0 ml/L
T6	Difenoconazole 25% EC	1.0 ml/L
T7	Tricyclazole 75% WP+Mancozeb 75% WP	3.0gm/L(1 gm+2gm)
T8	Control	Water spray

3.14.2 Variety used for fungicidal evaluation:

Swarnamashuri (MTU7029) which is susceptible for rice blast disease is used for the *in vivo* evaluation of fungicides. The seed of variety Swarnamashuri was collected from University Research Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar for conducting field experiment on evaluation of fungicides during the kharif season of 2016

3.14.3 Parameters observed:

Physiological traits:

AUCTPC (Area Under Canopy Temperature Progress Curve)

AUCTDPC (Area Under Canopy Temperature Depression Progress Curve)

AUSDC (Area Under Spad Decline Curve)

Morphological traits:

Panicle length

Panicle number/sq.m

Yield Attributes:

Seed / panicle

Panicle weight

Thousand seed weight

Unfilled grain / panicle

Yield

3.14.4 Observations and their procedures:

Observations of the characters under study were recorded for comparing the effect of fungicides. For each observation, fifteen randomly selected plants were tagged from each plot and used further for observations. The mean values of the recorded data were taken as the actual values of the respective characters.

3.14.4.1 Canopy temperature:

The canopy temperature was recorded using hand-held infrared thermometer, starting from 110th days after transplanting within 10 days interval for 3 times. Then Canopy temperature over the period of observation was converted to Area Under Canopy Temperature Progress Curve (AUCTPC) and was calculated with formula given by (Rosyara *et al*., 2009)

$$AUCTPC = \sum_{i=1}^{n-1} [\{(C_{(i+1)} + C_i)/2\} (T_{(i+1)} - T_i)]$$

Where, C_i is the Canopy Temperature on ith date, T_i is the date on which Canopy temperature was measured and n is the number of scoring days.

Ambient temperatures were measured using the handheld thermometer instantly after five readings in each plot. Deviation of temperature of plant canopies in comparison to ambient temperature (air temperature - canopy temperature), also known as canopy temperature depression (CTD) was calculated and it has been a good criterion for screening heat stress tolerance (Reynolds *et al*., 1998). Similar to canopy temperature, canopy temperature depression for all the 3 days were found out and Canopy Temperature depression over the period of observation was converted to Area Under Canopy Temperature Depression Progress Curve (AUCTDPC) and was calculated with the same formula given by Rosyara *et al.,* (2009)

$$AUCTDPC = \sum_{i=1}^{n-1} [\{(C_{(i+1)} + C_i)/2\} (T_{(i+1)} - T_i)]$$

Where, C_i is the Canopy Temperature Depression on ith date, T_i is the date on which Canopy temperature depression was measured and n is the number of scoring days.

3.14.4.2 Spad reading:

Spad reading was recorded selecting the particular leaves within a 10 days interval from tagged plant with the help of Chlorophyll meter (model: KONICA MINOLTA SPAD – 502 plus). Five readings were taken and the average was considered as the spad value for that plant. Area Under Spad Value Decline Curve (AUSDC) was estimated to record the accumulated effect of stay green trait of the plant using the formula similar to AUDPC given by Rosyara *et al.,* (2007), which is as follows.

$$AUSDC = \sum_{i-1}^{n-1}[\{(X_i + X_{(i+1)}/2\}\ (t_{(i+1)} - t_i)]$$

Where, X_i is the spad value on ith date, t_i is the ith day and n is the number of scoring days.

3.14.4.3 Length of panicle (cm):

The length of panicle was measured in centimeter from the base ofrachis to tip of the panicle. The length of five sampled panicles was measured and averages were worked out.

3.14.4.4 Panicle number / sq.m :

Total number of panicle per sq.m of each plot was counted and averages were worked out of three replication of each treatments.

3.14.4.5 Grains / panicle:

Total number of grain per panicle was recorded from the 15 panicle of each plot after harvesting and average was worked out. Then average from three replication of each treatments were calculated.

3.14.4.6 Panicle weight (g):

The weight of five sampled panicles was measured and averages were worked out. Then average from three replication of each treatments were calculated.

3.14.4.7 Thousand seed weight (g):

Randomly selected 1000-grains were counted from each plot and weight was recorded in gram (gm) after sun drying. Then average from three replication of each treatments were calculated.

3.14.4.8 Unfilled grain / panicle:

Total number of unfilled grain per panicle was recorded from the 15 panicle of each plot after harvesting and average was worked out. Then average from three replication of each treatments were calculated. Then percent unfilled grain in respect of total grains in a panicle was worked out.

.

3.14.4.9 Grain yield (t/ha):

The yield of grains obtained from each net plot was recorded in kilograms after sun drying of grains and the grain yield per plot was converted into tonnes per hectare. Then average from three replication of each treatments were calculated.

3.14.4.10 Disease severity index:

The severity of rice blast disease was assessed based on the relative lesion size & area covered as mentioned in IRRI for rice and its corresponding rating value on 0-9 scale given by Anonymous (1996).

Scale	Description
1	Small brown specks of pin-point size
2	Small, roundish to slightly elongated, necrotic grey spots, about 1 -2 mm in diameter, with a distinct brown margin. Lesions are mostly found on lower leaves.
3	Lesion type is same as in scale 2, but significant numbers of lesions are on the upper leaves.
4	Typical susceptible blast lesions, 3mm or longer, infecting less than 4% of the leaf area
5	Typical susceptible blast lesions, 3mm or longer, infecting less than 4-10% of the leaf area
6	Typical susceptible blast lesions, 3mm or longer, infecting less than 11-25% of the leaf area
7	Typical susceptible blast lesions, 3mm or longer, infecting less than 26-50% of the leaf area
8	Typical susceptible blast lesions, 3mm or longer, infecting less than 51-75% of the leaf area, many leaves dead
9	Typical susceptible blast lesions, 3mm or longer, infecting more than 75% of the leaf area

IRRI-Planning Breeding Programs for Impact

Percent disease index (PDI) was worked out by using the formula given by Wheeler (1969).

$$\textbf{PDI} = \frac{\textit{Sum of disease rating}}{\textit{Number of plants observed}} \times \frac{100}{\textit{Maximum scale}}$$

Area under Disease Progress Curve (AUDPC) was calculated with the formula given by (Das *et al*., 1992; Sharma *et al*., 2004)

$$AUDPC = \sum_{i=1}^{n-1} [\{(X_i + X_{(i+1)})/2\} (t_{(i+1)} - t_i)]$$

Where, X_i is the Rice blast severity on ith date, t_i is the ith day and n is the number of scoring days.

Data were taken from 15 plant of each plot for 4 times. 1st reading was taken before first spray and then at 10 days interval for 3 times.

3.15 Statistical Analysis

The field trials were conducted following Randomized Block Design field trials. The laboratory trials were conducted following Completely Randomized Design. The replicated data generated from different experiments were analysed statistically using statistical package of INDOSTAT and the ANOVA determined the probability for significant variation among the treatments.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Isolation of the pathogen

The pathogen was isolated in Potato Dextrose Agar (PDA) media (Section 3.7) and purified (Section 3.8). The pathogen produced white mycelial growth at first but later on it turned into black in colour. Sporulation was not observed in PDA even after 2-3 months of culture maintenance. PDA was also used for isolation of *Pyricularia grisea* by Motlagh and Javadzadeh, 2010) and Priya Vanaraj *et al*. (2013).

4.2 Establishment of Koch's postulates

In order to prove the pathogenic nature of *P. grisea* producing blast disease in rice Koch's postulates tests were conducted on leaves of the rice plants by detached leaf technique (Section 3.9). The pathogen was isolated in PDA and at first white coloured and then blackish fungal growth was observed in the media. The leaves started to produce symptoms from 4th day onwards; initially the spots were small, yellow, round to oval. At later stage the spots became enlarged and spindle shaped having ash coloured centre. The spot was similar in appearance with the rice blast spots found in the field. The developed spots when kept in moist chambered condition, from those spots typical 3 celled conidia of *Pyricularia grisea* was observed under the microscope. The re-isolated pathogen from the artificially developed spots in the laboratory was producing similar type of growth which was found in first isolation of the pathogen *in vitro* (Plate 1). In this way Koch's postulates was established.

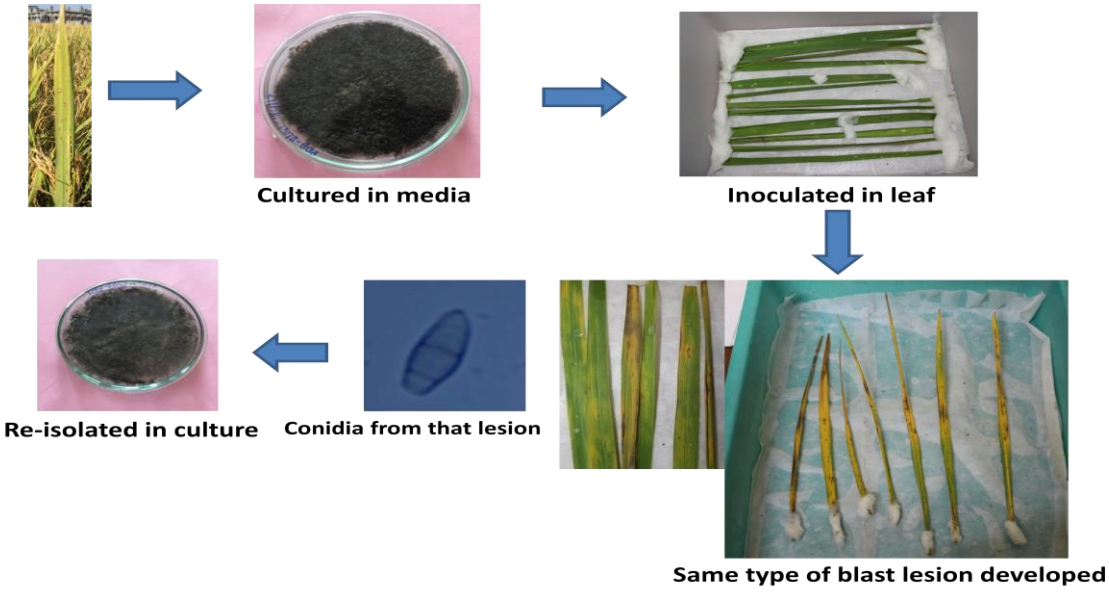


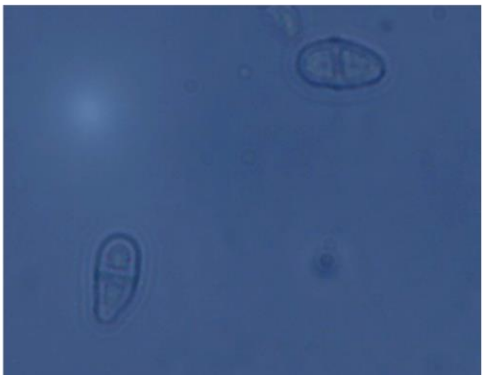
Plate 1: Establishment of Koch's Postulates

4.3 Sporulation of the pathogen

For sporulation of the pathogen, rice grain media was used (Section 3.10). This result confirms the findings of Sun *et al.* (1989) who studied the effects of 17 media on 41 isolates of *P. oryzae.* They found that, corn meal and rice straw agar media were most conducive for sporulation of *Pyricularia oryzae*. The culture first became black and only conidiophores were observed at that time. Upto 30 days the culture remained black. Then it started to change colour and at 55 -60 days, it became whitish and at that time the conidia of *Pyricularia* was observed under the microscope (Plate 2).

Culture in the initial stage

Culture in the sporulation stage

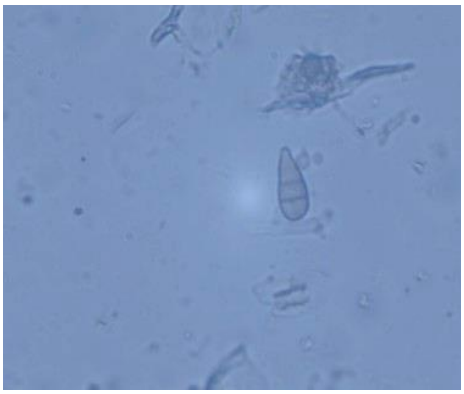


Conidia in culture

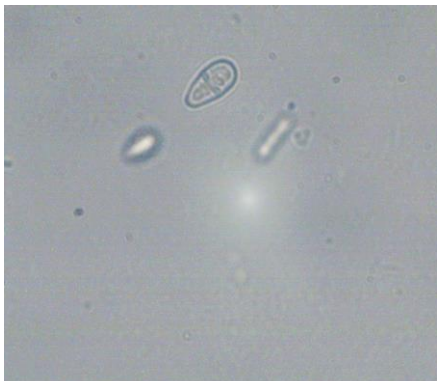
Plate 2: Growth and sporulation of *Pyricularia grisea* in sterilized rice grains

4.4 Morphological characterization of the pathogen

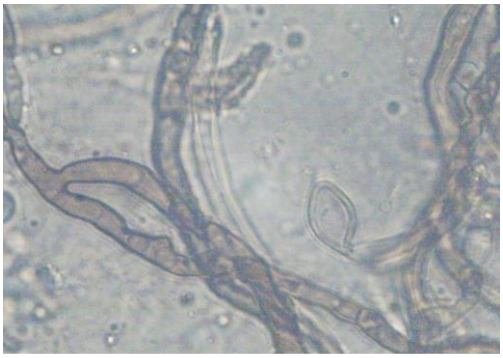
Length and breadth of the conidia of blast pathogen (*Pyricularia grisea*) was measured from both rice grain media and from lesion developed by inoculation of that culture in the rice leaves (section 3.10). The size of the conidia was much higher from leaf sample than in the media. The size of conidia measured about 17.96 - 26.64 µm × 7.36 - 9.22 µm (average 22.42 × 8.59 µm) and 12.06 - 19.95 µm × 5.38 - 9.06 µm (average 16.45 × 7.46 µm) from leaf sample and media, respectively (Plate 3). This result is in confirmity with Tochinai and Shimamura (1932) who classified 39 isolates into nine forms on the basis of cultural characteristics. On steamed rice straw, the conidia of the isolates belonging to four forms were short, the mean value ranged from 19.3 to 22.8 µm. The conidia of other five forms were long, the mean value ranged from 26.8 to 29.9 µm. Mijan Hossain (2000) also observed that mycelium in cultures was first hyaline in colour, then changed to olivaceous, 1 – 5.2 µm in width, septate and branched. The spore measurements were 15 – 22 µm × 4 – 7 µm (Average, 17.4 µm × 5.2 µm). Mostly 2 celled conidia were found from rice grain media and 3 celled conidia were found in infected leaf sample.



Conidia of Pyricularia from leaf



Conidia of Pyricularia from media



Conidiophores of the pathogen

Plate 3: Conidia and Conidiophores of the pathogen

4.5 Cultural characterization of the pathogen

4.5.1. Growth of the pathogen in different media

Table 2: Growth of *Pyricularia grisea* in Different Media

Media	Growth (mm)			AUGPC
	2 DAI	4 DAI	6 DAI	
PDA	28.33	47.77	71.00	147.10
OMA	28.50	45.53	71.33	145.37
RPA	14.17	26.00	59.33	99.50
WRA	15.17	29.03	47.33	91.53
MEA	19.00	32.17	44.67	95.83
SEM ±	2.3203	1.8353	2.0385	3.0176
CD (5%)	7.3111	5.7830	6.4235	9.5087
CV (%)	19.106	8.805	6.012	4.511

The pathogen was grown in different media and the growth was noted at 2, 4 and 6 days after inoculation and AUGPC was also calculated (Section 3.12). From the table (Table 2) it was found that highest growth of 28.50 mm and 71.33 mm was recorded in Oat meal agar (OMA) medium at 2 and 6 days after inoculation, respectively whereas at 4 days after inoculation highest growth was achieved in Potato dextrose agar (PDA) medium (47.77 mm). The second highest growth at 2 and 6 days after inoculation of 28.33 mm and 71 mm respectively was recorded in PDA whereas second highest growth of 45.53 mm at 4 days after inoculation was recorded by OMA (Plate 4). Growth in PDA and OMA was statistically *at par*. Highest Area Under Growth Progress Curve (AUGPC) of 147.10 was recorded by PDA which is immediately followed by OMA (145.37) whereas the lowest AUGPC of 91.53 was recorded by was White Rice Agar (WRA) medium (Fig 2). So, PDA and OMA were found to be the suitable media for *Pyricularia grisea* here. This result is in agreement with Ravindramalviya (2014) who studied that PDA media supported maximum mycelial growth of *P. grisea* after 168 hr of incubation. This result is in accordance with the findings of Mahdieh S (2013) who reported that PDA culture medium could provide the best medium for *P.*

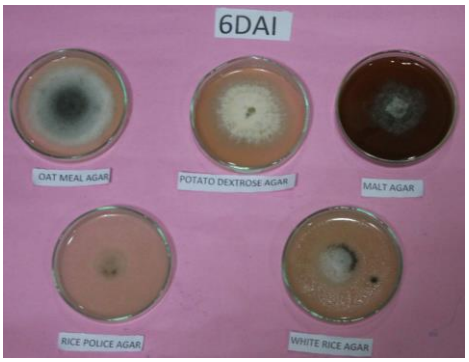
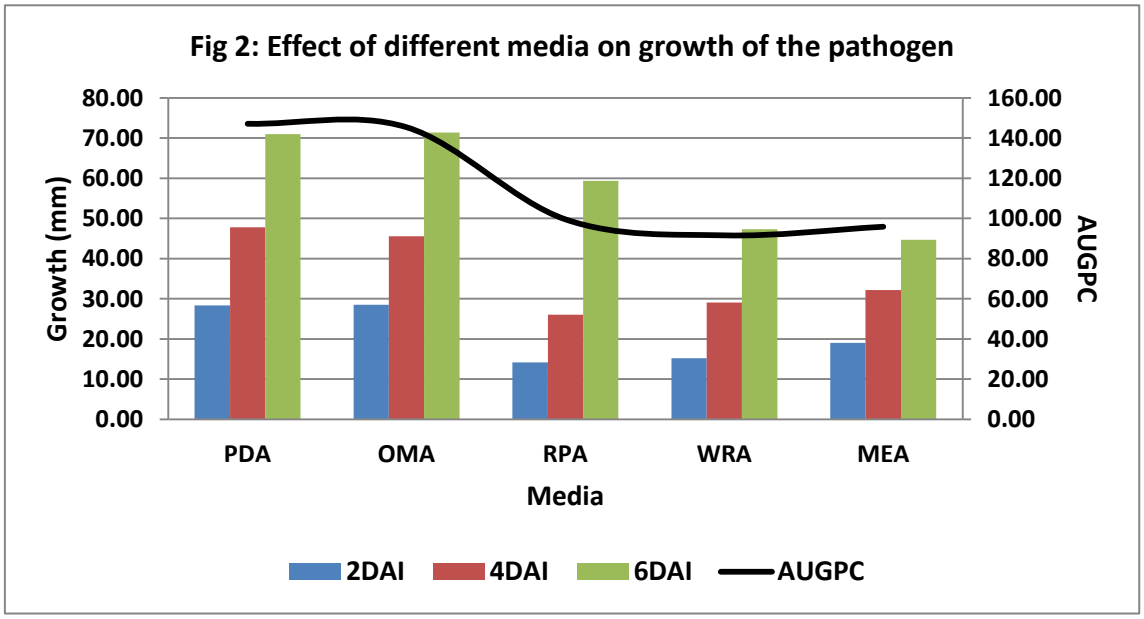


Plate 4: Growth of the pathogen in different media at 6 days after inoculation

oryzae vegetative growth, regardless of light condition.



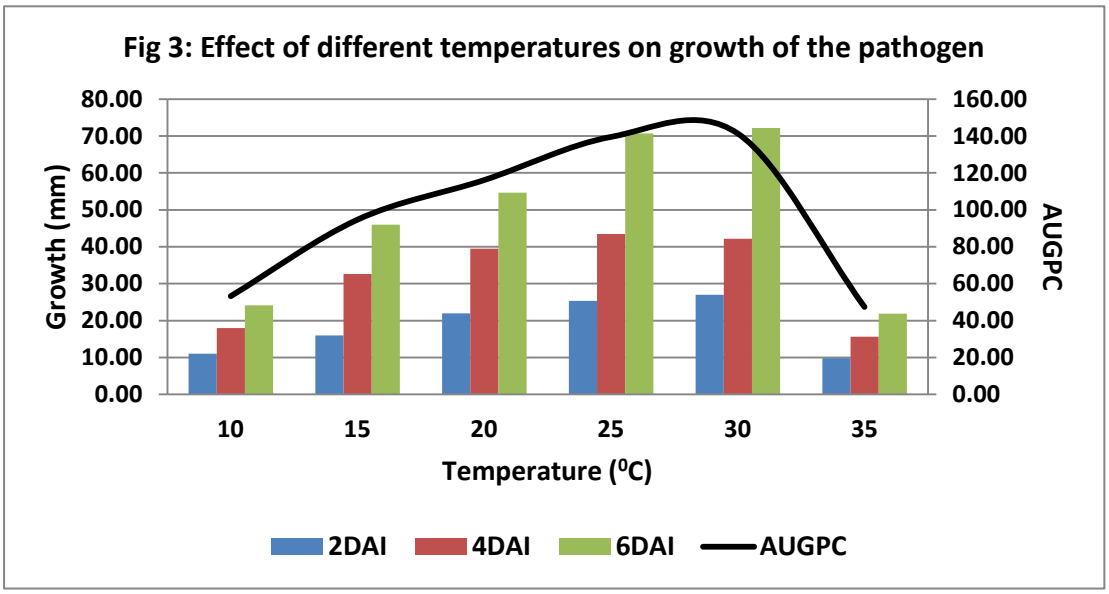
4.5.2 Growth of the pathogen in different temperatures

Table 3: Growth of *Pyricularia grisea* in different temperatures

Temperature (^{0}C)	Growth (mm)			AUGPC
	2DAI	4DAI	6DAI	
10	11.07	18.00	24.17	53.23
15	16.00	32.67	46.00	94.67
20	22.00	39.47	54.67	116.13
25	25.33	43.50	70.67	139.50
30	27.00	42.17	72.17	141.33
35	9.83	15.67	21.87	47.37
SEM ±	2.6701	1.5934	1.4679	2.4837
CD (5%)	8.2274	4.9099	4.5231	7.6530
CV (%)	24.946	8.649	5.269	4.358

The pathogen was grown in different temperatures and the growth was noted at 2, 4 and 6 days after inoculation and AUGPC was also calculated (Section 3.13). From the table (Table 3) it was found that highest growth of 27 mm and 72.17 mm was recorded at 30^{0}C at 2 and 6 days after inoculation, respectively whereas at 4 days after inoculation highest growth was achieved in 25^{0}C (43.50 mm). The second highest growth at 2 and 6 days after inoculation of 25.33 mm and 70.67 mm respectively was recorded in 25^{0}C whereas second highest growth of 42.17 mm at 4 days after inoculation was recorded in 30^{0}C (Plate 5). Growth in 25^{0}C and 30^{0}C was statistically at par. Highest Area Under Growth Progress Curve (AUGPC) of 141.33 was recorded at 30^{0}C which is immediately followed at 25^{0}C (139.50) whereas the lowest AUGPC of 47.37 was recorded at 35^{0}C (Fig 3). At 35^{0}C, the growth was black in

colour. In this temperature huge amount of conidiophores were produced. This may be due to the fact that fungus produces conidia in stress condition. So, 25^{0}C and 30^{0}C were found to be the suitable media for *Pyricularia grisea* here. This result is in confirmity with Awoderu *et al.* (1991), Okeke *et al.* (1992) and Arun kumar and Singh (1995) who reported that optimum temperature for the mycelial growth of *P. grisea* is 25 to 30^{0}C. This result also confirms the findings of Okeke *et al*. (1992) who noted that the growth of *P. grisea* was optimum at 28^{0}C, moderate at 23^{0}C and minimum at 15^{0}C and growth was inhibited at a temperature of 37^{0}C.



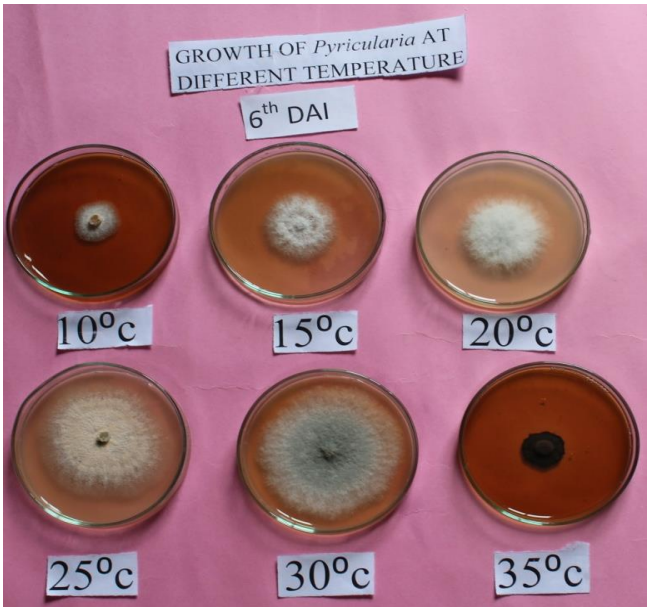


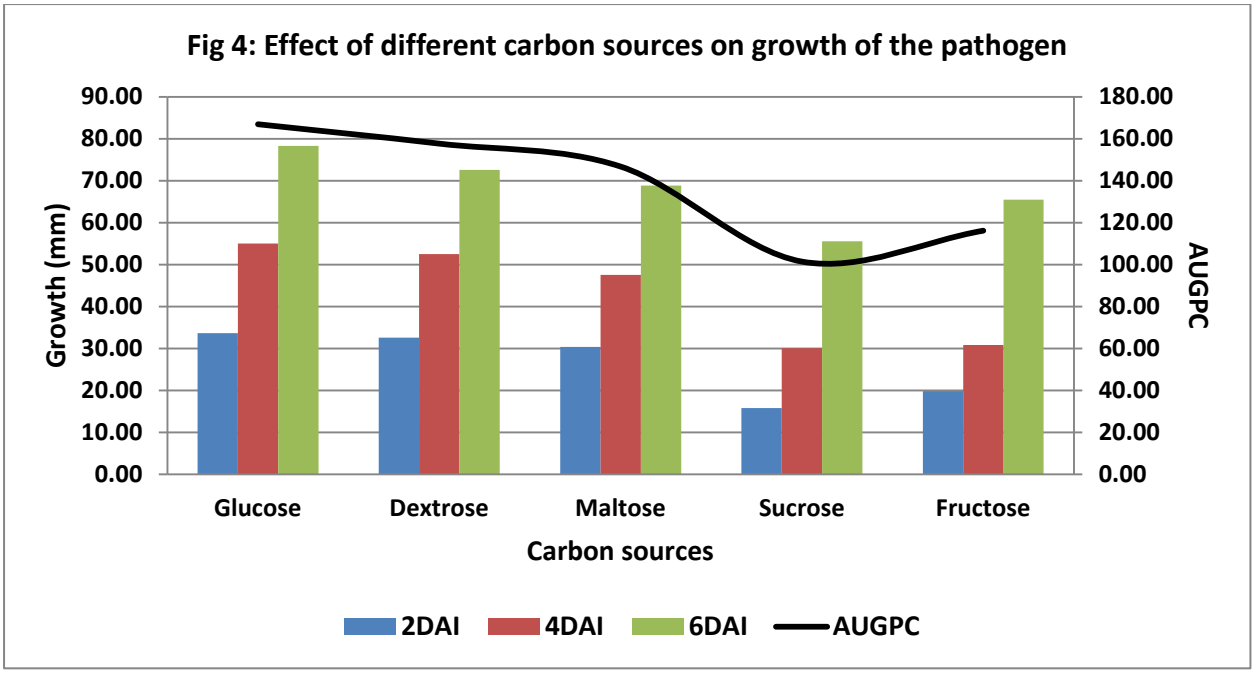
Plate 5: Growth of the pathogen in different temperatures at 6 days after inoculation

4.5.3 Growth of the pathogen in different carbon sources

Table 4: Growth of *Pyricularia grisea* in different carbon sources

Carbon sources	Growth (mm)			AUGPC
	2DAI	4DAI	6DAI	
Glucose	33.60	55.00	78.30	166.90
Dextrose	32.59	52.47	72.60	157.65
Maltose	30.37	47.53	68.82	146.72
Sucrose	15.75	30.07	55.57	101.38
Fructose	19.83	30.80	65.50	116.13
SEM ±	0.8022	1.0510	1.8904	2.5958
CD (5%)	2.5279	3.3119	5.9568	8.1796
CV (%)	5.258	4.217	4.804	3.264

The pathogen was grown in different carbon sources as mentioned in section 3.14. From the above table (Table 4), it was found that highest growth in all the three days of observation was obtained by glucose (33.60 mm, 55 mm and 78.30 mm at 2, 4 and 6 DAI, respectively) whereas the second highest growth in all the three days was achieved by dextrose (32.59 mm, 52.47 mm and 72.60 mm at 2, 4 and 6 DAI, respectively) [Plate 6] The AUGPC was also recorded highest in glucose (166.90) and second highest was in dextrose (157.65). The lowest AUGPC of 101.38 was recorded by sucrose (Fig 4). This may be due to the fact that glucose is the simplest form of carbon source and the fungus finds it very easy to utilize this simple source of carbon for its good growth and thus the fungus growth is highest in this source of carbon. This result is in confirmity with the findings of Otsuka *et al*. (1957) who reported that Sucrose, glucose, maltose, fructose, lactose and xylose were the most suitable carbon sources for all the 47 isolates of *Pyricularia grisea* they tested. Similar type of results were also found by Otani (1953) who reported that carbon compounds like maltose, sucrose, glucose, inulin and mannitol as well as organic acids such as succinic acid were the best carbon sources whereas, lactose and galactose were not suitable for *Pyricularia grisea*.



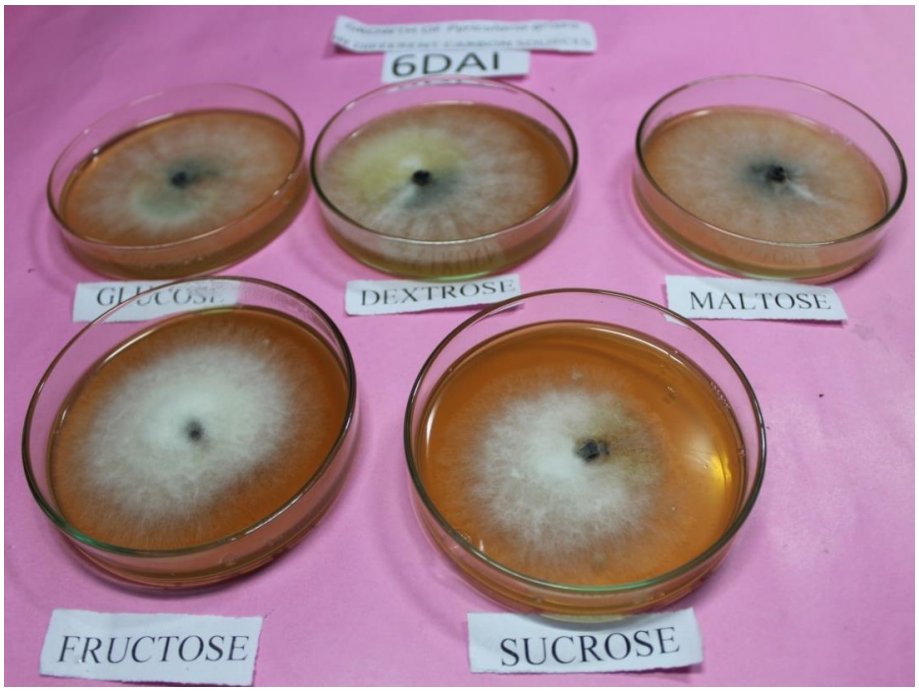


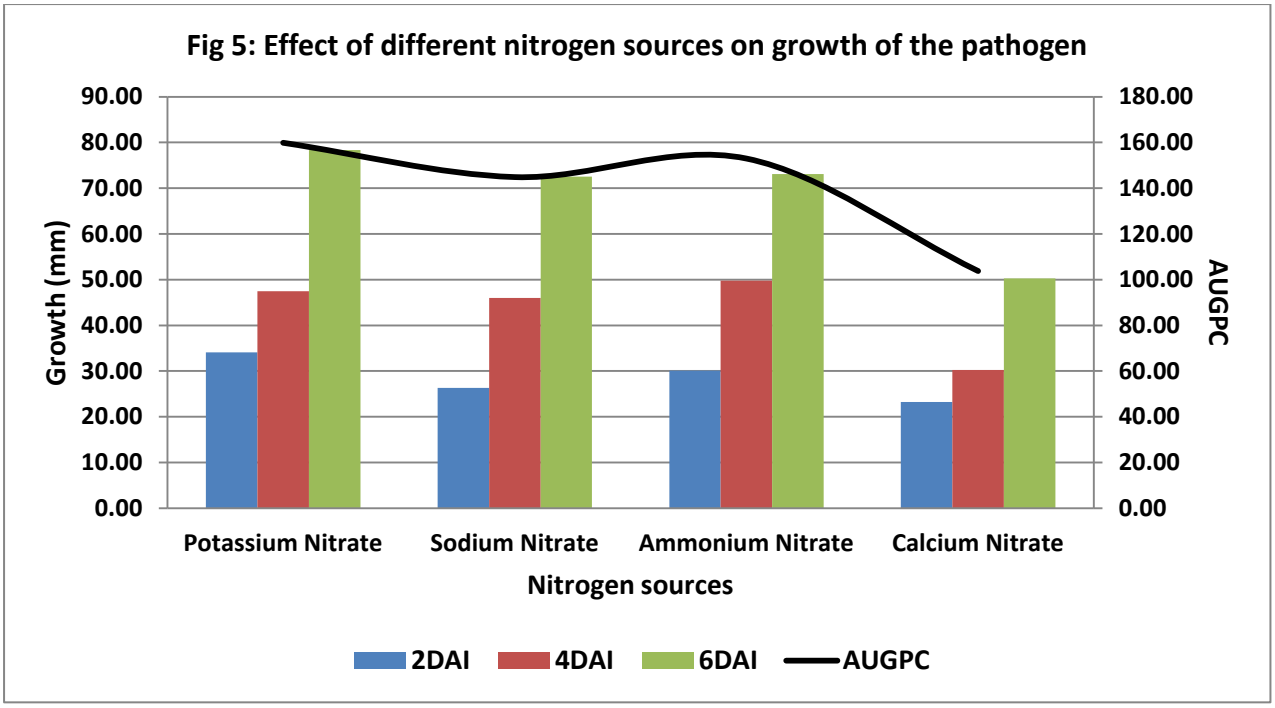
Plate 6: Growth of the pathogen in different carbon sources at 6 days after inoculation

4.5.4 Growth of the pathogen in different nitrogen sources

Table 5: Growth of *Pyricularia grisea* in Different nitrogen sources

Nitrogen sources	Growth (mm)			AUGPC
	2 DAI	4 DAI	6 DAI	
Potassium Nitrate	34.10	47.43	78.33	159.87
Sodium Nitrate	26.33	46.00	72.53	144.86
Ammonium Nitrate	30.07	49.80	73.10	152.97
Calcium Nitrate	23.27	30.23	50.27	103.77
SEM ±	1.8016	1.1908	2.1123	3.6124
CD (5%)	5.8752	3.8835	6.8885	11.7806
CV (%)	10.972	4.756	5.336	4.458

The pathogen was grown in different nitrogen sources as mentioned in section 3.15. From the table (Table 5) it was noticed that highest growth of 78.33 mm was recorded in potassium nitrate at 6 days after inoculation. This is followed by the growth in ammonium nitrate where 73.10 mm growth was recorded at 6 DAI (Plate 7). Growth progress was also highest in potassium nitrate which is indicated by an AUGPC of 159.87. Second highest AUGPC of 152.97 was recorded in potassium nitrate whereas the lowest AUGPC of 103.77 was recorded by calcium nitrate (Fig. 5). So, potassium nitrate was considered to be the best nitrogen source for the growth of the fungus. This result is in confirmity with Otani (1952) who found that among the nitrogenous compounds, KNO_3, $NaNO_3$, glycine, L – alanine, asparatic acid and asparagine markedly accelerated the growth of the isolates of *P. grisea* that he was using. Apparao (1956) also got similar type of results where he found that asparagine, peptone, $NaNO_3$ and KNO_3 supported good growth of *P. grisea.* He told that nitrate could be utilized by the pathogen and obtained the growth. One of the reasons for good growth in Nitrate nitrogen sources may be that nitrate may be the easiest form of nitrogen that can be utilized by the pathogen and thus all the forms of nitrate used here supported good growth of *Pyricularia grisea*. Our result is in partial agreement with the results of Vikram Pal (2014) who studied that maximum mycelial growth of *P. grisea* was reported in barium nitrate followed by Ammonium nitrate. But he noted that minimum mycelial growth was found in Sodium nitrate and Potassium nitrate over control. None of nitrogen sources induced the sporulation of *P. grisea*.



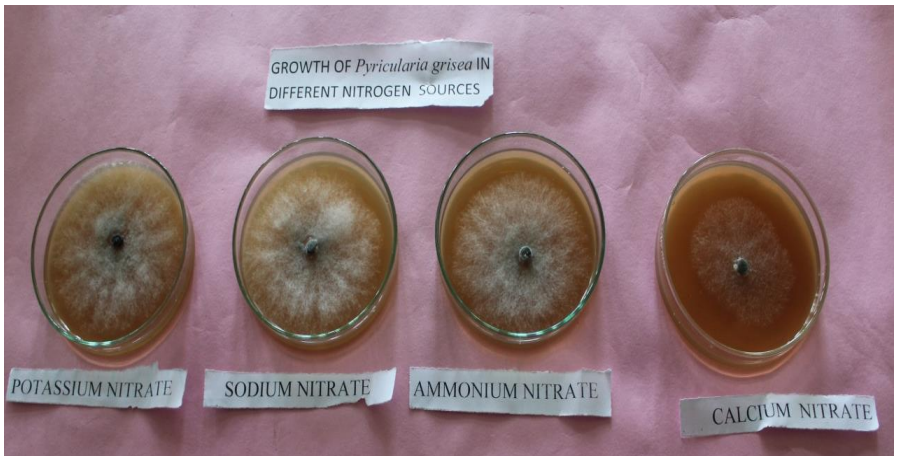


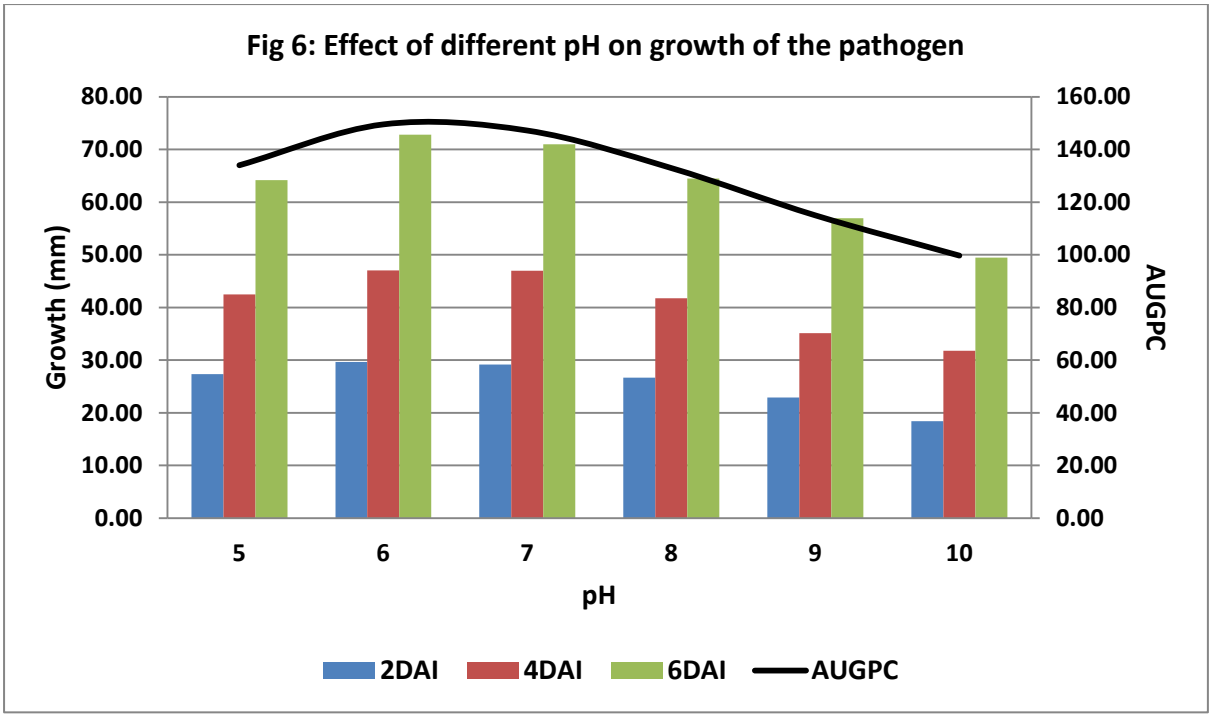
Plate 7: Growth of the pathogen in different nitrogen sources at 6 days after inoculation

4.5.5 Growth of the pathogen in different pH levels

Table 6: Growth of *Pyricularia grisea* in different pH levels

Media	Growth (mm)			AUGPC
	2DAI	4DAI	6DAI	
5	27.33	42.50	64.17	134.00
6	29.67	47.02	72.83	149.52
7	29.20	46.98	71.00	147.18
8	26.67	41.77	64.50	132.93
9	22.90	35.13	56.93	114.97
10	18.43	31.80	49.47	99.70
SEM ±	1.4404	2.5022	2.6996	3.9231
CD (5%)	4.4382	7.7100	8.3183	12.0883
CV (%)	9.707	10.605	7.404	5.238

The pathogen was grown in different pH conditions (section 3.16) to see the best pH requirement for its growth. From the results (Table 6) it was seen that the fungus. Best growth was recorded in pH 6 which is indicated by highest growth of 29.67 mm, 47.02 mm and 72.83 mm at 2, 4 and 6 DAI, respectively. Second best growth was achieved at pH 7 as second highest growth was recorded in this pH (Plate 8). The growth in both these pH are statistically at par. Highest AUGPC of 149.52 was recorded at pH 6 which is closely followed by pH 7 with an AUGPC of 147.18 whereas the lowest AUGPC of 99.70 was recorded in pH 10 (Fig. 6). At pH 9 and pH 10 drastic decrease in growth was recorded. So, it can be said that very high alkaline condition is not preferred by the fungus. The best growth of the fungus can be achieved at light acidic to neutral condition. This result is in agreement with the findings of Ravindramalviya (2014) who studied that the mycelial radial growth of *P. grisea* was significantly high at pH 6 followed by pH 7.0. Similar finding was observed by Mijan Hossain (2000) who studied that mycelial growth of *P. grisea* increased with increase in pH from 3.5 to 6.5. The pathogen showed maximum mycelial growth at pH 6.5. Our result is also in accordance with Pal (2014) who studied that the mycelial growth of *P. grisea* was maximum at pH 7 followed by pH 6. The least mycelial growth was recorded at the pH 9. pH 6 and pH 7 was found suitable for both growth and sporulation.



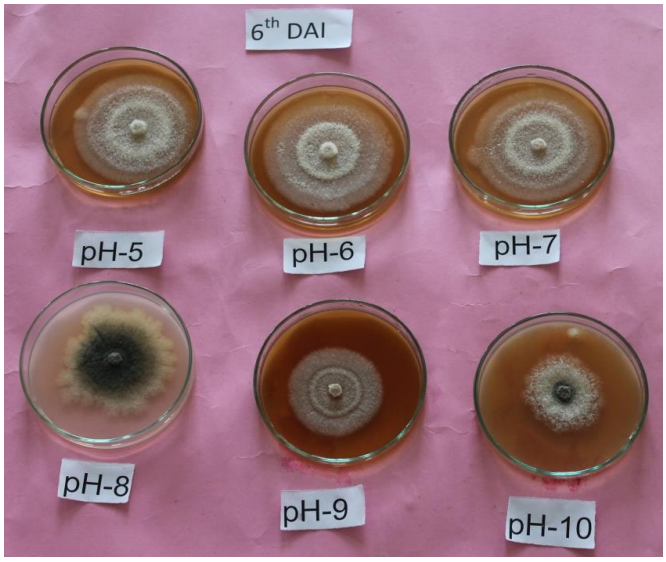


Plate 8: Growth of the pathogen in different pH at 6 days after inoculation

4.6 Testing of some new fungicides against *Pyricularia grisea* under *in vitro* conditions

Table 7: *In vitro* testing of different new fungicide molecules against *Pyricularia grisea* (% inhibition over control)

Treatments	Fungicide concentration (ppm)						ED 90 (ppm)
	50	100	200	300	400	500	
T1	62.87 (52.46)	64.75 (53.58)	68.82 (56.06)	71.26 (57.58)	77.25 (61.51)	88.82 (70.47)	1311.13
T2	45.55 (42.45)	58.08 (49.65)	72.34 (58.27)	74.29 (59.53)	85.39 (67.53)	100.00 (90.00)	450.47
T3	58.08 (49.65)	67.27 (55.10)	76.21 (60.81)	80.44 (63.75)	85.67 (67.76)	100.00 (90.00)	424.32
T4	28.14 (32.04)	58.64 (49.98)	74.37 (59.58)	77.64 (61.78)	85.03 (67.24)	100.00 (90.00)	399.82
T5	21.56 (27.67)	47.90 (43.80)	60.56 (51.10)	74.25 (59.51)	76.05 (60.70)	85.07 (67.27)	602.42
T6	22.83 (28.54)	52.50 (46.43)	59.88 (50.70)	69.82 (56.68)	84.91 (67.14)	100.00 (90.00)	460.77
T7	37.13 (37.54)	61.12 (51.43)	66.47 (54.62)	74.81 (59.87)	85.13 (67.32)	100.00 (90.00)	446.98
SEM ±	1.0248	0.3674	1.1338	0.8088	1.0083	0.3600	-
CD (5%)	3.1085	1.1144	3.4390	2.4533	3.0583	1.0920	-
CV (%)	4.599	1.273	3.513	2.342	2.661	0.743	-

(Figures in parenthesis are angular transformed value)

From Table 7, it was found that at 50 ppm concentration highest inhibition of 62.87% of the fungus was recorded by *in vitro* treatment with Azoxystrobin 23% SC (T1) followed by 58.08% inhibition in T3 (Azoxystrobin 23% SC + Difenoconazole 25% EC). The highest inhibition of 67.27%, 76.21%, 80.44%, 85.67% and 100% was recorded in 100, 200, 300, 400 and 500 ppm, respectively by T3 which is followed by T4 (Tebuconazole 50% + Trifloxystrobin 25% WG) with 58.64%, 74.37%, 77.64%, 85.03% and 100% inhibition, respectively (Fig 7; Plate 9). Lowest ED 90 value of 399.82 was recorded by T4 which is followed by 424.32 in T3 (Fig. 8). That means the dose for those two treatments should be around 0.4 g/litre of water for 90% control of the pathogen in the field. So, T3 and T4 is considered to be the best treatment for management of the pathogen under *in vitro* condition. The present findings is in agreement with Ghosh, 2015 who found that under *in vitro* conditions, Tricyclazole 75WP, Azoxystrobin18.2% + Difenoconazole 11.4% SC and Azoxystrobin 23SC were the most effective fungicides against *P. oryzae*. This result is also in accordance with Chander Mohan *et al.* (2013) who found that under *in vitro* conditions Tilt (Propiconazole), Amistar top (azoxystrobin + difenoconazole), Score (difenconazole) and Folicur (tebuconazole) were found significantly effective in growth inhibition of *Pyricularia*

grisea. Singh *et al*. (2014) also reported similar type of results 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}. Our results also in confirmity with Kunova *et al.* (2013) who 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.

Fig 7: *In vitro* effect of diffferent fungicide treatments on growth of the pathogen

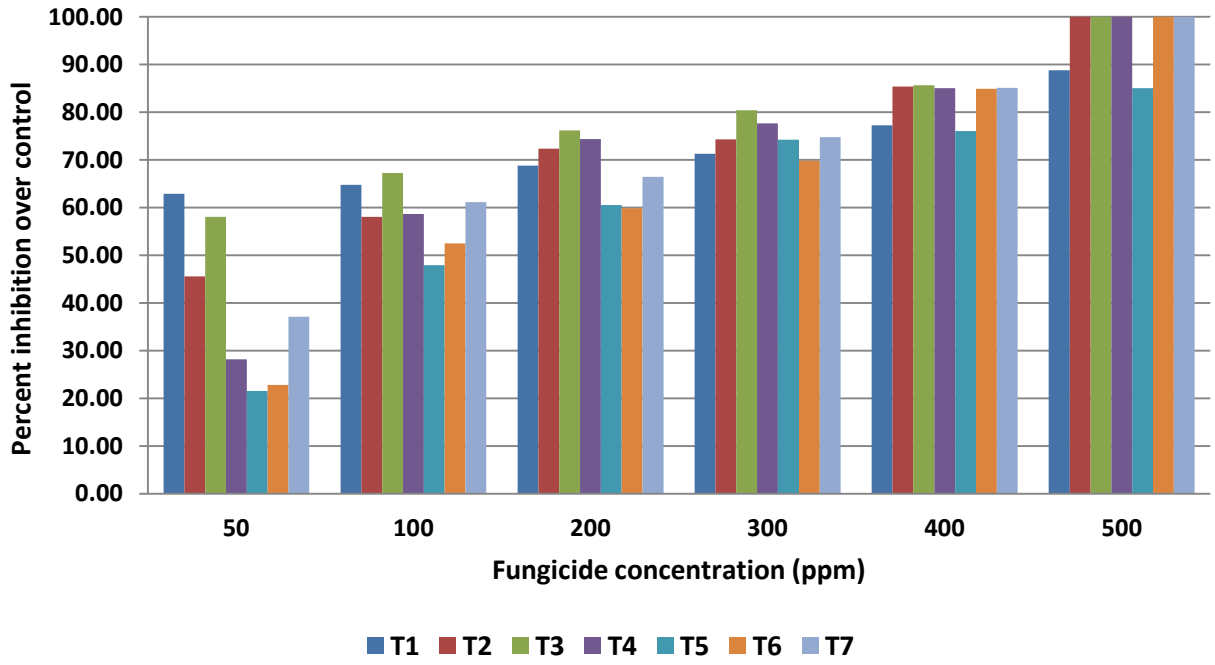
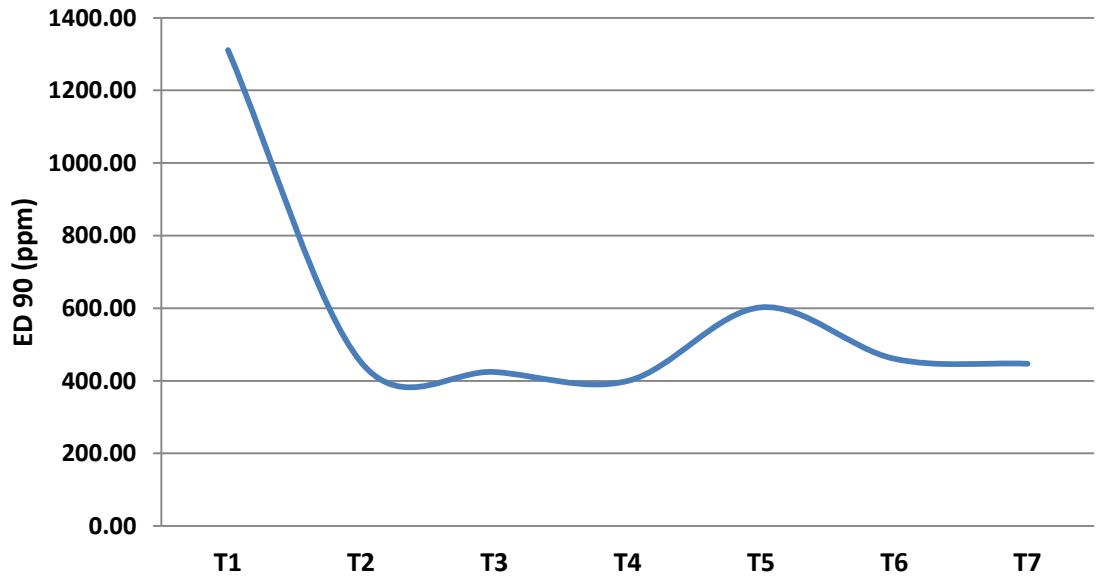
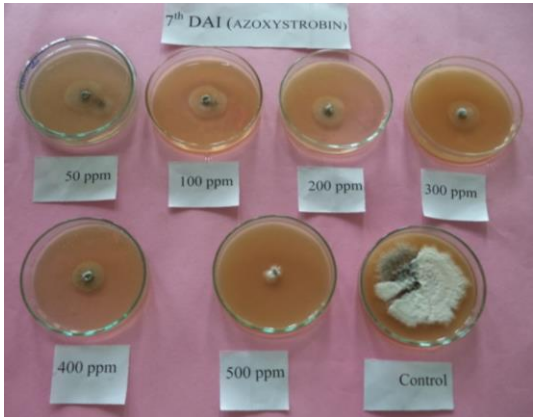
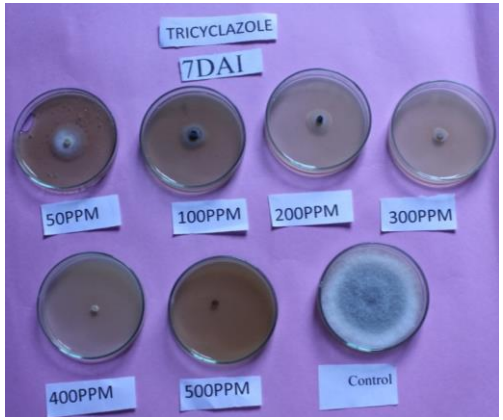
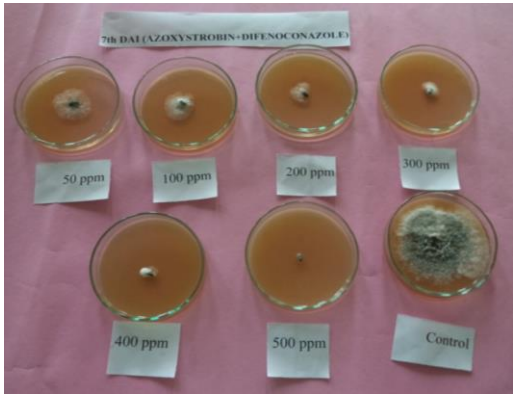


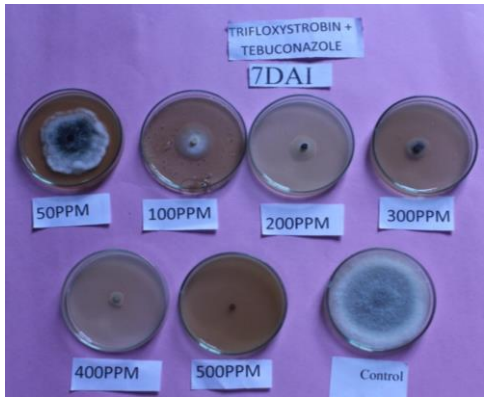
Fig 8: ED 90 values of different treatment under *in vitro* condition

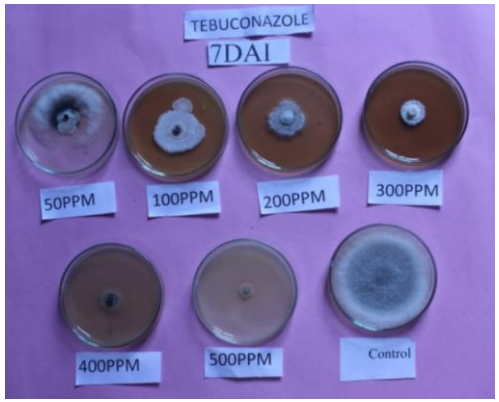












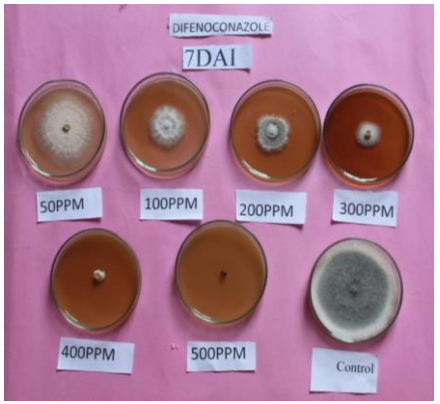


Plate 9: *In vitro* evaluation of different fungicides against *Pyricularia grisea* at 7 days after inoculation

4.7 Effect of different new fungicide molecules on leaf blast disease of rice under field conditions

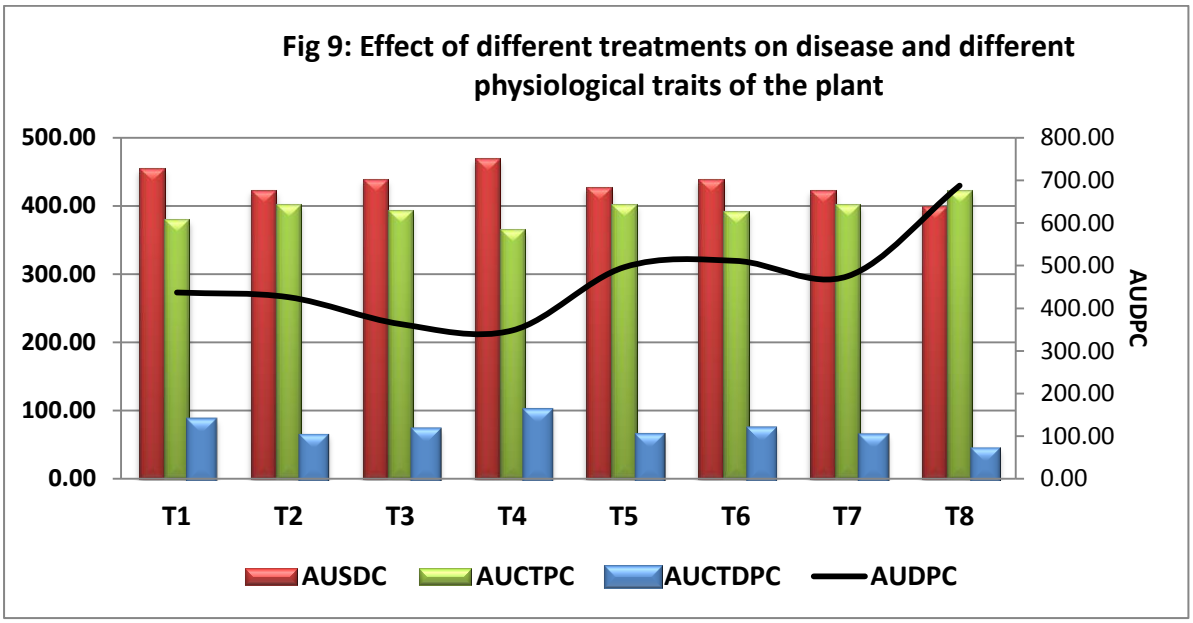
4.7.1 Effect of different treatments on leaf blast disease of rice and different physiological parameters of the plant

Table 8: Effect of different treatments on disease and different physiological traits of rice plants under field condition

Treatments	AUDPC	AUSDC	AUCTPC	AUCTDPC
T1	437.04	455.28	379.72	85.78
T2	425.93	422.10	402.72	62.78
T3	362.96	439.02	394.11	71.39
T4	348.15	469.77	365.67	99.83
T5	496.30	427.11	402.06	63.44
T6	511.11	438.58	392.72	72.78
T7	475.31	422.71	402.39	63.11
T8	687.65	399.20	422.50	43.00
SEM ±	16.4277	3.3986	1.9657	1.9657
CD (5%)	49.8283	10.3085	5.9623	5.9624
CV (%)	6.079	1.356	0.861	4.846

The above table (Table 8), indicates that lowest Area Under Disease Progress Curve (AUDPC) of 348.15 was recorded by T4 i.e. 3 sprayings with Trifloxystrobin + tebuconazole which is closely followed by 3 sprayings with Difenocoazole + Azoxystrobin (T3) with AUDPC of 362.96. Highest AUDPC of 687.65 was recorded by T8 i.e. control. Highest Area under spad decline curve (AUSDC) of 469.77 was recorded by T4 i.e. the plants in this treatment have more green leaves than any other treatments whereas the lowest AUSDC of 399.20 was recorded by T8. Lowest Area under canopy temperature progress curve (AUCTPC) of 365.67 and highest Area under canopy temperature depression progress curve (AUCTDPC) of 99.83 was recorded by the best treatment i.e T4 and these two traits were highest and lowest respectively in Control (T8) with a value of 422.50 and 43.00, respectively (Fig. 9). This indicates that the less diseased plants have more vigourous growth and canopy temperature inside the plot is minimum whereas the temperature difference between the plot and ambient temperature is maximum in that treatment. This trait is reverse in the highest diseased plot i,e, control. Similar results are also achieved by Ghazanfar *et al.* (2009) as they reported that application of fungicides Armure (propiconazole + difenoconazole), Rabicide (tetrachlorophthalide) and Score (difenoconazole) showed the best results with blast disease percentage 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%. Debashis Dutta *et al.* (2012) further confirms the present findings

by finding out that fungicides viz., Nativo 75 WG (tebuconazole + trifloxystrobin), Gain 75 WP, Score 250 EC (Difenoconazole), Hexacon Super 5% SC, and Tilt 25 EC (Propiconazole) against rice blast on MTU 7029 rice variety were very effective in the management of rice blast disease but, Nativo, Gain and Score proved effective in all the three weeks in reducing the disease more in 3rd week with 10.15%, 12.85% and 11.46%.



4.7.2 Relationship between AUDPC, AUSDC, AUCTPC and AUCTDPC

Negative correlation 61.6% and 62% was found between AUDPC with AUSDC and AUCTDPC, respectively in the plots (Fig. 10, 11) whereas positive correlation of 62% was found between AUDPC and AUCTPC (Fig. 12). It indicates that less diseased plant have more green leaves and vice versa. This is obvious because we are dealing here with the leaf blast disease of rice and the disease affects the leaves of the plants more. So, in more diseased plants the chlorophyll of the leaves are destroyed and the greenness of the leaves are reduced and thus AUSDPC of more diseased plots are less. This situation is just opposite in case of less diseased plots. So, we find a negative correlation between AUDPC and AUSDC of the plots. Similarly in less diseased plants canopy temperature depression was more as there is huge growth of the plant canopies which does not allow entry of extra sunlight inside the plant canopy and micro climate inside the plants are much more cooler than the normal atmospheric temperature. The positive correlation between AUDPC and AUCTPC suggests that more diseased plants have higher temperature inside the plant canopies as heavily diseased plants are less vigourous in growth and normal sunlight penetrates more inside the heavily diseased plant canopies and thus making the plant canopies more hotter. Wakiyama (2002) found that rice plants with high chlorophyll content are having low canopy temperature than the

plant with low chlorophyll content and this finding support the present experiment findings. All these result are also in agreement with Ashajyothi (2016) who noticed that two physiological traits like AUCTDC and AUSDC were negatively correlated with AUDPC. These results suggest that disease severity decrease with the decline in canopy temperature. The chlorophyll content was reduced with the increase in disease severity showing a negative correlation between AUDPC and AUSDC. Due to the necrosis of affected leaves, stems and other plant parts the chlorophyll content reduced in plant. Ashajyothi (2016) also found out that there is a positive correlation between AUDPC and AUCTPC which was evident from her results which implies increase in canopy temperature results in increase in disease severity. The decline in chlorophyll content is due to the increased disease severity resulting in negative correlation between AUSDC and AUDPC. This can be interpreted for the result that high chlorophyll content is responsible to lower the canopy temperature of the plants. Due to the fact disease severity is less as low temperature does not favour disease development.

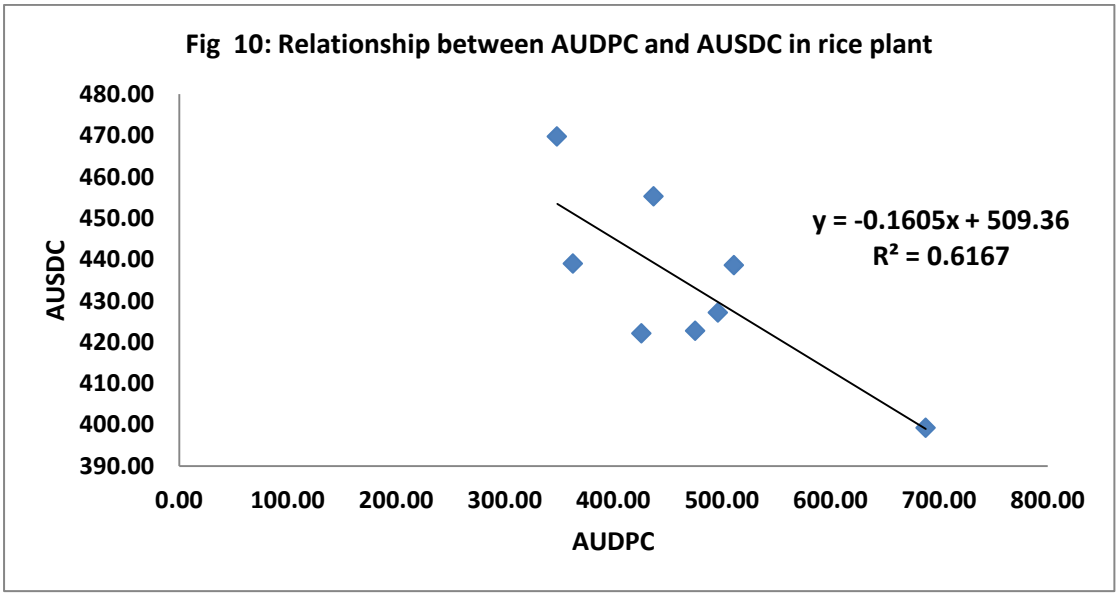


Fig 10: Relationship between AUDPC and AUSDC in rice plant

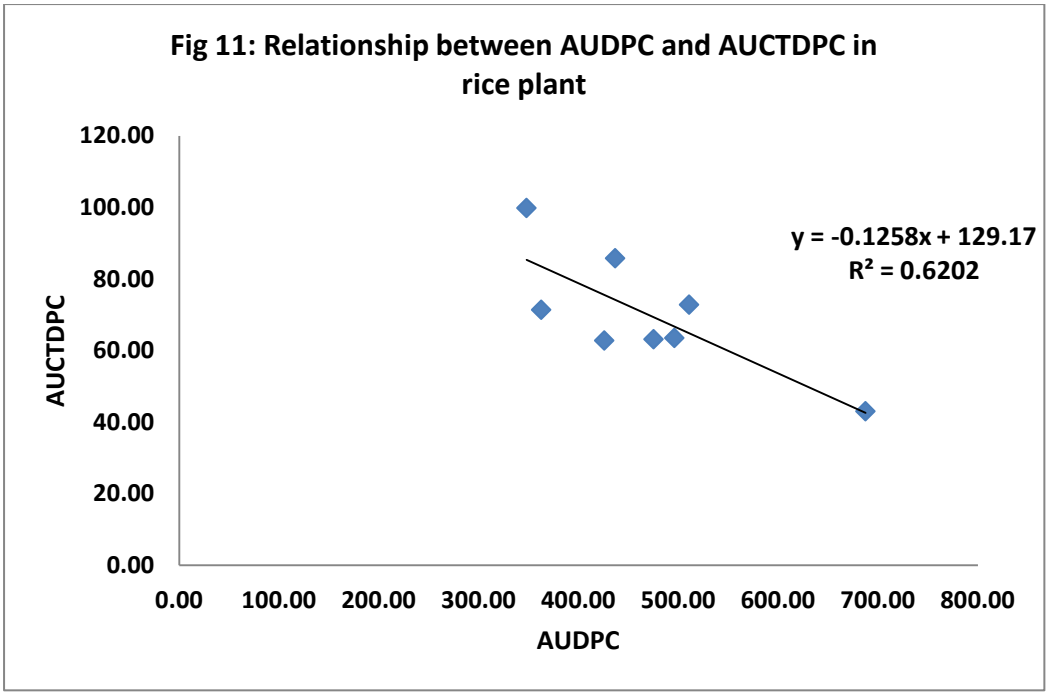


Fig 11: Relationship between AUDPC and AUCTDPC in rice plant
120.00
100.00
80.00
60.00
40.00
20.00
0.00
AUCTDPC
y = -0.1258x + 129.17
R² = 0.6202
0.00
100.00
200.00
300.00
400.00
500.00
600.00
700.00
800.00
AUDPC

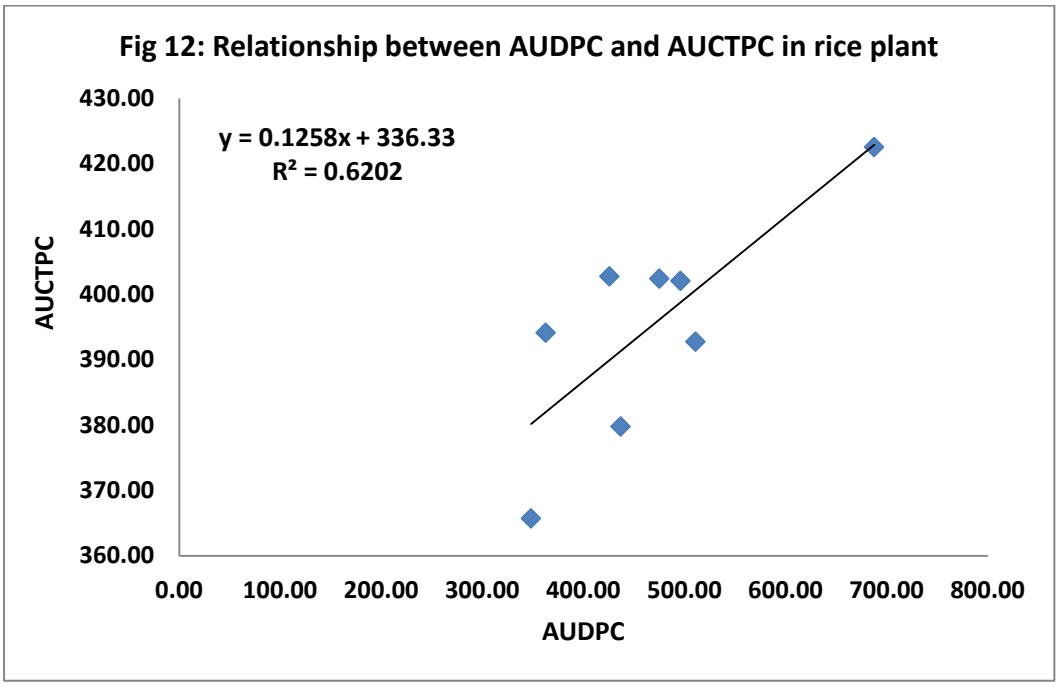


Fig 12: Relationship between AUDPC and AUCTPC in rice plant
430.00
420.00
410.00
400.00
390.00
380.00
370.00
360.00
AUCTPC
y = 0.1258x + 336.33
R² = 0.6202
0.00
100.00
200.00
300.00
400.00
500.00
600.00
700.00
800.00
AUDPC

4.7.3 Different yield attributes of rice

Table 9: Effect of different treatments on different yield attributes of rice

Treatments	Test weight (g)	Panicle No./m^2	Panicle weight (g)	Panicle length (cm)	Grains /Panicle	Unfilled grain (%)	Yield (t/ha)	Cost: Benefit Ratio
T1	22.58	266.63	2.98	22.71	147.60	6.72 (15.02)	3.83	1:0.29
T2	21.39	186.96	2.71	21.63	131.40	6.75 (14.80)	3.50	1:0.42
T3	21.97	234.76	2.95	22.11	144.13	6.06 (14.24)	3.67	1:0.54
T4	23.37	283.63	3.12	22.80	153.53	5.36 (13.39)	4.07	1:0.62
T5	22.01	173.15	2.79	20.87	130.47	5.84 (13.75)	3.53	1:0.48
T6	22.13	228.92	2.89	21.59	143.53	7.43 (15.80)	3.63	1:0.38
T7	21.37	182.71	2.71	20.87	129.33	5.53 (13.48)	3.47	1:0.38
T8	21.26	152.97	2.38	20.06	115.33	9.77 (18.21)	2.43	1:0.11
SEM ±	0.4168	5.6419	0.0564	0.501	1.7829	1.0091	0.0867	-
CD (5%)	1.2642	17.1130	0.1712	1.5196	5.4079	3.0608	0.2630	-
CV (%)	3.280	4.570	3.470	4.021	2.255	11.781	4.271	-

(Figures in parenthesis are angular transformed value)

From the above table (Table 9), it is also clearly seen that all the traits are best in T4 (3 sprayings with Tebuconazole 50% + Trifloxystrobin 25% WP). In this treatment test weight was maximum i.e. 23.37, panicle no. per m^2 was 283.63, panicle weight was 3.12 g, panicle length was 22.80, grains /panicle was 153.53, unfilled grain was 5.36% which is the lowest among all the treatments and highest yield of 4.07 t/ha was recorded (Fig. 13, 14, 15). This treatment is closely followed by T1 (3 sprayings with azoxystrobin 23% SC) with the second highest yield of 3.83 t/ha and T3 (3 spraying with Azoxystrobin 23% SC + Difenoconazole 25% EC) with the next highest yield of 3.67 t/ha. T1 and T3 are statistically *at par* in respect of yield. The lowest yield of 2.43 t/ha was recorded in control (T8) [Plate 10]. A negative correlation of 82% is found out between the disease and the yield of the crop (Fig. 16). That means more yield is achieved in less diseased plots and vice versa. So, T4 can be recognized as the best treatment. Highest cost: benefit ratio of 1:0.62 was also found in the best treatment i.e. T4 which is followed by T3 (1:0.54). Similar type of finding was recorded by Ganesh *et al.* (2012) who found that Tricyclazole sprayed rice plots produced highest yield and highest benefit:cost ratio.

Fig 13: Effect of different treatments on different yield attributes of rice

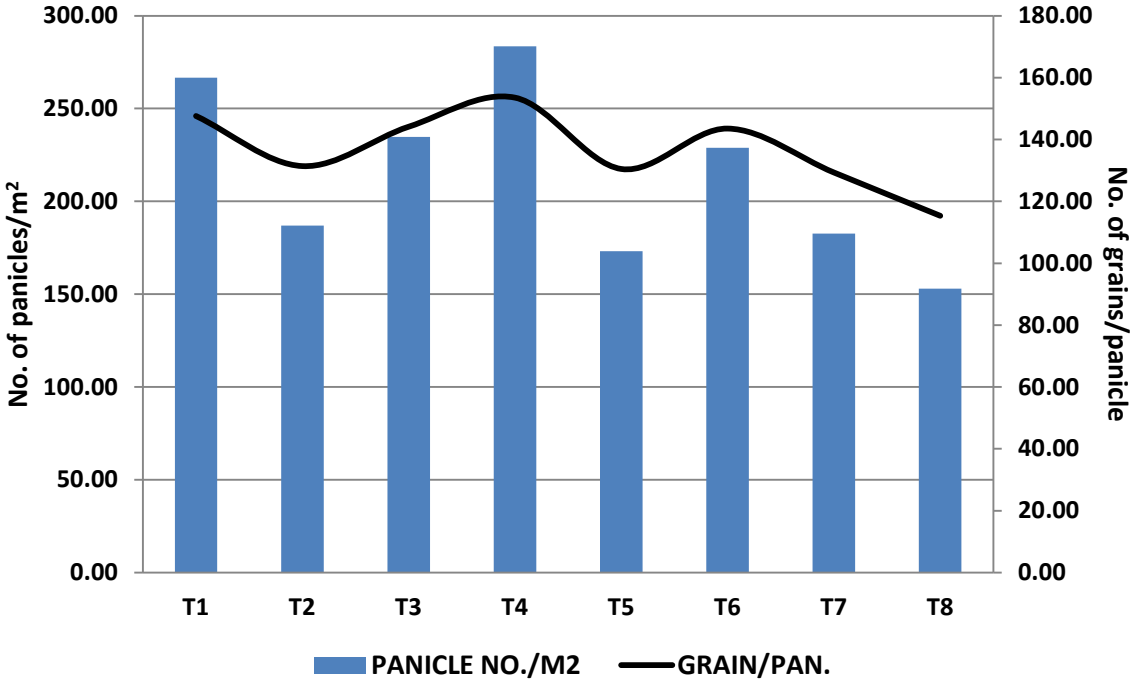


Fig 14: Effect of different treatments on different yield attributes

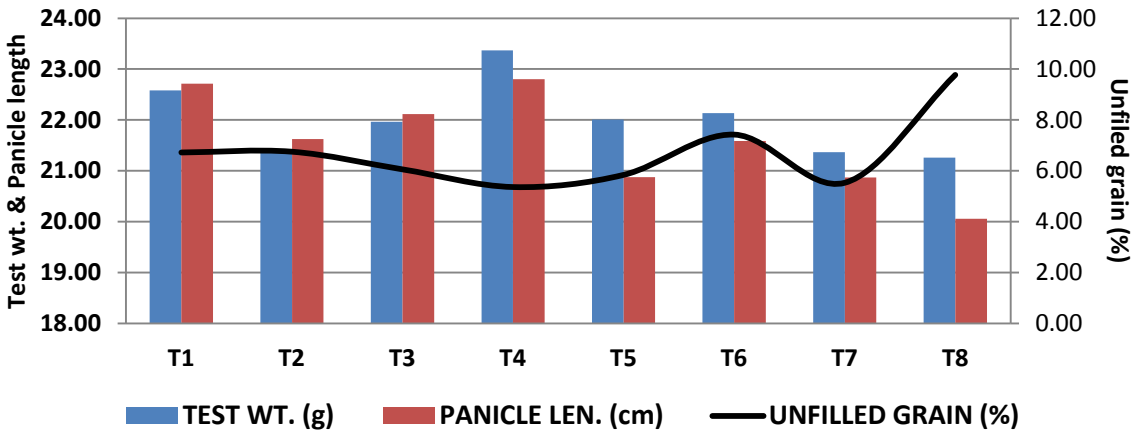


Fig 15: Effect of different treatments on yield and yield attributes of rice

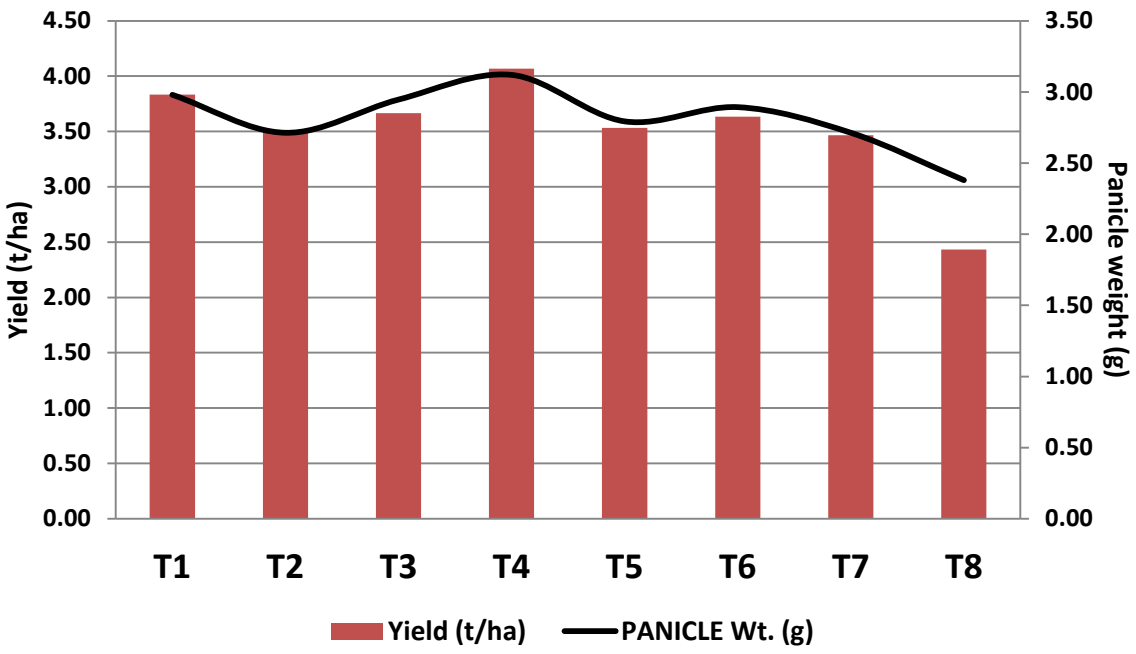


Fig 16: Relationship between AUDPC and yield of rice

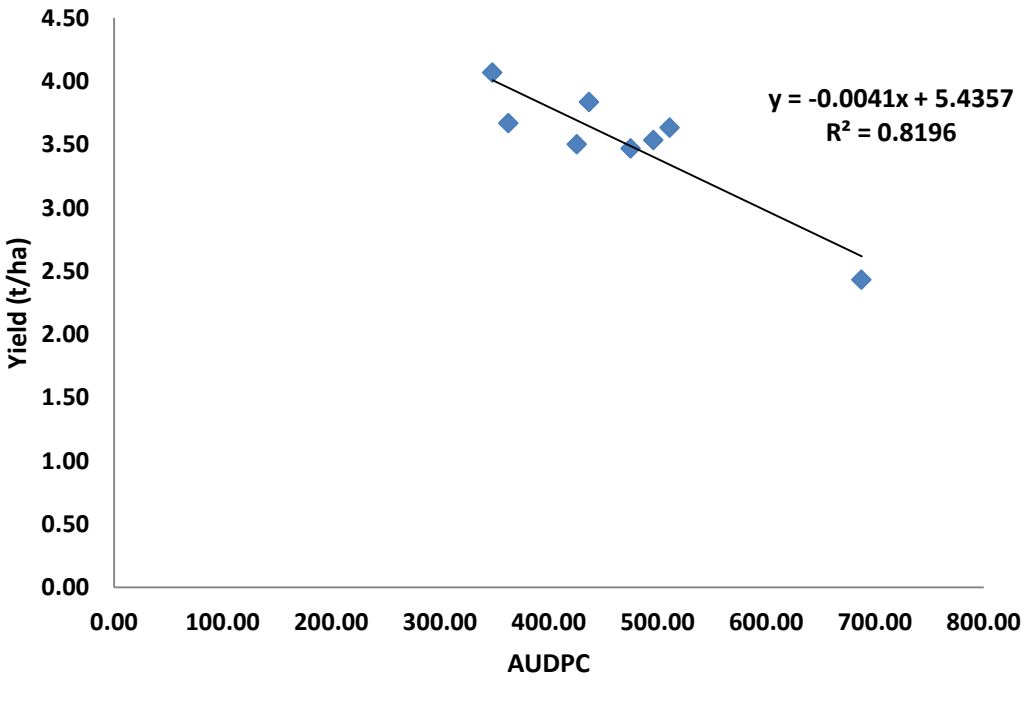


Plate 10 A: Photographs of field experiment during data taking

Rice field at initial stage

Characteristic symptoms of leaf blast produced by *Pyricularia grisea* in rice

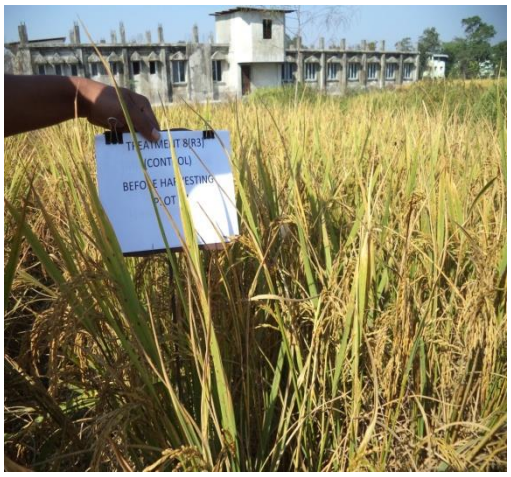
Rice field infected with leaf blast

Plate 10 B: Photographs of field experiment

Best treatment (T4)

Second best Treatment (T3)

Third Best treatment (T1)



Control (T8)

Close view of the control plot

Plate 10 C: Photographs of field experiment

CHAPTER 5

SUMMARY AND CONCLUSION

The present investigation was carried out in the field as well as in the laboratory condition. From the investigation it can be said that the leaf blast disease of rice can be effectively controlled by chemical management practices. The investigation has some good findings which are summarized as follows:

1. The pathogen was isolated from the infected leaf sample in the field in PDA and the isolated pathogen was inoculated in the healthy leaves. Same type of symptom was noticed. It was re-isolated again in the same media and the growth was similar to the earlier growth in the media.
2. No conidia were produced in all the media tested. Only in sterilized rice grain the conidia were produced after 55-60 days. Length and breadth of the conidia of blast pathogen (*Pyricularia grisea*) was measured and the size was much higher from leaf sample than in the media. The size of conidia measured about 17.96 - 26.64 µm × 7.36 - 9.22 µm (average 22.42 × 8.59 µm) and 12.06 - 19.95 µm × 5.38 - 9.06 µm (average 16.45 × 7.46 µm) from leaf sample and media, respectively. Mostly 2 celled conidia were found from rice grain media and 3 celled conidia were found in infected leaf sample.
3. Out of 5 media tested, best growth was achieved in PDA (71 mm) and OMA (71.33 mm) six days after inoculation and the AUGPC being 147.10 and 145.37 respectively.
4. Out of 6 temperatures tested, best growth was achieved in 25^0C (70.67 mm) and 30^0C (72.17 mm) six days after inoculation and the AUGPC being 139.50 and 141.33 respectively.
5. Out of 5 carbon sources tested, best growth of the was achieved in Glucose (78.30 mm) which is followed by Dextrose (72.60 mm) six days after inoculation and the AUGPC being 166.90 and 157.65 respectively.
6. Out of 4 nitrogen sources tested, best growth of the fungus was achieved in Potasssium nitrate (78.33 mm) which is followed by ammonium nitrate (73.10 mm) six days after inoculation and the AUGPC being 159.87 and 152.97 respectively.
7. Out of 6 nitrogen sources tested, best growth of the fungus was achieved in pH 6 (72.83 mm) which is followed by pH 7 (71 mm) six days after inoculation and the AUGPC being 149.52 and 147.18 respectively.

8. Under *in-vitro* condition, all the chemicals tested showed good growth inhibition over control. The lowest ED 90 value of 399.82 ppm was found in T4 (Tebuconazole 50% + Trifloxystrobin 25% WP).
9. In field condition, the lowest AUDPC of 348.15 was recorded by 3 sprayings with Tebuconazole 50% + Trifloxystrobin 25% WP (T4) which is closely followed by 3 times spray with Azoxystrobin 23% EC + Difenoconazole 25% EC (T3) with an AUDPC of 362.96. These two treatments are at par with each other. The highest AUDPC of 687.65 was recorded in control (T8).
10. Highest AUSDPC of 469.77 was found in T4 which is followed by T1 (Azoxystrobin 23% SC) and T3 with a value of 455.28 and 439.02, respectively. It indicates that more green leaves were there in these three treatments. A negative correlation of 61% was found in between AUDPC and AUSDPC. It may be explained by the fact that less disease is reflected by more green leaves of the plant.
11. Coolest canopy was found in T4 with lowest AUCTPC of 365.67 among all the treatments. Second lowest AUCTPC of 379.72 was found in T1. A positive correlation of AUDPC with AUCTPC was recorded.
12. Canopy temperature depression was just opposite of the canopy temperature within the plant. More depression was found in best treatments which is reflected by the result that highest AUCTDPC of 99.83 was recorded in T4 which is followed by T1 (85.78). A negative correlation of 62% was found in between AUDPC and AUCTDPC.
13. Yield as well as all the yield parameters like test weight, number of panicles per m^2, panicle weight, panicle length, grains/panicle were recorded highest in T4 which is followed by T1 and T3. Number of unfilled grain was also in the lower side in those treatments. Highest yield of 4.07 t/ha was achieved in T4 which is followed by T1 and T3 with a yield of 3.83 t/ha and 3.67 t/ha respectively. The lowest yield of 2.43 t/ha was recorded in control.
14. A negative correlation of 82% between AUDPC and yield was found out.

CHAPTER 6

FUTURE SCOPE OF RESEARCH

1. Suitable medium for fast sporulation of blast pathogen (*Pyricularia grisea*) may be found out.
2. Different strains of the pathogen from different places may be isolated.
3. Molecular characterization of those strains of the pathogen can be done.
4. Response of defense related biochemical parameters in the plant may be found out when the plant is challenged against the pathogen.
5. Varietal testing against the pathogen with different varieties may be done.
6. Some bio control agent can be included in the treatments and integrated as well as eco-friendly approach for the management of this disease may be found out.

BIBLIOGRAPHY

Ahn SW and Mukelar A. 1986. Rice blast management under upland conditions. *In: Progress in upland Rice Research, proceedings of a symposium at Jakarta*, IRAI, Los Banos, Phillipines. 363 - 373.

Alam MS. 1998. Evaluation of rice cultivars for resistance to *Diopsis longicornis* (Diptera: Diopsidae). *Journal of Economic Entomology.* **81**(3):934-936.

Anonymous. 2013. Agricultural Statistics at a glance. Ministry of Agriculture, India.43-44.

Aoki Y. 1955. On physiological specialization in the rice blast fungus *Pyricularia oryzae* Madras, India 117.

Apparao A. 1956. Studies on the blast disease of paddy, *Ph.D. Thesis*, University of Madras, India. 117.

Arshad HM, Khan, IJA and Jamil FF. 2008. Screening of rice germplasm against blast and brown spot disease. *Pakistan* Journal of *Phytopathology*. **20**: 52-57.

Arunkumar and Singh RA. 1995. In vitro evaluation of fungicides against *Magnaporthe grisea* isolated from rice, finger millet and pearl millet. *Indian Phytopathology*. **48**: 167-188.

Arun KS, Sachin U and Ajay S. 2011. Field evaluation of insecticides and fungicides for the control of whorl maggot, *Hydrellia philippina* and rice blast caused by *Pyricularia grisea.* **48** (3):280-281.

Arunkumar, H and Singh, R.A. 1995. Differential response of *Pyricularia grisea* isolates from rice, finger millet and pearl millet to media, temperature, pH and light. *Indian Journal of Mycology and Plant pathology.* **25**: 238 – 242.

Ashajyothi M. 2016. Resistance studies on sheath blight of rice and its chemical management, M.Sc. Thesis, UBKV, Pundibari, Coochbehar, West Bengal

Attere AF and Fatokun CA. 1983. Reaction of *O. glaberrima* accessions to rice yellow mottle virus. *Plant Disease*. **67**: 420–421.

Awodera VA and Esuruoso OF. 1975. Reduction in grain yield of two rice varieties infected by rice blast disease in Nigeria. *Nigerian* Agricultural *Journal.* **11**:170-173.

Awoderu VA, Esuruoso OF and Adeosun OO. 1991. Growth and conidia production in rice NG – 5 / IA – 65 of *Pyricularia oryzae* Cav. *in vitro. Journal of the Basic Microbiology*. **31:** 163 – 168.

Baker B, Zambryski P, Staskawicz B and Dinesh-Kumar SP. 1997 Signaling in plant-microbe interactions. *Science*. **276**: 726-733.

Barnwal MK, Singh VK, Sharma RB and Singh BN. 2012. Field evaluation of rice genotypes for resistance and new fungicides for control of blast (*Pyricularia oryzae*). *Indian Phytopathology*. **65**(1): 56-59.

Bastiaans L, 1991. Ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis of rice due to leaf blast. *Phytopathology*.**81**: 611-615.

Behera B, Senapati S, Swain NC and Mahanta IC. 2008. Prediction of some rice diseases using aerospore count and weather parameters. *Journal of Plant Protection and Environment*. **5**(1): 111-123.

Bhatt JC, Singh RA. 1992. Blast of rice. Plant diseases of international importance Volume I. *Diseases of cereals and pulses*. 80-115..

Bonman JM, Estrada BA and Banding JM. 1989. Leaf and neck blast resistance in tropical lowland rice cultivars. *Plant Disease.* **73**:388-390.

Bonman JM, Vergel de Dios TI, Bandong JM and Lee EJ. 1987. Pathogenic variability of monoconidial isolates of *Pyricularia oryzae* in Korea and in the Philippines. *Plant Disease.* **71**: 127-130.

Bonman JM. 1992. Blast. *In: Compendium of Rice Diseases (Eds. RK Webster, PS Gunnel), The American Phytopathological Society*, St. Paul Minnesota, USA: 14-16.

Boumas G. 1985. Rice Grain Handling and Storage.*Elsevier Science Publishers* B.V.9-10.

BPS. 2010. Informasi data luaspanen, produksitanamanpadiseluruhprovinsi. Jakarta :Badan Pusat Statistik.

Castilla N, Savary S, Veracruz CM and Leung H. 2009.*Rice Blast: Rice Fact Sheets*. International Rice Research Institute. pp. 1-3.

Chakrabarti NK and Wilcoxson RW. 1970. Effect of light on sporulation of *Pyricularia oryzae*. *Phytopathology*. **60**: 1171-1172.

Chander Mohan, Kaur, Amrinder, Raheja and Sandeep. 2013. *In vitro* evaluation of different fungicides against *Pyricularia grisea*, *Plant Disease Research*. **26**(2): 178

Chaudhary RG and Vishwadhar K. 1988. Epidemiology of rice blast and effect of date of sowing under upland conditions of Arunachal Pradesh. *Indian Phytopathology*. **41**(4): 552-557.

Chaudhary RG. 1986. Role of adjuvants in rice blast control under high rainfall conditions. *Oryza*. **23**(3): 196-197.

Chiba K, Tominaga T, and Urakawa F. 1996. Occurrence and control of rice blast disease in northern region of Iwate Prefecture in 1995. Annual Report of the Society of Plant Protection of North Japan. **47**: pp. 8-10

Chin KM. 1975.Fungicidal control of the rice blast disease. *Mardi Research Bulletin*. **2**(2): pp. 82-84.

Choi WB, Chun SJ and Lee YH.1987. Host range of Korean isolates of *Magnaporthe grisea. Korean Journal of Plant Pathology*. **12**(4): 453-454.

Clerk GC. 1974. Crops and their diseases in Ghana. Ghana Publishing Corporation, Tema: p. 144

Correa-Victoria FJ and Zeigler RS. 1993. Pathogenic variability in *Pyricularia grisea* at a rice blast "hot spot" breeding site in eastern Colombia. *Plant Disease*.**77**: 1029-1035.

Cortesi P and Giuditta L. 2003.Epidemiologiadell'elmintosporiosi e delbrusone e difesa del riso. *Informatore Fitopatologico*, **53**(2): 41–51.

Couch BC and Kohn LM. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia.* **94**:683-693.

Cruz MFA, da Prestes AM and Maciel JLN. 2009. Sporulation of *Pyricularia grisea* on culture media and light regimes. *Ciencia Rural*. **39**(5):1562-1564.

Dean RA. 1997. Signal pathways and appressorium morphogenesis. *Annual review of phytopathology*.**35**(1):211.234.PMid:15012522.http://dx.doi.org/10.1146/annurev.phyto.35.1.211

Debashis Dutta, Supradip Saha, Ray DP and Bag MK. 2012. Effect of different active fungicides molecules on the management of rice blast disease. *International Journal of Agriculture Environment & Biotechnology*. **5**(3): 247-251. Department of Agriculture. 2015. http://agricoop.nic.in

Dey U, Harlapur SI, and Dhutraj DN. 2013.Bioassay of fungicides, botanicals, bioagents and Indigenous Technology Knowledge (ITK) against *Pyricularia oryzae* Cav. causal agent of blast of rice. *Journal of Plant Diseases and Protection*. **8**(1):102-106.

Du Xinfa, Sun Shuyan, Zheng Zhong and Tao Rong Xiang. 1995. Evaluation of culture techniques for stimulating sporulation of *Pyricularia. Acta Agricultural Zhejiangensis.* **7**: 408 – 472.

Dubey SC. 2005. Efficacy of fungicides against blast (*Pyricularia grisea*) of rice (*Oryza sativa*). *Indian Journal of Agricultural Sciences.***75** (6):367-368.

Dubey SC. 1995. Evaluation of different fungitoxicant against blast of rice. *Journal of Research in Plant Disease.* **10**: 38 – 41.

El-Kazzaz MK, Osman ZH, Sehly MR and Badr SS. 1990. Chemical control of rice blast disease. *Agricultural Research Journal.* **68**: 491-500.

Enyinnia T. 1996. Effect of two systemic fungicides on the blast control in rainforest zone of Nigeria. *International Journal of Pest Management*. **42**: 77- 80.

FAO. 1995. World Rice Information, issue No.1. FAO, Rome, Italy.

FAO-Food and Agriculture Organization. 2009. FAOSTAT Database FAO, Rome, www.faostat.fao.org (accessed June 2010).

Faure J and Mazaud F. 1996. Rice quality criteria and the European market. In: *Proceedings of the 18th Session of the International Rice Commission*, 5-9 September 1996, Rome, Italy:121-131.

Filippi MC and Prabhu AS. 1997. Integrated effect of host plant resistance and fungicidal seed treatment on rice blast control. *Brazil Plant Disease.* **81**:351-355.

Fomba SN and Taylor DR. 1994. Rice blast in West Africa: it nature and control. *In: R.S, Zeigler, S.A Leong and P.S. Teng, (eds.). Rice Blast Disease.CAB International, Wallingford, UK, in association with IRRI, Manila, Philippines*. Fungi in rice seeds (*Oryza sativa* L.). *Summa Phytopathologica.* **27**(4): 403-409.

Food and Agriculture Organization (FAO). 2012. Food and Agricultural organization of United Nations. Website: http://FAOSTAT.fao.org [8th May 2014]

Froyd JD, Paget CJ, Guse LR, Dreikorn BA AndPafford J. 1976. Tricyclazole a new systemic fungicide for control of *Pyricularia grisea* on rice. *Phytopathology*. **66**: 1135-1139

Fukuda H, Matsuhashi M, Sato K and Fukaya T. 2004. The 2003 outbreak of rice panicle blast in Akita Prefecture and its major cause.*Annual Report of the Society of Plant Protection of North Japan*. **55**: 32-36.

Ganesh NR, Gangadhara NB, Basavaraja NT and Krishna NR. 2012. Fungicidal management of leaf blast disease in rice. *Global Journal of Biochemistry and Biotechnology*. **1** (1): 18-21.

Garcia Fernandez M. 2001. *Pyricularia* and other diseases in rice. *Agricultura Revista Agropecuaria*. **70**: 827, 358-359.

Gashaw G, AlemuT and Tesfaye K.2014. Morphological, physiological and biochemical studies on *Pyricularia grisea* isolates causing blast disease on finger millet in Ethiopia. *Journal of Applied Biosciences*.**74**: 6059- 6071.

Georgopoulos SG and Ziogas BN, 1992. Principles and methods for control of plant diseases. *Athens*: 236.

Ghazanfar MU, Wakil W, Sahi, ST and Saleem Y. 2009. Influence of various fungicides on the management of rice blast disease. *Mycopath.* **7**(1)**:** 29-34.

Gohel NM, Chauhan HL and Mehta AN. 2008. Bio-efficacy of fungicides against *P. oryzae* the incitant of rice blast. *Journal of Plant Disease Sciences*. **3**(2):189-192.

Gohel NM and Chauhan HL. 2009 . Evaluation of various bioagents against *Pyricularia oryzae* Cavara, the incident of rice blast. *Green Farming*. **2**(5):582-583.

Gouramanis GD. 1995. Biological and chemical control of rice blast disease (*Pyricularia oryzae*) in Northern Greece. CIHEAM. *Options Mediterraneennes*. **15**.

Groth DE. 2006. Azoxystrobin rate and timing effects on rice head blast incidence and rice grain and milling yields. *Plant Disease*. **90** (8):1055-1058.

Hajano JU, Lodhi AM, Pathan MA, Khanzada MA and Shah GS. 2012. *In Vitro* evaluation of fungicides, plant extracts and Bio-control agents against rice blast Pathogen *Magnaporthe oryzae* Couch. *Pakistan Journal of Botany*.**44** (5): 1775-1778.

Hajimo K. 2001. Rice Blast Disease.*Pesticide Outlook*.23-25.

Hamer JE, Howard RJ, Chumley FG and Valent B. 1988. A mechanism for surface attachment in spores of a plant pathogenic fungus.*Science*. **239** (4837): 288-290. PMid: 17769992 http://dx.doi.org/10.1126/science.239.4837.288.

Haq IM, Adnan MF, Jamil FF and Rehman A. 2002.Screening of rice germplasm against *Pyricularia oryzae* and evaluation of various fungitoxicants for control of disease. *Pakistan Journal Pythopathology*. **14**:32-35.

Hawksworth DL. 1990. CMI Descriptions of Fungi and Bacteria. August 1990, 111, Issue 2. *Mycopathologia*. **111**:109.

Heaton JB. 1964. Rice blast disease (*Pyricularia oryzae* Cav) of the Northern Territory. *The Australian Journal of Science*. **27**(81).

Hebert TT. 1971. The perfect stage of *Pyricularia grisea*. *Phytopathology*.**61**: 83-87.

Hollier CA, Groth DE, Rush MC and Webster RK. 1993. Common Names of Plant Diseases. The American Phytopathological Society, St. Paul, MN.

Hossain MM and Kulakarni S. 2001. *In vitro* evaluation of fungicides and neem based formulations against blast of rice. *Journal of Maharashtra Agricultural University*. **26** (2):151-153.

Huang YT, Yu CM, Tsai WH, Tai LH and Huang TY. 1980. Epidemiology of rice blast.III. Dependences of infection rate of leaf blast on dew, temperature and relative humidity. *Plant Protection Bulletin Taiwan*. **22**:307-320.

IPNI. International Plant Nutritional Institute. 2013. http://sap.ipni.net/article/andhra-pradesh

Iwadate Y, Chiba K, Sasaki N and Tominaga T. 2004.Outbreak of rice panicle blast disease in Iwate prefecture in 2003 and effect of its suppression using blasticides. *Annual Report of the Society of Plant Protection of North Japan*. **55**: 11-15.

Jabeen R, Iftikhar T and Batool H. 2012. Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae* PV. *Oryzae*causing BLB disease in rice. **44** (1): 261-265.

Jamal-u-Ddin H, Lodhi AM, Pathan MA, Khanzada MA and Shah GS. 2012. *In-vitro* evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen *Magnaporthe oryzae* Couch. *Pakistan Journal of Botany*. **44**(5): 1775-1778.

Jia Y, Adams SAM, Bryan GT, Hershay HP and Valent B. 2000. Direct interaction of resistance genes products confers rice blast resistance. *EMBO Journal*. **19**: 4004-4014.

John VT, Thottapilly G, Ng Q, Aluri K and Gibbons JN. 1985. Varietal reaction to rice yellow mottle virus disease. FAO plant protection bull. **33**:109-111.

Jones M, Heinrichs E, Johnson D and Riches C. 1994. Characterization and utilization of *Oryza glaberrima* in the upland rice breeding programmed. *In: WARDA(ED) WARDA Annual report for 1993, WARDA,* Côted'Ivoire, 3-1.3

Joshi MS and Mandokhot AM. 2002. Efficacy and economics of tricyclazole 75 WP in control of leaf blast of rice. *Annals of Plant Protection Sciences*. **10**(2):392-393.

Kapoor AS, Prasad R and Sood GK. 2004. Forecasting of rice blast in Kangra district of Himachal Pradesh. *Indian Phytopathology*. **57**(4):440-445

Kapoor Pooja and Abhishek Katoch. 2014. Past, present and future of rice blast management. *Plant Science Today*. **1** (3): 165-173 http://dx.doi.org/10.14719/pst.2014.1.3.24.

Kato H. 2001. Rice blast disease. Pesticide Outlook February 2001:23-25.

Khush GS, Kinoshita T. 1991. Rice karotype, marker gene and linkage groups. *In: Khush GS, Toenniessen GH (eds) Rice biotechnology, CABI Int, Wallingford, UK*: 83–108

Kim CK. 1994. Blast management in high input, high yield potential temperate rice ecosystems. In: *R. S. Zeigler, S. A Leong., P. S Teng. (eds.) Rice Blast Disease. CAB International, Wallingford, UK, in association with IRRI, Manila, Philippines*: 451-464.

Kim CK and Kim CH. 1991.Predicting rice blast outbreaks in Korea.Rice blast modeling and forecasting Selected papers from the International Rice Research Conferences-27-31 August 1990, Seoul Korea Republic. pp. 53-67.

Kim YS, Oh JY, Hwang BK and Kim KD. 2008. Variation in sensitivity of *Magnaporthe oryzae* isolates from Korea to edifenphos and iprobenfos. *Crop protection*. **27**: 1464-1470.

Kishan Lal, Ramesh Singh Yadav and Satish Chand.2015. In-vitro management of rice blast caused by *P. oryzae* with *Pseudomonas Fluorescens* & Fungicides, *The Ecoscan.* **9**(3 & 4):1001-1006.

Kole C. 2006. Cereals and millets (Vol. 1): Springer. http://dx.doi.org/10.1007/978-3-540-34389-9

Kumbhar CT. 2005. Evaluation of new fungicide formulations against blast disease of rice. *Karnataka Journal of Agricultural Sciences.* **18** (1):184-185.

Kunova A, Pizzatti C and Cortesi P. 2013. Impact of Tricyclazole and Azoxystrobin on growth, sporulation and secondary infection of the rice blast fungus (*Pyricularia oryzae). Pest Management Science.* **69**(2):278-284.

Leong SA. 2004. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter? Presentation at the Beaumont Texas A&M Research and Extension Center, July 8, 2004. *In: http://www.ars.usda.gov /research/projects/projects.*

Li JB, Yang J, Liu L, He HM, He XH, Zhu YY and Li CY. 2011. Geographic distribution of avirulence genes in rice blast fungus in Yunnan province, China. *Journal of Resources and Ecology*. **2**(2): 181-185.

Linares OF. 2002. African rice (*Oryza glaberrima*) history and future potential. *Proceeding of the National Academy of America*; *National Academy of Sciences*. **99** (25): 16360-16365.

Ling KC. 1980. Studies on rice diseases. In:*Rice Improvement in China and other Asian Countries.* International Rice Research Institute and Chinese Academy of Agricultural Science. pp. 135-148.

Linscombe LS. 2006. Rice cultivar designated cheniere. Available at http//www.freepatentsonline.com/741725.htm. Accessed 03/03/10.

Liu YF, Chen ZY, Hu M, Li Lian and Liu YZ. 2004. Distribution of *Magnaporthe grisea* population and virulence of predominant race in Jiangsu Province, China. *Rice Science*. **11**(3): 324-330.

Luo YPS, Tang NG, Febellar DO and Te Beest. 1998. Risk analysis of yield losses caused by rice leaf blast associated with temperature changes above and below for five Asian countries. *Agricultural Ecosystem& Environment*. **68**:197-205.

Manandhar HK, Jorgensen HJL, Mathur SB and Petersen VS. 1998. Seed borne infection of rice by *Pyricularia oryzae* and its transmission to seedlings. *Plant Disease*. **82**(10):1094-1099.

Manandhar HK. Jorgensen HJL, Mathur S.B and Petersen VS. 1998. Resistance to rice blast induced by ferric choride, di-potassium hydrogen phosphate and salicylic acid. *Crop protection*. **17** (4): 323-329.

Manibhushan Rao K, Joe Y and Krishnan P. 1989. Rice blast: epidemiology and application of its techniques in disease management. *Review-of-tropical-plant-pathology Techniques and plant quarantine.* **6**: 119-149.

Manibhushan Rao K. 1994.Rice Blast Disease. 1st Ed., Daya Publishing House, Delhi.1.

Mbodi Y, Gaye S and Diaw S. 1987. The role of tricyclazole in rice protection against blast and cultivar improvement. *Parasitica*. **43**: 187-198.

Meena BS. 2005. Morphological and molecular variability of rice blast pathogen *Pyricularia grisea* (Cooke) Sacc. M.Sc. Thesis. University of agricultural sciences, Dharwad.

Mahdieh S, Hosseini-Moghaddam and Jalal Soltani. 2013. An investigation on the effects of photoperiod, aging and culture media on vegetative growth and sporulation of rice blast pathogen *Pyricularia oryzae. Progress in Biological Sciences.* **3**(2):135 143.

Mijan Hossain MD. 2000. Studies on Blast disease of rice caused by *Pyricularia grisea* (Cooke) Sacc. in upland areas. M.Sc. Thesis, University of Agricultural Sciences, Dharwad. 52 – 53.

Minami E and Ando I. 1994. Analysis of blast disease resistant induced by probanazole in rice. *Journal of Pesticide Science.* **19**: 79 - 83.

Monma Y, Kanagawa M and Sasahara M. 2004. Outbreak of rice blast disease and its causal factors in Miyagi prefecture in 2003.*Annual Report of the Society of Plant Protection of North Japan*. **55**: 16-19.

Motlagh MRS and Javadzadeh A. 2010.Evaluation of the reaction of *Alismaplantago aquatica* and some rice cultivars to *Curvularia lunata* in the north of Iran. *Journal of Food, Agriculture and Environment*. **8**: 3-4.

Muralidharan K and Rao G. Venkata. 1980. A simple method of forecasting of outbreak of rice blast. *Indian Phytopath*. **33**(4):560 – 564.

Naidu VD and Reddy GV. 1989. Control of blast (BI) in main field and nursery with some new fungicides. *Review of Palaeobotany and Palynology*. **69**: 209.

Naik VKB and Jamadar MM. 2014. *In- vitro bioassay of different fungicides against blast of pearl millet caused by Pyriculariagrisea* (Cooke.) Sacc. *Karnataka Journal of Agricultural Sciences*. **27** (1): 88-90.

Nasruddin and Amin. 2013. Effect of Difenoconazole and difenokonazole + propikonazole against the rice blast disease, *Journal of Agricultural Science* (Toronto)**. 5**(1):160-167.

Netam RS, Tiwari RKS, Bahadur AN and Shankar Deo. 2014. *In vitro* and *in vivo* efficacy of fungicides against *Pyricularia grisea* causing finger millet blast disease. *International Journal of Plant Protection*. **7** (1): 137-142.

Nicholas J and Talbot. 2003. On the trail of a cereal killer: Exploring the Biology of *Magnaporthe grisea*. *Annual Review of Microbiology*. **57**:177–202.

Nishikado Y. 1917. Studies on rice blast fungus. *I. Berichted. oharaInst.f. landw. Forsch*. **1:** 179 – 219.

Nutsugah SK. 1997. Survey of rice diseases/pests in Northern Ghana.*In*: *Proceedings of the 6thAnnual WARDA IPM.TF Meetings*, March 2-5, 1997. Bouaké, Côte d.Ivoire.

Nutsugah SK. 1997. Survey of diseases/pests of upland/hydromorphic rice in Northern Ghana. *Proceedings of the SARI Annual In-house Review*, March 24-26, 1997, Nyankpala, Ghana.

Nutsugah SK and Twumasi JK. 2001. Fungal Diseases of rice in Ghana: Knowledge and research needs. *In*: S. *Sreenivasa prasad and R. Johnson, (eds.).Major fungal*

diseases of rice recent advances.Kluwer Academic Publishers. The Netherlands. pp. 321-329.

Nutsugah, S.K., J.K. Twumasi, Y Chipili, and S Sreenivasa prasad. 2008. Diversity of the rice blast pathogen in Ghana and strategies for resistance management. *Plant Pathology Journal.* **7**(1): 109-113.

Oduro KA. 2000. Checklist of Plant Pests in Ghana. Diseases. Ministry of Food and Agriculture. **1**, p. 105.

Oerke EC and Dehne HW. 2004. Safeguarding production--losses in major crops and the role of crop protection. *Crop Protection.* **23**(4): 275-285.

Okeke B, SegigleMurandI F, Steiman R and Sage L. 1992. Investigation on cultural and cellulolytic activity in *Pyricularia oryzae* Cav. *Agronomie*. **12**: 325 – 329.

Ono K and Nakazato K. 1958. Morphology of the conidia of Py*ricularia* from different host plants produced under different conditions. *Annals of the Phytopathological Society of Japan.* **23**: 1 – 2.

Otani Y. 1953. Growth factors and nitrogen sources of *Pyricularia oryzae* Cav. *Annals of the Phytopathological Society of Japan.* **17**: 9 – 15.

.Otsuka H, Tamari K and Agaraswara N. 1957. Biochemical classification of *Pyricularia oryzae* Cav. (1-4) in Japan. *Journal of Agricultural Chemistry Society*. **31**:791 –798

Ou SH. 1985. Rice Diseases, CAB International Mycological, Institute Kew, Survey, UK..

Ou SH and Nuque FL. 1985. Rice Diseases, second ed. Commonwealth Mycological Institute, Kew, Surrey, UK.

Ou SH. 1985. Rice Diseases, 2nd edn. *Common wealth Mycological Institute*, Kew, UK. p. 380.

Padmanabhan SY. 1974. Fungal Diseases of Rice in India. 1st Ed. Indian council of Agriculture Research, New Delhi. 15.

Padmanabhan SY, Chakrabarti NK, Mathur SC and Veeraraghavan J. 1970.Identification of pathogenic races of *Pyricularia oryzae* in India. *Phytopathology.* **60**: 1574 – 1577.

Padmanabhan SY. 1965. Estimating losses from rice blast in India. *In: the rice blast disease: Johan Hopkins Press, Baltinoie, Maryland.* pp. 203-221.

Padmanabhan SY. 1965. Studies on Forecasting outbreaks of blast disease of rice. Central Rice Research Institute, Cuttack. pp. 117-129.

Pal V. 2014. Studies on leaf blast of rice caused by *Pyricularia grisea* (Cooke) Sacc. and their management. M.Sc. Thesis**,** Department of Plant Pathology College of Agriculture, Rewa 486001 Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh.

Pena FA, Perez LM, Cruz CMV and Ardales EY. 2007. Occurrence of rice blast fungus, *Pyricularia grisea* in the Philippines. *JIRCAS Working Report*. **53**: 65-70.

Perezsendin M, De LA and Barrios GJ. 1982. Biological aspects of *Pyricularia oryzae*. *Ciencias de la Agricultural.* **12**: 111-113.

Perini VM, Castr, H, Santos G, Aguiar RS, Leao EU and Seixas PTL. 2011. Evaluation of the preventive and curative effect of the essential oil of citronella grass in the control of *Pyricularia grisea*. *Journal of Biotechnology and Biodiversity*. **2**(2): 23.

Perrott RF and Chakraborty S. 1999.Pyriculariagrisea causes blight of buffel grass (*Cenchrus ciliaris*) in Queensland, Australia. *Tropical Grasslands.* **33**:201-206.

Peterson LG. 1990. Tricyclazole for control of *Pyricularia oryzae* on rice: the relationship of the mode of action and disease occurrence and development. *Pest management in rice conference held by the Society of Chemical Industry*, London, UK,4-7 June 1990. pp. 122-130.

Pinnschmidt HO, Teng PS and Luo Y. 1994. Methodology for quantifying rice yield effects of blast. In: *Zeigler RS, Leong SA, Teng PS, editors. Rice blast disease. Wallingford, Oxon (United Kingdom)*: CAB International, Los Baños (Philippines): International Rice Research Institute. pp. 381-408.

Prabhu AS, Filippi MC and Zimmermann FJP. 2003. Cultivar response to fungicide application in relation to rice blast control, productivity and sustainability. *Pesquisa Agropecuária Brasileira. Brasília*. **38**:11-17.

Prabhu AS and Morais OP. 1986. Blast disease management in upland rice in Brazil. *In progress in upland Rice Research. In: Proceedings of the 1985 Jakarta Conference*. pp. 383-382.

Prajapati KS, Patel RC and Pathak AR. 2004. Field evaluation of new fungicides against blast of rice. *Pesticide Research Journal.* **16** (2): 26-28.

Prasanna kumar and Veerabhadraswamy 2014**.** Appraise a combination of fungicides against blast and sheath blight diseases of paddy (*Oryza sativa* l.) *Journal of Experimental Biology and Agricultural Sciences.* **2**(1).

PriyaVanaraj, Kandasamy S, Ambalavanan S, Ramalingam R and Sabariyappan R. 2013. Variability in *Pyricularia oryzae* from different rice growing regions of Tamil Nadu, India. *African journal of Microbiology Research*. **7**(26):3379-3388.

Ram B, Khadka, Sundar M, Shrestha, Hira K. Manandhar and Gopal BKC. 2012. Study on Differential Response of *Pyricularia grisea* Isolates from Rice, Finger Millet and *Panicum* sp. with Local and Alien Media, and Their Host Range. *Nepal Journal of Science and Technology*. **13(**2): 7-14.

Ram T, Majumder TND, Mishra B, Ansari MM and Padmavathi G. 2007. Introgression of broad spectrum blast resistance genes into cultivated rice (*Oryza sativa* sp. *indica*) from wild rice *Oryza rufipogon*. *Current Science*. **92** (2):225-230.

Ravindramalviya. 2014. Studies on integrated approaches for the management of leaf blast of rice caused by *Pyricularia grisea* (Cooke) Sacc**.** M.Sc. Thesis**,** Department of Plant Pathology College of Agriculture, Rewa (M.P.) Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh.

Reddy APK and Bonman JM. 1987. Recent epidemics of rice blast in India and Egypt. *Plant Disease*. **71** : 850.

Reddy APK and Satyanarayana K. 1988. Evaluation of new organic fungicides for the control of blast disease of rice. *Pesticides*. **22**(9): 21-26.

Reversat G and Destombes, D. 1995. Resistance to *Heterodera sacchari* in rice.*Nematologica.***41**: 333-334. [Abstr.]

Reynolds MP, Singh RP, Ibrahim A, Ageeb OAA. 1998. Evaluating the physiological traits to complement empirical selection for wheat in warm environments. *Euphytica*. **100**: 85–94.

Rossman AY, Howard RJ, Valent B. 1990). *Pyricularia grisea*, the correct name for the rice blast fungus. *Mycologia.* **82**:509–512.

Rosyara UR, Khadka K, Subedi S, Sharma RC and Duveiller E. 2009. Field resistance to spot blotch in three spring wheat populations. *Journal of Plant Pathology*. **91**(1): 113-122.

Rosyara UR, Pant K, Duveiller E and Sharma R.C. 2007. Variation in chlorophyll content is not associated with undesirable physio morphological traits spot blotch resistance under natural epiphytotic conditions.*Journal of the Australasian Plant Pathology Society*. **36**: 245- 246

Saifulla M and Seshadri VS. 1992. Chemical control of rice seedling blast. *Plant Protection Bulletin Faridabad*. **44**(3): 45-47.

Sano Y, Sano R and Morishima H. 1984.Neighbour effects between two occurring rice species, *Oryza sativa* and *O. glaberrima. Journal of Applied Ecology*. **21**: 245-254.

Scardaci SC. 2003.A new diseases in California. University of California. Davis: Agronomy Fact Sheet Series 1997-2. Retrieved 2010-10-20.

Seebold KW, Datnof JLE, Correa-Victoria FJ, Kucharek TA and Snyder GH.2004. Effects of Silicon and fungicides on the control of leaf and neck blast in upland rice. *Plant Disease.* **88**: 253-258.

Senadhira D, Dhanapala MP and Sandanayake CA. 1980.Progress of rice varietal improvement in the dry and intermediate zones of Sri Lanka.Proceedings of the Rice Symposium, 1980. Dept. of Agriculture, Sri Lanka.

Sesma A and Osbourn AE. 2004. The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature*. **431** (7008): 582-586. PMID:15457264 http://dx.doi.org/10.1038/nature02880.

Shafaullah K, Khan MA, Khan NA and Yasir Mahmood. 2011. Effect of epidemiological factors on the incidence of paddy blast (*Pyricularia oryzae*) disease. *Pakistan Journal of Phytopathology*. **23**(2): 108-111.

Sharma PK, Upamanyu S and Srivastava A. 2011. Field evaluation of insecticides and fungicides for the control of whorl maggot (*Hydrellia philippina*) and rice blast (*Pyricularia grisea*). *Oryza*. **48**(3):280-281.

Sharma TR, Madhav MS, Singh BK, Shanker P, Jana TK, Dalal V, Pandit A, Singh A, Gaikwad K, Upreti HC and NK Singh. 2005. High-resolution mapping, cloning and molecular characterization of the Pi-kh gene of rice, which confers resistance to *Magnaporthe grisea*. *Mol. Genet. Genomics*. **274**:569–578.

Silue D and Notteghem J. 1991.Resistance of 99 *Oryza glaberrima* varieties to blast. Int. Rice Res. News. **16**, pp. 13–14.

Silva GB, Prabhu AS, Filippi MCC, Trindade MG, Araujo LG and Zambolim L. 2009.Genetic and phenotypic diversity of *Magnaporthe oryzae* from leaves and panicles of rice in commercial fields in the State of Goias, Brazil. *Tropical Plant Pathology*. **34** (2): 71-76.

Singh G and Prasad CS. 2007. Evaluation of fungicides against blast in Basmati rice.*Ann. Pl. Prot. Sci*. **15**(2) : 514-515.

Singh NI. 1988. The fungal air-spora of Imphal, its seasonal fluctuations and relationship with occurrence and intensity of major crop diseases. *International Aerobiology Newsletter*. **28**: 15-17.

Singh SR, Sunder DS, Dodan DS and Ram L. 2011. Sources of resistance to blast and its management through chemicals. *Journal of Mycology and Plant Pathology*. **4**(3):422-425.

Singh S, Mohan C and Pannu PPS.2014. Bio-efficacy of different fungicides in managing blast of rice caused by *Pyricularia grisea*. *Plant Disease Research.* **29** (1): 16-20.

Sirkant Kulkarni and Govindu HC. 1976. Studies on the blast disease of ragi in Karnataka. Physiological studies of leaf and neck isolates of *Pyricularia setariae* Nishikado. *Mysore Journal of agricultural Sciences.* **10**: 627-631.

Sood GK and Kapoor AS. 1997. Efficacy of new fungicides in the management of rice blast. *Plant Disease Research.* **12**:140-142.

Sotodate K, Hirano M, Ichimori T and Hirano Y. 1991. Occurrence and control of rice blast disease in Iwate Prefecture in 1990. *Annual Report of the Society of Plant Protection of North Japan*. **42**: 8-10.

Srinivas Prasd M, Sheshu Madhav M, Laha GS, Ladhalakhmi D, Krishnaveni D, Mangrauthia SK, Balachandran SM, Sundaram RM, Arunakranthi B, Madhan Mohan K, Ratna K, Madhavi V Kumar and Viraktamath BC. 2011. Technical Bulletin No. 57. Directorate of Rice Research (ICAR), Rajendranagar, Hyderabad-500030, A.P, India, pp. 1-50.

Srivastava D, Shamim MD, Kumar D, Pandey P, Khan NA and Singh SN. 2014. Morphological and molecular characterization of *Pyricularia oryzae* causing blast disease in rice (*Oryza sativa*) from North India. *International Journal of Scientific and Research Publications.* **4** (7): 2250-3153.

Stahl W. 1955. Report of the Plant Diseases Conference, Hawkesbury Agricultural College, NSW. pp. 296-308.

Sun GC, Sun SY and Shen ZT. 1989. Conditions for sporulation of rice blast fungus. *International Rice Research Newsletter*. **14**: 12 – 13.

Sundravadana S, Kuttalam S, Alice D andSamiyappan R. 2008.Fungicidal activity of Azoxystrobin against *Pyricularia grisea* (Cooke) Sacc. and its controlling activity against rice blast.*Archives of Phytopathology and Plant Protection*. **41**(8):608- 615.

Suprapta DN and Khalimi K.2012. Pengembangan agen hayati untuk mengendalikan penyakit blas, memacu pertumbuhan dan meningkatkan hasil tanaman padi. Laporan Penelitian Riset Invensi Udayana. Universitas Udayana, Denpasar. pp. 42.

Supriya Devi and Sharma GD. 2010. Blast Disease of Rice Caused by *Magnaporthe grisea*, Assam University. Biological and Environmental Sciences. *Journal of Science and Technology*. **6**(1):144-154.

Swamy HN, Syed S and Kumar MD.2009. Evaluation of new fungicides against rice blast in cauvery delta. *Karnataka Journal of Agricultural Sciences*. **22**(2): 450-451.

Sy AA, Albertini L and Hamant C. 1977.Effect of the pH on mycelial growth, conidia formation and conidial germination of *Pyricularia oryzae. Bulletin de Toulouse Histoire naturella de Toulouse.* **113**: 200 – 211.

Teng PS. 1994. The epidemiological basis for blast management. *In: Zeigler R.S and Leong, S.A. (eds.) - Rice Blast Disease. CAB International, Wallingford, UK.*

Thomas KM and Krishnaswamy CS. 1948. The control of chief disease of rice as a means of increasing production. *Madras Agriculture Journal*. **34**:1-8

Tirmali AM and Patil BK. 2000. Evaluation of new fungicidal formulations for the control of blast disease of rice. *J. Maharashtra Agricultural Universities.* **25**: 122-221.

Tirmali AM, Latake SB and Bendra NJ. 2001. Evaluation of new fungicides for control of blast disease of rice. *J. Maharashtra Agricultural Universities.* **26**: 197-198.

Tochinai Y and Shimamura M. 1932. Studies on the physiological specialization in *Pyricularia oryzae* Br. et. Cav. *Annals of the Phytopathoglocial Society of Japan*. **26** : 60

Trimali AM, Gupta, Ruchi, Patni CS and Mishra KK. 2004. Integrated management of leaf blast of rice. Souvenir and abstact. Nat. Sym. On crop surveillance. Disease forecasting and management. *Indian Phytopathological Society*.**19**(21): 69.

Tripathi SK, Patel RP, and Tiwari KL. 1997. Influence of weather factors on rice blast (*Pyricularia oryzae*) and its chemical control. *Anaerobic digestion plants science*. **10** (2): 89- 93.

Tripathi SK. 2000. Evaluation of fungicide against leaf blast disease of rice. *Oryza*. **37**(2): 75-76.

Tripathi SK and Jain AK. 2005. Evaluation of bio-pesticides and fungicides for leaf blast and seed discolouration of rice. *Plant Protection Bulletin Faridabad*. **57**(1/2): 20-22.

Tsai WH, Yang YZ, Leo SC and Chien CC. 1981.Low volume chemical control of rice blast in China. *Journal of Agricultural Research*. **30**:187-191

Tuite J. 1969. *Plant Pathological Methods, Fungi and Bacteria.* Burges Publishing Company, USA. 239.

Twumasi JK. 1996. Survey on rice diseases in Southern Ghana. *Proceedings of the Annual WARDA IPM Task Force Meeting.*March 20-22, 1996. Bouaké, Côte d.Ivoire.

Twumasi JK. 1998. *Country Report from Ghana in Workshop on Rice Crop Protection in Africa* .December 8-10, 1998, NRI, Chatham Maritime, Kent, UK.

Twumasi JK and Adu-Tutu KO. 1995.Weeds and fungal diseases of rice in selected inland valleys in Ghana. *Proceedings of the Annual WARDA IPM Task Force Meeting,* March 15-17, 1995. Monrovia, Liberia

Varier M, Maiti D and Shukla VD. 1993. Efficacy of combination of fungicide formulations on management of rice-blast (*Pyricularia oryzae*) in rainfed upland. *Indian Journal of Agricultural Sciences* .**63**:386-389.

Varma CKY and Santhakumari P. 2012. Management of rice blast through new fungicidal formulations. *Indian Phytopath*. **65**(1): 87-88.

Veeraraghavan J and Padmanabhan SY. 1965. Conidiation of *Pyricularia oryzae* in different solid media. *Current Science*. **47**:441-445.

Veeraraju V and Prasad NN. 1972, Effect on nitrogen fertilization on inhibitor level and blast disease resistance in rice. *Annamalai University Agricultural Research*. **4** (5): 82-87

Vijaya M. 2002. Field evaluation of fungicides against blast disease of rice. *Indian Journal of Plant Protection*. **30**(2): 205-206.

Vijaya M. 2003. Influence of weather parameters in relation to rice blast. *JNKVV Research Journal*. **37**(1): 107-108.

Von Braun, J. 2007a. "The World Food Situation: New Driving Forces and Required Actions." Food Policy Report. Washington, DC: IFPRI. HTTP://WWW.IFPRI.ORG/PUBS/FPR/PR18.PDF.

Wakiyama, Y. 2002. Infrared remote sensing for canopy temperature in paddy field and relationship between leaf temperature and leaf colour. *Journal of Agricultural Meteorology*. **58**: 185-194.

Webster RK and Gunnell PS. 1992. Compendium of Rice Diseases. *The American Phytopathological Society*, St. Paul, MN.86.

Xia TQ, Correll JC, Lee FN, Marchetti MA and Rhoads DD. 1993.DNA fingerprinting to examine microgeographic variation in the *M. grisea* (*P. grisea)* population in two rice field in Arkansans. *Phytopathology*. **83**: 1029 – 1035.

Yaduraju NT and Rao AN. 2013. Implications of weeds and weed management on food security and safety in the Asia-Pacific region. *Proc. of 24th Asian-Pacific Weed Science Society Conference*.22-25 October, 2013, Bandung, Indonesia.13-30.

Yaegashi, H and Nishihara, N. 1976.Production of the perfect stage in *Pyricularia* from cereals and grasses.*Annals of the Phytopathological Society of Japan.* **42**: 511-515.

Yaegashi H and Udagawa S. 1978.The taxonomical identity of the perfect stage of *Pyricularia grisea* and its allies. *Canadian Journal of Botany*. **56**: 180-183.

Yamini Varma CK and Santhakumari P. 2012. Management of rice blast through new fungicidal formulations. *Indian Phytopath*. **65** (1): 87-88.

Yang H, Yang XH, Lu CM and Wang YY. 2011. The correlation analysis between blast resistance and genetic diversity of 39 Yunnan glutinous rice varieties. *Journal of Yunnan Agricultural University.* **26**(1): 1-5.

You MP, Lanoiselet V, Wang CP, Shivas RG, Li YP and Barbetti MJ. 2012. First report of rice blast (*Magnaporthe oryzae*) on rice (*Oryza sativa*) in Western Australia. *Plant Disease*. **96**(8): 1228.

Zeigler RS, Leong SA and Teng P. 1994. Rice blast disease: International Rice Research Institute, Manila, Philippines.

Zhu YY. Fang H, Wang YY, Fan JX, Yang SS, Mew TW and Mundt CC. 2005. Panicle blast and canopy moisture in rice cultivar mixtures. *Phytopathol.* **95**: 433-438.