SCREENING OF RICE VARIETIES AGAINST *PYRICULARIA ORYZAE* AND EFFECT OF DIFFERENT FUNGICIDES ON RICE BLAST DISEASE

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SEPTEMBER 2019

SCREENING OF RICE VARIETIES AGAINST *PYRICULARIA ORYZAE* AND EFFECT OF DIFFERENT FUNGICIDES ON RICE BLAST DISEASE

A Thesis presented by

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to

The Postgraduate Committee of the Yezin Agricultural University as a requirement for the degree of Master of Agricultural Science in the Subject of Plant Pathology

Yezin Agricultural University

SEPTEMBER 2019

The thesis attached here to, entitled "**SCREENING OF RICE VARIETIES AGAINST *PYRICULARIA ORYZAE* AND EFFECT OF DIFFERENT FUNGICIDES ON RICE BLAST DISEASE**" was prepared under the direction of the supervisor of the candidate supervisory committee and has been approved by all members of that committee as a requirement for the degree of **Master of Agricultural Science (Plant Pathology)**.

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This thesis represents the original work of the author, except where otherwise stated. It has not been submitted previously for a degree at this or any other University.

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DEDICATED TO MY BELOVED PARENTS,
U NYUNT HLAING AND DAW KYIN SHWE

ACKNOWLEDGEMENTS

I wish to express my sincere and deep gratitude to Dr. Nang Hseng Hom, Rector, Yezin Agricultural University (YAU), for providing an opportunity as an on-deputation candidate. I would like to express my sincere appreciation to Dr. Soe Soe Thein, Pro-rector (Academic) and Dr. Kyaw Kyaw Win, Pro-rector (Administration), YAU for their administrative support and valuable suggestions to this study.

I wish to express my deep sincere gratitude to Dr. Tin Aye Aye Naing, Professor and Head, Department of Plant Pathology, YAU for inspiration guide at every step, valuable comments, and encouragement which support me not only physical but also mental strength to complete my research study and critical reading of this thesis**.**

I would like to express my special gratitude to external examiner, Dr. Ei Ei Khaing, Staff Officer, Postharvest Research Institute, Department of Agriculture, (DOA) for her valuable suggestions and criticism to improve my thesis.

It gives me a great pleasure to express my whole hearted thanks and sincere gratitude to my supervisor, Dr. Seint San Aye, Associate Professor, Department of Plant Pathology, YAU for her keen interest, inspiring suggestions, constant supervision through my research study and comments on this thesis.

I wish to express my deep and sincere gratitude to the supervisory committee member, Dr. Htay Htay Oo, Associate Professor, Department of Agronomy, YAU for her valuable comments and suggestions during preparation and submission of the thesis.

I owe thanks from the bottom of my heart to the supervisory committee member, U Nay Lin Oo, Lecturer, Department of Plant Pathology, YAU for his productive discussion, and kind cooperation throughout my study and writing my thesis.

I would like to extend my special thanks to Daw Myint Myint San (Retd. Head of Plant Pathology Section), and Daw Si Si Myint, Head of Plant Pathology Section, Department of Agricultural Research (DAR) for their moral support and encouragement to complete this study.

I appreciate to all teachers at Department of Plant Pathology, YAU for their suggestions and encouragement throughout my study. I wish express my special

thanks to technical staff member of Department of Plant Pathology, YAU for their help in my work.

I would like to express my sincere thanks to my friends and postgraduate students at Department of Plant Pathology who kindly help in my lab works and course study.

I greatly acknowledge to Mitsubishi Corporation and YAU-JICATCP for giving financial support. My special thanks are extending to the Department of Plant Breeding, Physiology and Ecology, Yezin Agricultural University for providing local rice varieties and YAU rice lines.

Words are not enough to express my heartfelt gratitude to my beloved parents U Nyunt Hlaing and Daw Kyin Shwe and my sisters and my best friend for their never ending love, encouragement, moral and financial support to complete this study.

ABSTRACT

The experiments were conducted at the Department of Plant Pathology, Yezin Agricultural University (YAU) from December 2017 to March 2019. The objectives are to find out resistant rice varieties against rice blast disease caused by *Pyricularia oryzae* and to evaluate the effectiveness of different fungicides to control rice blast disease. A total of seven isolates of *P. oryzae* were collected from Nay Pyi Taw Union Territory and Ayeyarwaddy Region, and studied for pathogenicity. The isolate, *Po1*, from Nay Pyi Taw Union Territory was more virulent than all other isolates. In the evaluation of resistance of 33 local rice varieties and 32 YAU rice lines to rice blast disease with Randomized Complete Block design consisting 3 replications, 4 varieties were found to be moderately susceptible and 27 varieties susceptible. The rest two varieties, Shwe Thwe Yin and Naung Ta Moe Se, were observed to be highly susceptible. Of 32 tested YAU rice lines, 13 were found as moderately susceptible, and 18 as susceptible ones. One line, YAU-1211-22-2-1 was highly susceptible. Among tested varieties and lines, 5 varieties namely Manaw Thu Kha, Paw San Hmwe, Ayar Padae Thar, Bu Toyl and Lone Phyu, and 16 lines were found to have quantitative resistance to rice blast disease. In the evaluation of eight fungicides on the mycelial growth of *P. oryzae* under Completely Randomized design with 4 replications, the IC_{50} values of Isoprothiolane, Thiophanate methyl, Carbendazim, Mancozeb, Kasugamycin and Tricyclazole were 2.03 ppm, 3.47 ppm, 3.56 ppm, 105.76 ppm, 125.49 ppm and 126.91 ppm, respectively, and they were not significantly different with each other but were significantly lower compared to Dicarboximide (557.95 ppm) and Copper oxychloride (3384.60 ppm). In the evaluation of effectiveness of eight fungicides at minimum inhibition concentrations under Randomized Complete Block design with 4 replications *in vivo*, all fungicides tested were not different on the percent disease index of rice blast disease. Tricyclazole gave minimum AUDPC value and was significantly lower compared to other fungicides tested. Tricyclazole, Mancozeb, Kasugamycin, Dicarboximide and Carbendazim treated plants were significantly lower infection rate compared to the rest fungicides treated plants. Reduction percent in plant height of Tricyclazole treated plants was 27.7 % and was significantly lesser than plants treated with the rest fungicides except plants treated with Mancozeb and Carbendazim. This study showed the potential contribution of Tricyclazole fungicide for reducing rice blast disease.

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CHAPTER I
INTRODUCTION

Rice (*Oryza sativa* L.) belonging to the family Gramineae is the staple food crop for more than 50 % of the world's population (Gowda, Venu, Roopalakshmi, Sreerekha & Kulkarni, 2003). To keep up food demand from population growth, rice supply must be doubled by 2050 (Food and Agricultural Organization [FAO], 2009). In Myanmar, agriculture is the backbone of economy and rice is a major calorie intake among cereal crops. In 2018, total sown area of rice was 7.26 million hectares and the Union average yield was 3.92 metric tons per hectare. The Union target yield of rice is 5 tons ha^{-1} (Ministry of Agriculture, Livestock and Irrigation [MOALI], 2018). There is a yield gap between Union average yield and Union target yield. To decrease yield gap has become vital to match the rising caloric demand of the population growth for this staple food crop. To reach Union target yield, there are many constraints such as biotic and abiotic stresses.

Among biotic stresses, rice blast disease is a major biotic stress affecting rice yield and is being intensively studied due to the economically important threat to world rice production (Ou, 1985). Rice blast disease is caused by the fungus *Pyricularia oryzae* [Telemorph: *Magnaporthe oryzae* (Hebert) Barr.,] (Rossman, Howard & Valent, 1990). It can cause yield loss up to 70-80 % at high mean temperature, above 85-89 % relative humidity, presence of dew, drought condition and high nitrogen fertilizer application (Piotti et al., 2005). Blast disease can be detected in irrigated lowland rice, rain-fed upland rice, or deep-water rice fields (Rao, 1992). Rice blast disease threats all growth stages of rice and the pathogen infects all aerial parts of the plant and causes lesions on them, including the leaf blade, leaf sheath, ligule, collar region, stem, panicle (rachilla and neck) and grain (hull), and restricts yield potential under environments favoring the disease (Shindo, 1980).

Cultural practices such as planting resistant cultivars, applying fungicides, adjusting planting times, fertilizers and irrigations are the most usual approaches in managing rice blast disease. To manage the disease, the cultivation of resistant or tolerant varieties is the most economically and environmentally friendly way (Ghazanfar, Habib & Sahi, 2009). To detect the resistant levels of rice varieties, efficient artificial inoculation methods are important. Moreover, success of the

screening is influenced by inoculum quality and quantity, inoculation technique, and pre and post inoculation environmental conditions (Twizeyimana et al., 2007).

Breeding for disease resistant varieties has been operated for managing rice diseases and is one of the most economically important methods which contributed hugely to in rising world's rice productivity (Mew, 1991). Different breeding strategies are being approached to increase the durability of resistance in different rice-growing countries. At present, around 100 rice blast resistance genes have been known. Out of them, 45 % are from japonica cultivars, 51 % from Indica cultivars and the rest 4 % from wild species of rice (Sharma et al., 2012). Although many resistant varieties to *P. oryzae* have been developed, the resistance is not long lasting, because the pathogen adaptability in the fields makes single resistance gene break down after three to five years of the cultivar release (Lang, Luy, Ha & Buu, 2009).

Traditional rice varieties are valuable genetic sources in finding out the resistance to pests, diseases and abiotic stresses. Therefore, the traditional rice varieties resistant to a particular disease are being bred with modern varieties (Sanni et al., 2008). The analysis of resistance using agriculturally important local cultivars other than using the international differentials gives great value for the utilization of genes that exhibit a wide range of resistance (horizontal resistance) to a determined population (Bonman, Khush & Nelson, 1992). On the other hand, hybrid rice is extremely susceptible to rice blast disease because the reason is due to limitation of genetic diversity of its parents. The susceptibility of hybrid rice to rice blast causes huge yield damage (Xing et al. 2019). Hybrid rice is being bred with the blast resistant modern varieties for integration of sources of resistance genes into hybrid rice. YAU rice lines are achieved through the process of selection after crossing hybrid rice and modern resistant cultivars. Using resistant varieties produces effective and environmentally safe alternatives against chemical control in disease management.

Among several methods for the control of the disease, Prabhu and Fillippi (2006) as cited in Soares, Raphael, Bortolotto, Nora & Gruhn, (2014) stated that the use of fungicides is essential when the resistance of the cultivar is ineffective, and due to its short durability of vertical resistance and due to the gradual increase of cultivar susceptibility with partial resistance, both in highland rice and irrigated rice. Many fungicides are being used in different rice growing countries against blast disease,

including systemic fungicides such as benomyl, fthalide, edifenphos, iprobenfos, tricyclazole, isoprothiolane, probenazole, pyroquilon, felimzone (meferimzone), diclocymet, carpropamid, fenoxanil and metominostrobin, carbendazim, thiophanate methyl, non-systemic fungicides such as mancozeb, copper oxychloride, captan and antibiotics such as blasticidin and kasugamycin. The fungicides have efficiency to control leaf blast up to a range of 40 to 84 % (Swamy, Syed & Kumar, 2009).

The cultivation of resistant varieties and the use of fungicides have been recommended by some researchers for rice blast management (Faivre et al., 2011; Fang, Yan, Wang, Zhang & Ma, 2009; Kunova, Pizzatti, Bonaldi & Cortesi, 2014). Therefore, finding out the resistance of rice varieties to diseases remain as an important research work. At present, resistant varieties and chemical control are widely practiced by farmers. However chemical control is widely practiced, it is environmentally hazardous at high application rates. Sustainable management system of rice blast disease can be practiced through a combination of resistant cultivars and the use of proper dosage of fungicides. Therefore, the present study was carried out with the following objectives:

1. to find out resistant rice varieties against rice blast disease caused by *Pyricularia oryzae* and
2. to evaluate the effectiveness of different fungicides to control rice blast disease

CHAPTER II
LITERATURE REVIEW

2.1 Rice Blast

2.1.1 Occurrence and distribution

Rice blast disease has been recognized in 85 rice-growing countries (Wang, Bianco & Jia, 2014). Blast is considered the most destructive rice disease as the environmental conditions favored for disease occurrence and distribution. Rice blast disease has been reported in all the rice growing countries of the world, firstly in China from 1637, Japan from 1704, America from 1876, India from 1913, and Australia from 2011 (Shafaullah, Khan & Mahmood, 2011). It was found as destructive disease in West Africa, Iran (Mousanejad, Alizadeh & Safaie, 2010), Malaysia (Rahim, 2010) and Savanas of South America (Bonman, Bandong, Lee & Valent, 1986). Rice blast was reported for the first time in Africa in 1930 (Feakin, 1974). Climatic changes accompanying with the global warming could prompt its spread in to other parts around the world (Kohli, Mehta, Guzman, DeViedma & Cubilla, 2011). Rice varieties resistant to blast frequently lose their resistance within a few years because of shifts in strains of the fungal population (Huang, 2011).

In Myanmar, the occurrence of rice blast disease in Ayeyarwady (Central Agricultural Research Institute [CARI], 2000), leaf blast epidemic and neck blast around Yezin area on the variety IR50 in 2002-2003 cold and dry seasons (Naing, 2004), the occurrence of blast disease in 2013 early summer and 2014 rainy season in Nay Pyi Taw Union Territory (Aye et al., 2015), the leaf blast and neck blast occurrence in Aungban, Pindaya, Taunggyi, Kyaukme, Yezin, Lapputa and Bago during 2015 to 2018 rice growing season (Khaing, Win., Win. & Naing, 2018) and leaf blast disease incidence in Aungban research farm every year (Department of Agricultural Research [DAR], 2018) has been reported.

2.1.2 Economic importance

The disease is caused by a filamentous fungus *Pyricularia oryzae* and is reported from more than 85 countries of the world (Gilbert, Soanes & Talbot, 2004; Scardaci et al., 1997). Several rice blast epidemics have occurred in different parts of the world, resulting in 50 to 90 % of the grain yield losses (Agrios, 2005). In farmers' fields, neck blast is considered more destructive than leaf blast, because it is more

closely tied to yield losses (Zhu et al., 2005). Heavy yield losses caused by rice blast disease have been reported in many rice growing countries. Panicle infection causes complete yield loss (Ou, 1985). In the first recorded outbreak of blast in India in 1918, the loss in rice production was estimated at 69 %. Blast epidemics in Malaysia and the Philippines have caused yield reductions of 50-70 % (Supaad, 1980). In India, 75 % loss of grain occurred in 1950 in susceptible cultivars (Padmanabhan, 1965) while in the Philippines several thousand hectares suffered causing more than 50 % yield loss (Ou, 1985) and 40 % grain loss in Nigeria (Awodera & Esuruoso, 1975).

Yield reduction by neck blast infection is twice as severe as the leaf blast (Hwang, Koh & Chung, 1987). Under favourable environmental conditions, particularly temperature and humidity, rice blast can even cause total crop loss (Okeke, Murandi & Benoid-Guyod, 1992). In Nepal, the disease causes the 10-20 % yield reduction in susceptible varieties, but in severe case it goes up to 80 % yield reduction (Manandhar, Shrestha & Amatya, 1992). In rice-growing areas, a blast outbreak could cause the loss of about 35–50 % of rice yield, and in a serious outbreak of the disease, up to 100 % of yield could be lost (Warda, 1999).

Blast disease is the most devastating fungal disease of rice encountered by farmers in Nigeria. In rice-growing areas, a blast outbreak could cause the loss of about 35–50 % of rice yield, and in a serious outbreak of the disease, up to 100 % of yield could be lost (Warda, 1999). Rice blast is the most harmful fungal disease causing rice yield losses up to 70 to 80 % (Miah et al., 2013; Nasruddin & Amin, 2013).

2.1.3 Symptoms

Blast symptoms develop on all the aerial organs of the rice plant, mainly on the coleoptiles, leaf sheaths and leaf blades, neck of panicles, stem nodes and spikelet. Oval or diamond-shaped spots (5-15 mm long and 3-5 mm wide) with dark borders occur on the leaves. Often, the spots have yellow haloes. Spots develop quickly under moist conditions and produce large numbers of spores on both sides of the leaves. As they age, the spots become longer, the centers turn whitish grey and the borders become wider and red-brown. The spots join together and the leaves die. The foliar lesions reduce the leaf area available for photosynthesis and, when they are severe and occur in the early development stages, they are likely to destroy the whole tiller. Severely infected fields have a scorched appearance (Jackson, 2015).

Spores from the leaves infect the leaf sheath, stem and panicle and cause rots. There are several different types of rot: (i) collar rot appears at the junction of the leaf base and leaf sheath; this can kill the leaf; (ii) neck rot (also called "rotten neck") appears on the stem below the panicles (the flower heads) and can destroy the stem or result in pale-coloured grains that are partly filled, known as "whiteheads"; (iii) panicle rot occurs on the branches of the panicle so that it appears brown or black; (iv) node rot (slightly swollen parts of the stem where the leaves and tillers develop) occurs on the stem below the panicles, the rots become black-brown and dry and, if the stem breaks, the plant dies. Neck blast and node blast are characterized by a brown rot that disorganizes the tissues and prevents the migration of the nutrients that should ensure grain filling (Jackson, 2015).

2.1.4 Taxonomy and nomenclature

Teleomorph: Magnaporthe oryzae (Hebert) Barr
Kingdom : Fungi
Division : Ascomycota
Subdivision : Pezizomycotina
Class : Sordariomycetes
Subclass : Sordariomycetidae
Order : Magnaporthales,
Family : Magnaporthaceae
Genus : *Magnaporthe*
Species : *oryzae*
Anamorph : *Pyricularia oryzae* (Couch & Kohn, 2002)

The blast causal agent is an ascomycete fungus described on many graminaceous species and in its asexual form called *Pyricularia grisea* (Cke) Sacc, whose perfect stage was known as *Magnaporthe grisea* (Hebert) Barr. However, Couch and Kohn (2002) distinguished through multilocus gene genealogy and host preference - two clades within *M. grisea*. One, associated with the grass genus *Digitaria*, is named *M. grisea*, while the other, associated with *Oryza sativa* and other cultivated grasses, was described as a new species, *M. oryzae*. Thus, the correct name of the blast pathogen is currently *Magnaporthe oryzae* B.Couch [anamorph: *Pyricularia oryzae* Cavara] (Couch & Kohn, 2002). The asexual stage is the most common form of the fungus. It has been classified based on the anamorphic stage, *Pyricularia oryzae* (Deuteromycota: Hyphomycetes: Moniliales: Dematiaceae).

2.1.5 Infection process and disease cycle

A spore (conidium) landing on the rice organ surface initiates the infection. Conidium is attached to the host plant until it can germinate (Koga & Nakayachi, 2004). Thereafter, the conidium of *M. oryzae* develops germ tubes and appressorium. The conidium attachment and germination, and differentiation of the appressorium belong to the passive stage of the host-pathogen relationship since they occur for both compatible and incompatible host-pathogen combinations (Arase, Miyahara, Honda & Nozu, 1994).

In compatible interactions, the appressorium differentiates a peak, which penetrates the epidermal cells, allowing the pathogen to colonize the host tissues (Howard & Valent, 1996). At this stage, the active interaction between blast fungus and rice begins. Host plant resistance manifests itself either by preventing the subsequent hyphal growth inside the host cell through hypersensitive reaction, or by reducing the damaged cell and therefore the size of the lesions and their sporulating abilities, slowing epidemic development and finally leading to a partial resistance of rice to the blast fungus.

The disease is particularly serious in areas of frequent and prolonged showers and temperatures in the range of 24-28 °C. This is because the leaves need to be wet for 6-8 hours for spore germination. High humidity, close to 100 %, is needed for infection and spore formation. In upland areas, conditions are favourable to the disease because differences between day and night temperatures cause dew to form on the leaves and the overall temperatures are cooler. By contrast, in lowland tropical areas, leaf infection is less, but blast is still serious in seedling nurseries and on panicles (Jackson, 2015).

Spread occurs in irrigation water. Spores are spread short and long distances on air currents and wind. Survival between crops is in straw and stubble, in or on seed, volunteer rice plants, and alternative hosts, mostly grass species (Jackson, 2015).

2.1.6 Disease development

Several environmental factors can influence the infection rate and spread of the disease, including temperature, nitrogen levels, intermittent rain showers or drizzle airflow, high relative humidity and drought conditions. Blast susceptibility is inversely related to soil moisture. Plants grown under upland conditions are more

susceptible, while plants grown under lowland condition are more resistant. The pathogen requires free moisture for spore penetration. High relative humidity (90-92 %) is also reported to be essential for infection. Severe blast epidemics are usually associated with moist weather. Low solar radiation and cloudy skies are also good deeds to blast (Miah et al., 2017). Using 13-year data, Padmanabhan (1963) concluded that whenever the minimum temperature of 24 °C or below was associated with relative humidity of 90 % or above, the conditions were favourable to blast infection.

2.1.6.1 Source of inoculum

Infested seeds are a source of primary inoculum. Dead infested grains could serve as primary inoculum when placed on the field during seedling development (Long, Correll, Lee & TeBeest, 2001). Seed contamination and panicle symptoms are interrelated using naturally infested seeds as primary inoculum in field conditions (Manandhar, Jorgensen, Mathur & Petersen, 1998). They observed that sporulation of *M. oryzae* on infested seeds was favourably found at the embryonic end of germinating seeds. A seed lot with 21 % contamination led to <4 % seedlings with blast lesions. Tests employing different ways of covering seeds with soil and underwater seeding (no covering) pointed out that complete covering or seedings underwater induce a lower infection frequency (Manandhar et al, 1998). Guerber and Tebeest (2006) conveyed similar experiments in the USA, but no disease was observed when infested seeds were germinated under water. When infested seeds were sown in the field, the fungus was recovered from different seedling parts, including roots. These results clearly indicated that the fungus can survive on the grains used for seeding and could serve as primary inoculum (Miah et al, 2017).

2.1.6.2 Climatic conditions

Most severe blast disease occurs when more than a few days of continuous rains and average temperatures between 18-25 °C during the flowering stage of the crop followed by sunny, hot and humid days (Kohli, Mehta, Guzman, Deviedma & Cubilla, 2011). Under controlled growth chamber conditions, the highest blast intensity was observed at 30 °C which increased with a longer wet period, and low at 25 °C with a wet period of less than 10 hr (Cardoso, Reis & Moreira, 2008). However, at 25 °C and 40 hr of wetting, blast intensity exceeded to 85 % (Miah et al, 2017).

2.1.7 Management strategies

2.1.7.1 Cultural practices

Cultural practies are important control measures but will not provide complete eradication of the disease. Burning of crop residues reduces the over wintering inoculum in the field, but this may not prevent the inoculums coming from other sources (Zeigler, Leong & Teng, 1994). Seeding on very wet soil is recommended as this will reduce the transmission of disease from the seed to the seedling. Flooding is also recommended as a water management strategy to reduce rice blast compare to when under water stress (Manandhar et al., 1998). Rice is more susceptible to drought than other cereals due to its inability to regulate its transpirational water loss, a weakness that may accelerate rice blast attack (Kato et al., 2004).

The availability of water also affects the susceptibility of the host plant to *M. oryzae*. Rice grown under upland conditions is more susceptible than rice grown in flooded soil. Under upland conditions, susceptibility is increased further with increasing drought stress. Hence flooding the field in upland rice can decrease the severity of blast disease (Bonman, 1992).

Planting time has a marked effect on the development of blast within a rice crop. Early planting is recommended to control rice blast. In tropical upland rice, crops are sown early during the rainy season generally have a higher probability of escaping blast infection than late-sown crops. Early planting date can help susceptible cultivars escape from a severe infection of leaf blast but can be infected by the head blast at the onset of panicles. But, if susceptible cultivars are planted later in the season, the plants can be severely infected by both leaf and head blight. When epidemic starts early, in late sown plantings, plant growth and development are severely affected, leading to the death of many plants (Filippi & Prabu, 1997).

2.1.7.2 Nutrition management

The understanding of effects of nutrition management on interactions between rice and diseases is a base to inspire high-yield production system. Nutrition management is one of the most significant practices for a high production system that affect the response of rice to diseases, as well as the developmental pattern of the disease populations due to the change of environments. The ability of a crop plant to resist diseases is tied to optimal physical, chemical and mainly biological properties

of soils. Soils with high organic matter and high biological activity generally exhibit good soil fertility that prevents infection (Luong et al., 2003).

Nitrogen is essential for plant growth and development and is usually a limiting factor for high productivity. Long, Lee and TeBeest (2000) found an increase in blast lesion when the level of nitrogen was applied above the recommended rate. On the contrary, Snoeijers, Perez-Garcia, Joosten and DeWit (2000) observed that low nitrogen also led to disease increase resulting from weak plants that lacked sufficient defences against disease. Rice disease resistance is habitually affected through high nitrogen supply (Ballini, Nguyen & Morel, 2013).

Silicon (Si) is known as a "beneficial element" for plants. However, it is not an essential nutrient. The direct and indirect benefits of the element for crops, the especially grass is related to resistance to diseases, pests, and drought. Low Si uptake increase the susceptibility of rice to blast, and grain discoloration (Massey & Hartley, 2006). Similarly, Prabhu, Filho, Filippi, Datnoff and Snyder (2001) found that rice cultivar that accumulated more silicon on the shoots showed fewer incidences of rice blast.

Soil characteristics (alkaline pH, high concentration of salt, organic carbon, nitrogen and low concentration of potassium and phosphorus) also lead to rice blast disease (Maheshwari & Sharma, 2013).

2.1.7.3 Chemical control

Fungicides are chemical agents that inhibit or eliminate the growth of fungi or fungal spores. The chemical, physical and biological characteristics of a fungicide determine its suitability to control a determined disease. Fungicidal control is the most efficient control measure for diseases of some crops caused by fungi (Reis & Carmona, 2013).

Benomyl, Carbendazim, edifenphos and 0.25 % Mancozeb were effective against the blast disease (Venkata & Muralidharan, 1983). Application methods of Carbendazim such as mud balls, soil drench and foliar spray at the rate of 0.5 kg a.i ha^{-1} showed effective control of the disease (Tewari & Rao, 1983). Systemic fungicides are extensively applied at seedling to protect against leaf blast and more than 20 days before heading to protect against panicle blast (Miah et al., 2017). Many researchers around the world have been reported that the amount of fungicide,

composition, application method and timing of fungicide applied depends on the disease forecast or disease severity (Miah et al., 2017).

Fungicides application increases the yield of rice (Prabhu, Filippi & Zimmermann, 2003; Tirmali, Latake & Bendra, 2001). Hai, Kim, Du, Thuy and Thanh (2007) reported that spraying of Tricyclazole can improve 1000 grain weight of rice cultivar. Sachin and Rana (2011) also observed increase in grain yield with the application of Tricyclazole. Ganesh, Gangadhara, Basavaraja and Krishna (2012) used ten fungicides for management of rice blast and found that the percent disease index was significantly less (15.56) in Tricyclazole sprayed plots followed by kitazine (17.63) and ediphenphos (18.03). Ganesh et al. (2012) observed that Tricyclazole, kitazine and ediphenphos were found significantly superior in increasing the grain yield. Tricyclazole exhibited better protective than curative activity and epoxiconazole at 112.5 g a.i ha^{-1} provided over 75 % rice blast control efficacy, which was similar to Tricyclazole with 300 g a.i. ha^{-1} and better than Carbendazim with 562.5 g a.i. ha^{-1} as observed by Chen et al. (2013).

Kapoor and Katoch (2014) observed that seed dressing with Tricyclazole have been found to provide effective protection to seed up to 8 weeks after sowing from fungal pathogen *Magnaporthe oryzae*. The experiment of Magar, Acharya and Pandey (2015) showed that maximum disease control and the highest grain yield were recorded from Tricyclazole 22 % + Hexaconazole 3 % SC thrice at weekly interval starting from the booting stage and hence, recommended this fungicide against rice leaf and neck blast disease to have effective control and higher grain yield under field condition. The findings are in-line with Iqbal et al. (2014); Kumar and Veerabhadraswamy (2014) who reported that Tricyclozole was most effective in reducing the leaf blast severity.

2.1.7.4 Cultivar resistance

Use of resistant varieties would offer a better management compared to other control strategies. However, it may take a long time to develop a variety of the desired type that is resistant to rice blast (Zeigler et al., 1994). Miah et al., (2017) and Hasan et al., (2016) developed blast resistant rice varieties using marker-assisted backcrossing. Inducing the resistance to rice plant is also an eco-friendly strategy for rice blast control.

The host plant resistance is treated as the best tactic to control the rice blast disease. Hence the arrangements of different blast resistance genes which interact with each other to impart resistance, are a combination of best alleles of the targeted genes in a host plant in the rice blast breeding programs (Ramkumar et al., 2010). Zaw, Oo, Tun and Naing (2015) reported that among the tested rice genotypes, 51 genotypes, 9 genotypes, 7 genotypes were resistant, moderately resistant and susceptible to rice blast disease, respectively. Khaing et al. (2018) screened 57 cultivated varieties against rice blast isolates and revealed that among 57 test varieties, 42 varieties showed resistant reaction to tested four isolates. Khaing et al. (2018) evaluated the distribution of blast resistance genes in 57 released varieties by using 13 of allele specific SSR markers and pointed out that two varieties namely Manaw Thu kha and Mote Soe Ma Kyway Kyay line MMK 03-23-3, 12 varieties, five varieties, 13 varieties, six varieties possessed seven blast resistance genes, single blast resistance gene, five resistance genes, four resistance genes, three resistance genes and two resistance genes, respectively. Exploitation of resistance gene resources for rice breeding is one of the most significant strategies to control the disease (Miah et al., 2017).

The existence of a monogenic (or oligogenic) resistance to the blast fungus has been largely confirmed in the couplet *O. sativa-M. grisea* by many studies (Chen et al., 2004; Liu, Lu, Zeng & Wang, 2002; Wang et al. 1994; Sallaud et al., 2003; Zhou, Wang, Xu, Lei & Ling, 2004). Such an oligogenic system is responsible for a qualitative, complete and non-durable resistance. Genetic studies carried out in Japan by Kiyosawa and co-workers led to identification of 13 dominant genes (*Pi-a, Pi-b, Pi-i, Pi-k, Pi-kh, Pi-km, Pi-kp, Pi-t, Pi-ta, Pi-ta2, Pi-z, Pi-zt, Pi-sh*) against Japanese blast isolates (Kiyosawa & Ling, 2001). Many other resistance genes have been described but some of them are the same as earlier ones, others different (Ahn et al., 2000; Chen et al., 2004; Sallaud et al., 2003; Zhou et al., 2004).

When a rice plant lacks vertical resistance against a blast strain, post-infection pathogenic processes proceed with the development of lesions and production of new spores. The importance of the disease at this time depends on the ability of the variety to slow the epidemic either by reducing the size of the lesions or by reducing the production of new spores. This aptitude is performed by what Van der Plank (1963) termed ‘horizontal resistance’.

According to Van der Plank's (1963) terminologies, rice blast belongs to the 'compound interest' disease type, as many cycles of the pathogen occur during the same cycle of rice production. For such disease, control methods that try to reduce the apparent rate of progression of the disease are more efficient than those that seek to reduce or suppress the initial quantity of inoculum/disease. Therefore, horizontal resistance was described as more efficient than the vertical resistance in sustainable control of rice blast. However, pyramiding two or more vertical resistance genes can also lead to a durable resistance (Séréa et al. 2013). Accordingly, a number of reports have been stated for investigating resistant rice varieties against rice blast disease.

CHAPTER III
MATERIALS AND METHODS

3.1 Resistance of Rice Varieties to Rice Blast Disease

The experiment was conducted at the Department of Plant Pathology, Yezin Agricultural University (YAU) from December 2017 to January 2019.

3.1.1 Pathogenicity test

The experiment was conducted at the Department of Plant Pathology, Yezin Agricultural University from December 2017 to June 2018.

3.1.1.1 Collection of rice blast disease specimens

All rice blast diseased specimens were collected from irrigated lowland rice fields of Zeyarthiri Township, Nay Pyi Taw Union Territory and Myaungmya Township, Ayeyarwady Region from December 2017 to March 2018. Rice leaves with typical leaf blast symptoms were collected from Aungzeya village and Thitetkalay village in Zeyarthiri Township, Nay Pyi Taw Union Territory and from Kwellwal village, Kyarphoongon village, Pandotpin village, Ywarsoechaung village and Myaungmya Research Farm in Myaungmya Township, Ayeyarwady Region. The diseased specimens were air dried at room temperature to reduce leaf wetness and were transferred to the sterilized paper bags. The specimens were placed in desiccator containing silica gel for one week to maintain conidiogenesis on the lesion and then kept at -20 ºC for further use.

3.1.1.2 Isolation and preservation of the pathogen

The infected leaves were cut into 1 to 2 cm sections including typical blast lesions. Three to five cut sections were placed on the sterilized glass slide and the glass slide was placed on the moist filter paper in a Petri dish. The Petri dishes containing diseased tissues were incubated at room temperature overnight to induce sporulation. Conidial masses on the lesion were taken using a sterilized disposable-syringe and transferred to five places on the surface of 3 % water agar containing streptomycin (25 mg in 250 ml agar) to get single conidium. Single conidium was checked under microscope (10 ×) and marked. The agar plate was incubated at room temperature overnight. The germinated single conidium was transferred to Potato Dextrose Agar medium (PDA) and incubated at room temperature for 7 to 10 days.

For the preservation, small pieces of mycelia were cut from the edge of the 7 to 10 days old culture and then transferred onto sterilized filter paper laying on PDA media (Xia, Correll, Lee & Rhoads, 1993). When the mycelial growth covered the filter paper, the filter paper was removed and transferred aseptically to the sterilized Petri dishes, and dried in desiccator containing silica gel at room temperature for three weeks. Then, the filter paper discs were cut into small pieces about 3 to 5 mm^2, and placed in sterilized paper bag. Then, the paper bag was sealed and packed with aluminum foil and preserved at -20 ºC.

3.1.1.3 Preparation of test plants

Shwe Thwe Yin rice variety (IR50) supported by the Department of Agricultural Research (DAR) was used as susceptible variety. The seeds were surface-sterilized by soaking in 10 % sodium hypochloride solution for 10 minutes, rinsed three times with sterilized distilled water and soaked in water for 24 hours. And then, the seeds were incubated for next 24 hours and the germinated seeds were sown in plastic trays filled with sterilized field soil. Recommended agronomic practices were done.

3.1.1.4 Inoculum preparation and inoculation

The stock cultures preserved on sterilized filter paper discs were grown on potato dextrose agar media and incubated at room temperature for 10 days. After 10 days, the mycelia on the surface of the media were scrapped with a sterilized tooth brush and the plates were incubated in plastic box containing a thin layer of the distilled water and sealed with plastic film for five days to induce sporulation. The masses of conidia were harvested by adding some sterilized distilled water into the plates and by scrapping with a sterilized fine paint brush. And the conidial suspension was filtered through muslin mesh. The inoculum concentration was adjusted to 1×10^5 conidia ml^{-1} using haemocytometer. Tween 20 (0.02 % Tween 20 in 0.25 % gelatin) were added to the conidial suspension to enhance the adherence of conidia to rice leaves (Jia, Valent, & Lee, 2003).

Before inoculation, the trays containing 21-day-old rice seedlings of Shwe Thwe Yin variety (IR50) were placed in the Polystyrene Foam boxes containing 3 cm depth of soil saturated with water. Rice seedlings were inoculated by spraying 20 ml of conidial suspension per tray with hand sprayers (Khaing et al., 2018). After inoculation, the boxes were covered with black plastic sheets to maintain high relative

humidity for disease infection and development. Three days after inoculation, black plastic sheets were removed for one hour to allow gas exchange and then covered again. At six days after inoculation, black plastic sheets were totally removed. During incubation period, the temperature and relative humidity of crop conopy were recorded with Illuminance UV recorder (TR-74*i*).

3.1.1.5 Disease scoring and data analysis

At 8 days after inoculation, a 0-9 point rating scale was used for scoring disease severity against the pathogen. Test varieties with consistent rating, between 4 and 6 with overall average not higher than 5.5 will be denoted as quantitative resistance (International Rice Research Institute [IRRI], 2013). The disease rating scale is presented in Table 3.1. In pathogenicity test, disease rating scales were recorded from 25 plants for each isolate at 8 days after inoculation. Percent disease index (PDI) was calculated using the formula by Mckinney (as cited in Pal, Mandal & Naik, 2017).

$$\text{PDI}\ (\%) = \frac{\text{Sum of all disease rating}}{\text{Total number of leaves assessed} \times \text{maximum disease grade}} \times 100$$

3.1.2 Resistance of rice varieties to *Pyricularia oryzae*

This experiment was conducted during January, 2019.

3.1.2.1 Preparation of test plants

A total of 33 local rice varieties and 32 YAU rice lines, including Shwe Thwe Yin variety as susceptible check and Manaw Thu Kha variety as resistant check, were used. The varieties were supported by the Department of Plant Breeding, Physiology and Ecology, YAU were used and listed in Table 3.2. After sterilization of seeds, the seeds were spread on a layer of two moist papers. The moist papers were rolled up and placed in the incubator at 30 °C for 2 days. One kilogram of soil in each nursery tray was applied with 2 g of urea (150 kg ha-1) as basal dressing. The germinated seeds of each variety were sown at the rate of one germinated seed per cell in each line of the nursery tray. Each tray consists of 15 lines and 7 cells in one line. One line of each tested variety was one replication. Three replications were maintained for each test variety. Two lines of Shwe Thwe Yin variety as susceptible check and two lines of Manaw Thu Kha variety as resistant check were included in all trays. Watering and fertilization were done as necessary.

Table 3.1 Disease rating scale used for leaf blast (IRRI, 2013)

0-9 Scale	Disease Severity	Host response or Reaction
0	No lesions observed	Highly Resistance
1	Small brown specks of pin-point size or larger brown specks without sporulating center	Resistance
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Moderately Resistance
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves	Moderately Resistance
4	Typical susceptible blast lesions 2-3 mm or longer, infecting less than 4 % of the leaf area	Moderately Susceptible
5	Typical blast lesions infecting 4-10 % of the leaf area	Moderately Susceptible
6	Typical blast lesions infection 11-25 % of the leaf area	Susceptible
7	Typical blast lesions infection 26-50 % of the leaf area	Susceptible
8	Typical blast lesions infection 51-75 % of the leaf area and many leaves are dead	Highly Susceptible
9	More than 75 % leaf area affected	Highly Susceptible

Quantitative resistance = average score ≤ 5.5

Table 3.2 List of local rice varieties and YAU rice lines

No	Local rice varieties	No	YAU rice lines
1	Ant Paw	1	YAU-1215-S-S-S-40-2-1
2	Ayer Padae Thar	2	YAU-1215-73-2-3-1-1-1
3	Boke Thwin Phyu	3	YAU-1215-80-1-2-1-1-1
4	Bu Aung Ban	4	YAU-1201-1-2-1
5	Bu Toyl	5	YAU-1201-202-2-2 Y-7
6	IR-36	6	YAU-1201-90-2-4 (Y-19)
7	Japan Ni	7	YAU-1211-71-1-1 Y-22
8	Kauk Kyee	8	YAU-1214-183-35-1-1-1-1
9	Khao lami L-5	9	YAU-1201-121-3-1
10	Khao Lami L-7	10	YAU-1201-202-1-2 Y-11
11	Khao Lin L-35	11	YAU-1215-S-S-S-77-2-1
12	Khao Pi Paung	12	YAU-1215-S-S-S-55-2-1
13	Khao Pi Paung L-18	13	YAU-1214-183-3-4-1-1-1
14	Khauk Kham Tu	14	YAU-1201-90-2-2 Y-2
15	Khauk Kyi Shan Mo	15	YAU-1215-B-B-B-10-1-1
16	Khauk Mae Pan	16	YAU-1211-20-1-1 (Y50)
17	Khun Na Yar Po	17	YAU-1201-61-3-3
18	Kun Lone L-41	18	YAU-1214-S-S-S-77-1-1
19	Lone Phyu	19	YAU-1214-183-3-3-1-1-1
20	Ma Naw Tun	20	YAU-1211-22-2-1
21	Manaw Thu Kha	21	YAU-1211-223-3-2 (Y-15)
22	Muyinn Sabar	22	YAU-1214-183-3-1-2-1-1
23	Naung Ta Moe Se	23	YAU-1215-S-S-S-41-1-1
24	Nga Sar Kay	24	YAU-1211-118-2-1
25	Paw (1)	25	YAU-1214-183-3-1-1-1-1
26	Paw San Hmwe	26	YAU-1215-B-B-B-139-3-1
27	Sa Bong Thaw	27	YAU-1215-B-B-B-153-3-1
28	Shwe Thwe Yin	28	YAU-1211-223-3-1
29	Shwe War Yin	29	YAU-1215-B-B-B-168-1-1
30	Ta Yoke Hmwe	30	YAU-1201-187-1-2
31	Thu Kha-2	31	YAU-1211-116-3-4-Y-21
32	V15	32	YAU-1214-B-B-B-153-3-1
33	Yoe Wa		

3.1.2.2 Inoculum preparation and inoculation

The most virulent isolate from the pathogenicity test was used in this experiment. Inoculum preparation and inoculation were carried out as same as the procedures described in the section 3.1.1.4. The inoculated seedlings of trays were kept under the controlled environmental conditions. The temperature and relative humidity of crop canopy were recorded.

3.1.2.3 Experimental design, data recording and data analysis

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Disease severity on rice seedlings were examined and rated with a 0-9 point rating scale at 8 days after inoculation (IRRI, 2013). Disease response of rice varieties to rice blast disease was determined on the mean of three replications.

3.2 Effect of Different Fungicides on Rice Blast Disease

This experiment was conducted at the Department of Plant Pathology, Yezin Agricultural University from October 2018 to March 2019. Non systemic fungicides, systemic fungicides and antibiotics recommended for rice blast control were selected to test in this experiment. The descriptions of tested fungicides are presented in Table 3.3.

3.2.1 Evaluation of different fungicides on the growth of *P. oryzae in vitro*

In this experiment, antibiotic Kasugamycin and seven different fungicides *viz.*, Tricylazole, Copper oxychloride, Isoprothiolane, Mancozeb, Carbendazim, Thiophanate methyl and Dicarboximide were evaluated. Five different concentrations were used for all treatments and the efficacy was tested. Test concentrations of the fungicides are described in Table 3.4.

3.2.1.1 Preparation of fungicide amended media and inoculation

The stock culture of the most virulent isolate from pathogenicity test was sub cultured on potato dextrose agar media and incubated for 10 days. Poison food technique was done according to Grover and Moore (1962). The conical flasks containing 100 ml of PDA media were autoclaved and cooled down to 40ºC. The required quantities of each fungicide were mixed with 100 ml of PDA and shaken thoroughly. Then, about 20 ml of poisoned media were poured into 90 mm sterilized Petri dishes and allowed to solidify. Media without fungicide was used as control.

Table 3.3 Description of tested fungicides

No.	Trade name	Common name	Chemical name	Active ingredient	Mode of action
1	KASUMIN 2L	Kasugamycin	Kasugamycin	Kasugamycin 2%	Systemic
2	PYRICIDE 40 SC	Tricyclazole	Triazolobenzothiazole	Tricyclazole 40 % W/W	Systemic
3	BAYIN Copper oxychloride 50 % WP	Copper oxychloride	Copper	Copper oxychloride 50 %W/W	Non Systemic
4	FUGI ONE 40 EC	Isoprothiolane	Diisopropyl 1,3-dithiolan-2-ylidenemalonate	Isoprothiolane 40 % W/W	Systemic
5	DICOZEB 80 WP	Mancozeb	Mancozeb	Mancozeb 80 % W/W	Non Systemic
6	CARBEN 50 SC	Carbendazim	Carbendazim	Carbendazim 50 % W/V	Systemic
7	TOPSIN-M 70 % WP	Thiophanate-methyl	Thiophanate-methyl	Thiophanate-methyl 70 % W/W	Systemic
8	EXTRA-CAP Captan 50 % WP	Dicarboximide	N-(trichloromathylthio) cyclohex-4-ene-1,2-dicarboximide	N-(trichloromathylthio) cyclohex-4-ene-1,2 dicarboximide 50 % W/W	Non Systemic

Table 3.4 Five different concentrations of fungicides used in the *in vitro* study

Fungicides	Concentration (C) ppm				
	C1	**C2**	**C3**	**C4**	**C5**
Kasugamycin 2L	2.40	12.0	60.0	300.0	800
Tricyclazole 40 % SC	1.00	10.0	100.0	500.0	1000
Copper oxychloride 50 % WP	2.00	20.0	200.0	2000.0	20000
Isoprothiolane 40 % EC	1.00	10.0	100.0	1000.0	10000
Mancozeb 80 % WP	1.00	10.0	100.0	1000.0	10000
Carbendazim 50 % SC	0.01	0.1	0.5	1.0	10
Thiophanate methyl 70 % WP	0.10	1.0	5.0	7.5	10
Dicarboximide 50 % WP	1.00	10.0	100.0	1000.0	10000

The actively growing peripheral growth of 10 days old culture of fungus were cut with 5 mm diameter cork-borer and transferred to the center of each prepared Petri dish and then incubated at room temperature for 15 days. Four replications were maintained for each treatment.

3.2.1.2 Experimental design, data collection and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with four replications. The diameters (cm) of the colonies were recorded from reverse side of Petri dish at 15 days after incubation by measuring the radial growth of the fungus in two directions at right angle to each other and average diameter was calculated. Percent inhibition of mycelium growth was computed using the following formula described by Vincent as cited in Kulmitra, Kumar, Thejesha, Ghosh & Sahu (2017).

$$I\,(\%) = \frac{C - T}{C} \times 100$$

(Vincent, 1947)

Where, I = Percent inhibition of mycelium growth

C = Colony diameter in control plate (cm)

T = Colony diameter in fungicide treated plate (cm)

IC_{50} was calculated from the linear regression equation between the decimal logarithms of fungicide concentrations and the mycelium growth inhibition percent transformed into provit values according to Nakpalo et al. (2017). The data were analyzed by using Statistix (version 8) and the means were compared with least significant difference test (LSD) at 5 % level.

3.2.2 Effect of different fungicides on rice blast disease *in vivo*

3.2.2.1 Preparation of test plants

The susceptible rice variety, Shwe Thwe Yin was used in this experiment. The procedure for seed germination was as same as the section 3.1.2.1**.** The germinated seeds were sown by 20 germinated seeds per plastic pot filled with sterilized field soil. Watering and fertilization were done as recommended.

3.2.2.2 Inoculum preparation and inoculation

The most virulent isolate from pathogenicity test was used in this experiment. Inoculum preparation and inoculation were carried out in accordance with the

procedure described in section 3.1.1.4. Uninoculated plants were used as negative control. At 8 days after inoculation, the inoculated plants and uninoculated plants were transplanted individually to the plastic bags (20 cm × 30 cm) containing sterilized soil.

3.2.2.3 Application of tested fungicides

The same fungicides used in the experiment 3.2.1 were tested in this experiment. Minimum inhibition concentrations of eight fungicides were calculated by linear regression equation from the result of the previous study 3.2.1. The concentrations of fungicides are expressed in Table 3.5. At 5 days after transplanting, the first time of fungicide application was done on the inoculated plants as treated plants. The inoculated plants without fungicide application were regarded as positive control. These plants (positive control) and uninoculated plants (negative control) were sprayed with water. Second time of fungicide application was followed at 14 days after the first fungicide application.

3.2.2.4 Experimental design, data collection and statistical analysis

The experiment was laid out under Randomized Complete Block Design (RCBD) with four replications. Three plants were prepared for each replication.

Disease severity was recorded according to the scale of IRRI (2013). Data collections were done for five times at 7 days interval. First time of data collection was done just before the first fungicide application. And the other four times of data collection were done at 7 days interval after first time of fungicide application.

Percent disease index (PDI), Area Under Disease Progress Curve (AUDPC), and the infection rate (r) were calculated according to the following equations.

$$\text{PDI}\,(\%) = \frac{\text{Sum of all disease rating}}{\text{Total number of leaves assessed} \times \text{maximum disease grade}} \times 100$$

(Mckinney, 1923 as cited in Pal, Mandal & Naik, 2017)

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

Where, X_i = disease severity at the i^{th} observation,

t_i = the time in days at the i^{th} observation, n = the total number of observation

(Shaner and Finney, 1977 as cited in Devil & Chhetry, 2014)

$$\text{Infection rate } (r) = \frac{\log (x_2 - x_1)}{t_1 - t_2} \times 2.303$$

Where, $x_1$and x_2 = percent disease index at time t_1 and t_2

infection rate = r per unit per day (Vanderplank, 1963 as cited in Pandey, 2017)

Reduction percent in plant height was calculated based on the differences in the plant height of uninoculated control plants to the fungicide treated plants. The data were analyzed by using Statistix (version 8) and means were compared by Least Significant Difference test at 5 % level.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Resistance of Rice Varieties to Rice Blast Disease

4.1.1 Pathogenicity test

Seven isolates were recorded from diseased specimens of blast infected rice fields. Two isolates namely *Po1*, *Po2* were isolated from Aungzeya village and Thitetkalay village in Zeyarthiri Township, Nay Pyi Taw Union Territory. Five isolates namely *Po3*, *Po4*, *Po5*, *Po6* and *Po7* were isolated from Myaungmya Research Farm, Kwellawl village, Kyarphoongon village, Pandotpin village and Ywarsoechaung village, respectively, in Myaungmya Township, Ayeyarwaddy Region. The list of seven isolates is shown in Table 4.1 and their colonies are shown in Plate 4.1.

The percent disease indexes of seven isolates are shown in Table 4.2. The range of percent disease indexes was from 64.8 % to 94.6 %. All collected isolates were found to be virulent isolates but their virulence was different. Among seven isolates, the minimum percent disease index was found in *Po3* inoculated leaf and the maximum percent disease index was resulted from *Po1* isolate inoculated leaf. Therefore, *Po1* isolate was found to be the most virulent one and used in the next experiments. Virulence of seven isolates on Shwe Thwe Yin variety (IR50) is shown in Plate 4.2. During incubation period, the minimum and maximum temperatures of the crop canopy were observed to be 26 ºC to 32 ºC, and the relative humidity to be 80 to 88 %.

4.1.2 Resistance of rice varieties to *Pyricularia oryzae*

Reactions of tested local rice varieties and YAU rice lines to rice blast disease are shown in Table 4.3 and 4.4. Among 33 tested local varieties, 4 local rice varieties namely Manaw Thu Kha, Paw San Hmwe, Ayer Padae Thar and Bu Toyl showed moderately susceptible reactions to rice blast disease. Shwe Thwe Yin (IR50) and Naung Ta Moe Se produced highly susceptible reactions to rice blast disease. The remaining 27 varieties responded susceptible reactions to rice blast disease. Of tested local rice varieties, 12.1 %, 81.8 %, 6 % were found as moderately susceptible, susceptible and highly susceptible varieties, respectively (Table 4.3).

Table 4.1 List of *P. oryzae* isolates and their origin of collection

No	Isolate	Village	Township	Region
1	*Po1*	Aungzeya	Zeyarthiri	Nay Pyi Taw Union Territory
2	*Po2*	Thittetkalay		
3	*Po3*	Myaungmya Research Farm	Myaungmya	Ayeyarwaddy
4	*Po4*	Kwallwel		
5	*Po5*	Kyarphoongon		
6	*Po6*	Pandotpin		
7	*Po7*	Ywarsoechaung		

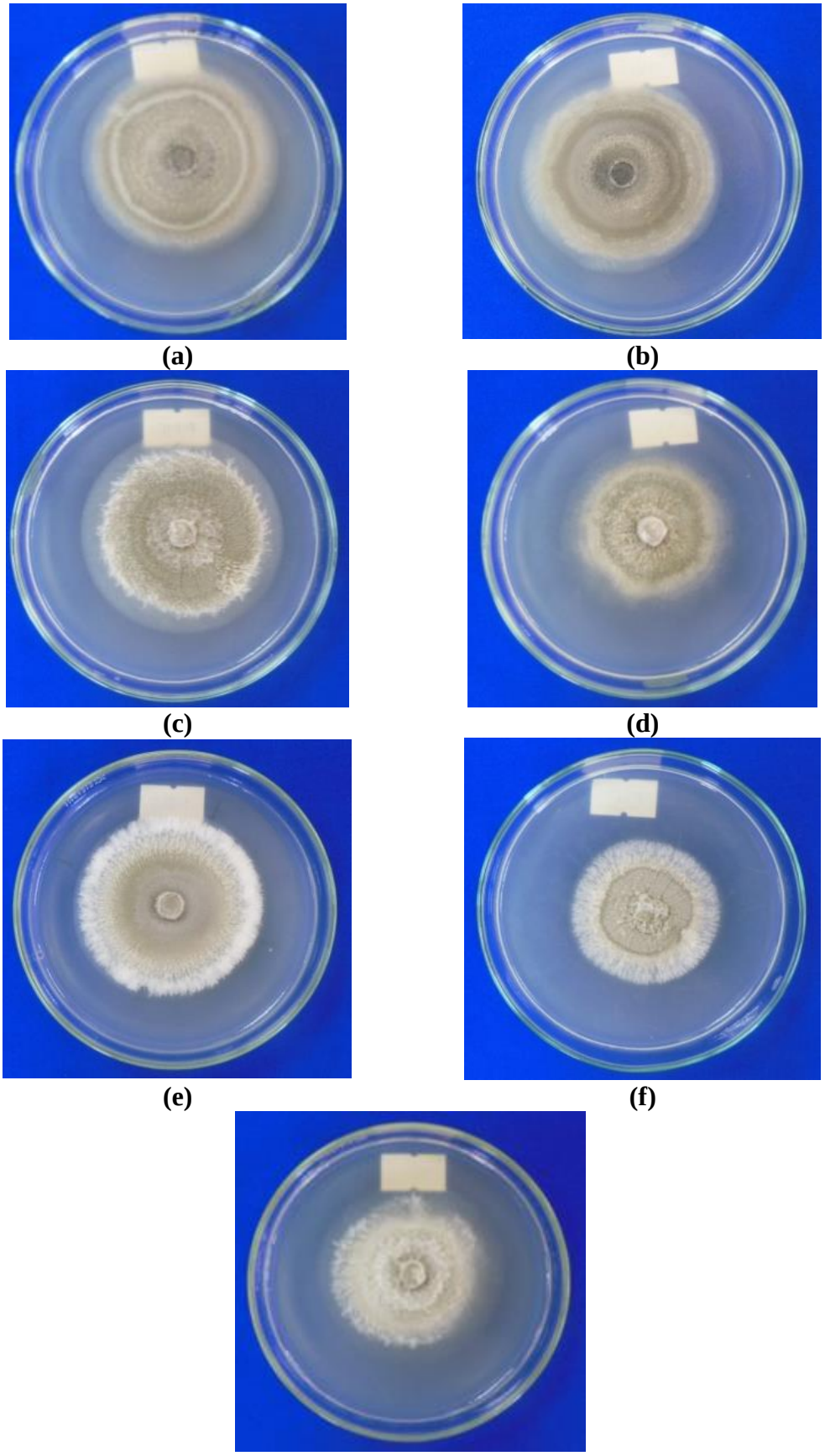


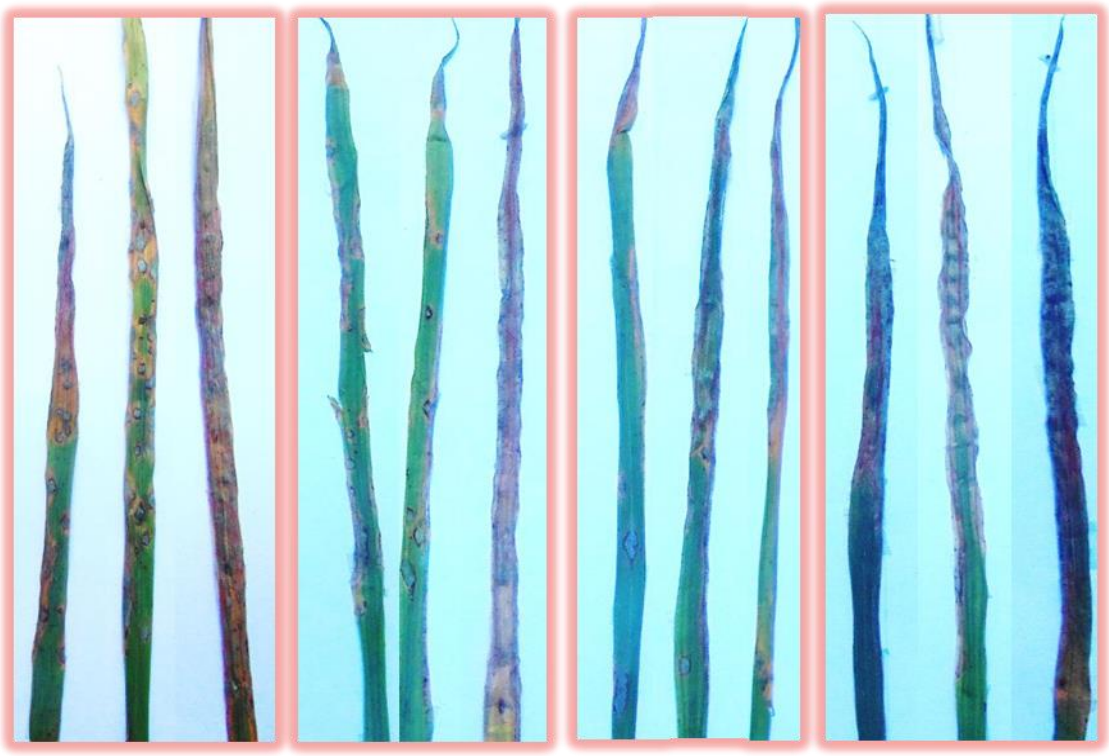
Plate 4.1 Ten days old colonies of seven isolates of *P. oryzae* on PDA media; (a) *Po1* (b) *Po2* (c) *Po3* (d) *Po4* (e) *Po5* (f) *Po6* (g) *Po7* isolates

Table 4.2 Percent disease index (PDI) by seven isolates on Shwe Thwe Yin variety (IR50)

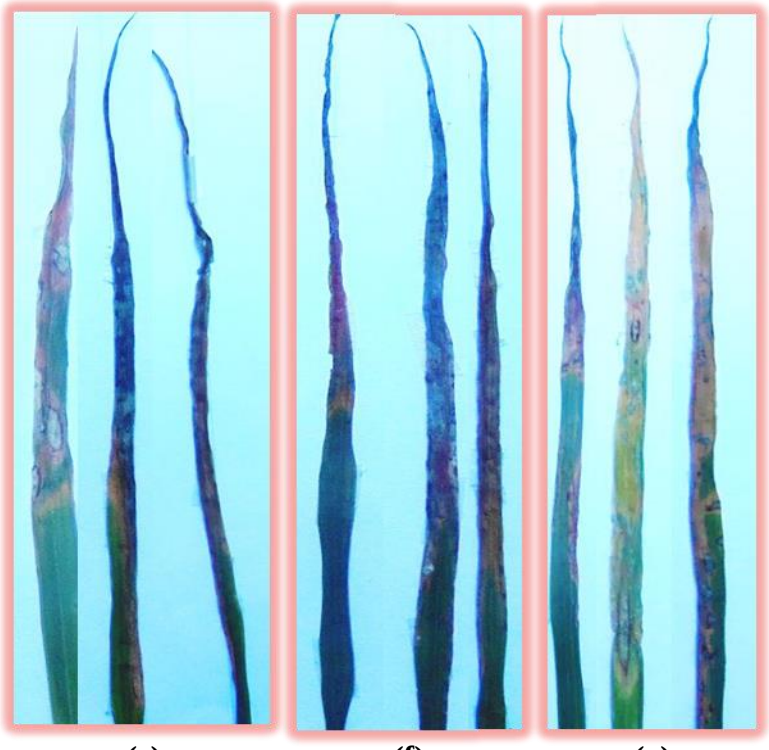
No.	Isolate	PDI (%) *
1	*Po1*	94.6 ± 1.17
2	*Po2*	86.8 ± 2.38
3	*Po3*	64.8 ± 2.26
4	*Po4*	86.7 ± 1.13
5	*Po5*	92.5 ± 2.44
6	*Po6*	93.7 ± 1.01
7	*Po7*	87.4 ± 1.51

* mean of 25 rice seedlings

Environmental condition - Temperature (26-32 ºC), Relative humidity (80-88 %)



(a) **(b)** **(c)** **(d)**



(e) **(f)** **(g)**

Plate 4.2 Virulence of seven isolates of *P. oryzae* (a) *Po1* (b) *Po2* (c) *Po3* (d) *Po4* (e) *Po5* (f) *Po6* (g) *Po7* on Shwe Thwe Yin variety (IR50)

Table 4.3 Reactions of rice varieties to rice blast disease at 8 days after inoculation

No	Local varieties	Average disease scale	Disease response or Reaction	Quantitative Resistance (R)[x] or Susceptible (S)[y]
1	Manaw Thu Kha (R Check)	5.4	Moderately Susceptible	R
2	Paw San Hmwe	4.7	Moderately Susceptible	R
3	Ayer Padae Thar	5.1	Moderately Susceptible	R
4	Bu Toyl	5.4	Moderately Susceptible	R
5	Lone Phyu	5.5	Susceptible	R
6	Sa Bong Thaw	5.6	Susceptible	S
7	Ma Naw Tun	5.7	Susceptible	S
8	Ta Yoke Hmwe	5.7	Susceptible	S
9	Thu Kha-2	5.7	Susceptible	S
10	Khao lami L-5	5.7	Susceptible	S
11	Bu Aung Ban	5.8	Susceptible	S
12	Ant Paw	5.8	Susceptible	S
13	Khauk Kham Tu	6.0	Susceptible	S
14	Shwe War Yin	6.0	Susceptible	S
15	Khao Pi Paung L-18	6.0	Susceptible	S
16	Khun Na Yar Po	6.1	Susceptible	S
17	Khao Pi Paung	6.3	Susceptible	S
18	Kauk Kyee	6.3	Susceptible	S
19	Khauk Mae Pan	6.3	Susceptible	S
20	Boke Thwin Phyu	6.4	Susceptible	S
21	Khauk Kyi Shan Mo	6.4	Susceptible	S
22	Khao Lami L-7	6.4	Susceptible	S
23	V15	6.5	Susceptible	S
24	Japan Ni	6.6	Susceptible	S
25	Paw (1)	6.6	Susceptible	S
26	Yoe Wa	6.7	Susceptible	S
27	Nga Sar Kay	6.8	Susceptible	S
28	Khao Lin L-35	7.0	Susceptible	S
29	IR-36	7.0	Susceptible	S
30	Kun Lone L-41	7.0	Susceptible	S
31	Muyinn Sabar	7.1	Susceptible	S
32	Naung Ta Moe Se	7.9	Highly Susceptible	S
33	Shwe Thwe Yin (S Check)	7.6	Highly Susceptible	S

[x] Disease score $\leq$ 5.5 = R

[y] Disease score > 5.5 = S

Table 4.4 Reactions of promising YAU rice lines to rice blast disease at 8 days after inoculation

No	YAU rice lines	Average disease scale	Disease response or Reaction	Quantitative resistant (R)[x] or Susceptible (S)[y]
1	Manaw Thu Kha (R Check)	5.4	Moderately Susceptible	R
2	YAU-1214-183-3-4-1-1-1	4.6	Moderately Susceptible	R
3	YAU-1215-B-B-B-168-1-1	4.6	Moderately Susceptible	R
4	YAU-1214-183-3-1-2-1-1	4.7	Moderately Susceptible	R
5	YAU-1214-183-3-1-1-1-1	4.8	Moderately Susceptible	R
6	YAU-1201-202-2-2 Y-7	4.9	Moderately Susceptible	R
7	YAU-1215-73-2-3-1-1-1	4.9	Moderately Susceptible	R
8	YAU-1215-B-B-B-139-3-1	5.0	Moderately Susceptible	R
9	YAU-1214-183-3-3-1-1-1	5.0	Moderately Susceptible	R
10	YAU-1201-90-2-2 Y-2	5.0	Moderately Susceptible	R
11	YAU-1211-20-1-1 (Y50)	5.1	Moderately Susceptible	R
12	YAU-1215-B-B-B-10-1-1	5.1	Moderately Susceptible	R
13	YAU-1201-90-2-4 (Y-19)	5.2	Moderately Susceptible	R
14	YAU-1214-S-S-S-77-1-1	5.4	Moderately Susceptible	R
15	YAU-1201-202-1-2 Y-11	5.5	Susceptible	R
16	YAU-1211-71-1-1 Y-22	5.5	Susceptible	R
17	YAU-1214-183-35-1-1-1-1	5.5	Susceptible	R
18	YAU-1201-121-3-1	5.6	Susceptible	S
19	YAU-1201-1-2-1	5.6	Susceptible	S
20	YAU-1215-B-B-B-153-3-1	5.7	Susceptible	S
21	YAU-1201-61-3-3	5.7	Susceptible	S
22	YAU-1201-61-3-3	5.7	Susceptible	S
23	YAU-1215-S-S-S-77-2-1	5.7	Susceptible	S
24	YAU-1214-B-B-B-153-3-1	5.8	Susceptible	S
25	YAU-1211-223-3-1	6.1	Susceptible	S
26	YAU-1201-187-1-2	6.3	Susceptible	S
27	YAU-1211-116-3-4-Y-21	6.3	Susceptible	S
28	YAU-1215-S-S-S-55-2-1	6.3	Susceptible	S
29	YAU-1211-118-2-1	6.7	Susceptible	S
30	YAU-1215-S-S-S-40-2-1	6.7	Susceptible	S
31	YAU-1215-80-1-2-1-1-1	6.7	Susceptible	S
32	YAU-1215-S-S-S-41-1-1	7.3	Susceptible	S
33	YAU-1211-22-2-1	7.5	Highly Susceptible	S
34	Shwe Thwe Yin (S Check)	7.6	Highly Susceptible	S

[x] Disease score ≤ 5.5 = R

[y] Disease score > 5.5 = S

Of 32 YAU rice lines, 13 YAU rice lines produced moderately susceptible reactions (score ranged from 4.6 to 5.4) to rice blast disease. Eighteen YAU rice lines showed susceptible reaction to rice blast disease and one YAU rice line showed highly susceptible reaction of score 7.5 to rice blast disease. Of tested YAU rice lines, 40.6 %, 56.3 % and 3.1 % were found as moderately susceptible, susceptible and highly susceptible varieties, respectively. Resistant YAU rice lines against rice blast disease were not found (Table 4.4). Although all of the tested varieties did not show qualitative resistance, some of the varieties seem to have quantitative resistance.

According to IRRI (2013), tested varieties with consistent rating, between 4 and 6 with overall average not higher than 5.5, may have a good level of quantitative resistance. In this experiment, the average scores of 5 local rice varieties and 16 YAU rice lines were found to be not higher than score 5.5. Therefore 15.2 % of tested local rice varieties, 5 varieties, and 50 % of YAU rice lines, 16 rice lines, may have a good level of quantitative resistance (Table 4.3 and Table 4.4). Quantitative resistance refers to the incomplete, partial resistance controlled by more than one recessive gene. Namrata, Bisen, Singh, Thakur and Loitongbam (2017) stated that quantitative resistance suppresses the growth and reproduction of *M. oryzae*, but it can cause disease when environment is conducive for blast so, it is more suited to low risk areas only. Quantitative resistance by quantitative trait loci (QTLs) are reported to durable for long time against a wide-range of pathogens, promising for sustainable rice production in the future (Song & Goodman, 2001).

In this experiment, susceptible check, Shwe Thwe Yin variety (IR50) showed susceptible reaction (score 7.6). Resistant check Manaw Thu Kha produced moderately susceptible reaction (score 5.4) to rice blast disease. The result was in disagreement with Aye et al. (2015) and Khaing et al. (2018), who found that Manaw Thu Kha was resistant to *P. oryzae* isolates. It might be that the reaction of Manaw Thu Kha could be governed by different pathogenicity of different isolates.

This experiment was conducted during January, 2019. During the experiment, the temperature and relative humidity were controlled at 22º to 30 ºC and 85 to 100 %, respectively. Importance of temperature and relative humidity on symptom expression and disease development has been described by some authors. Ribot et al. (2008) reported that the optimum temperature for spore germination in order to cause disease is 25-28 ºC. Castejon (2008) suggested that the first symptom appearance

during mid-tillering stage and disease development in later stages of crop growth occurred at a relative humidity of 95 % and an average temperature of 26 to 27 °C. Finding out resistant levels of tested rice varieties and promising YAU rice lines against rice blast disease was done by combining virulent pathogen and favorable environments for disease development under the controlled environmental conditions.

4.2 Effect of Different Fungicides on Rice Blast Disease

4.2.1 Evaluation of different fungicides on the growth of *P. oryzae in vitro*

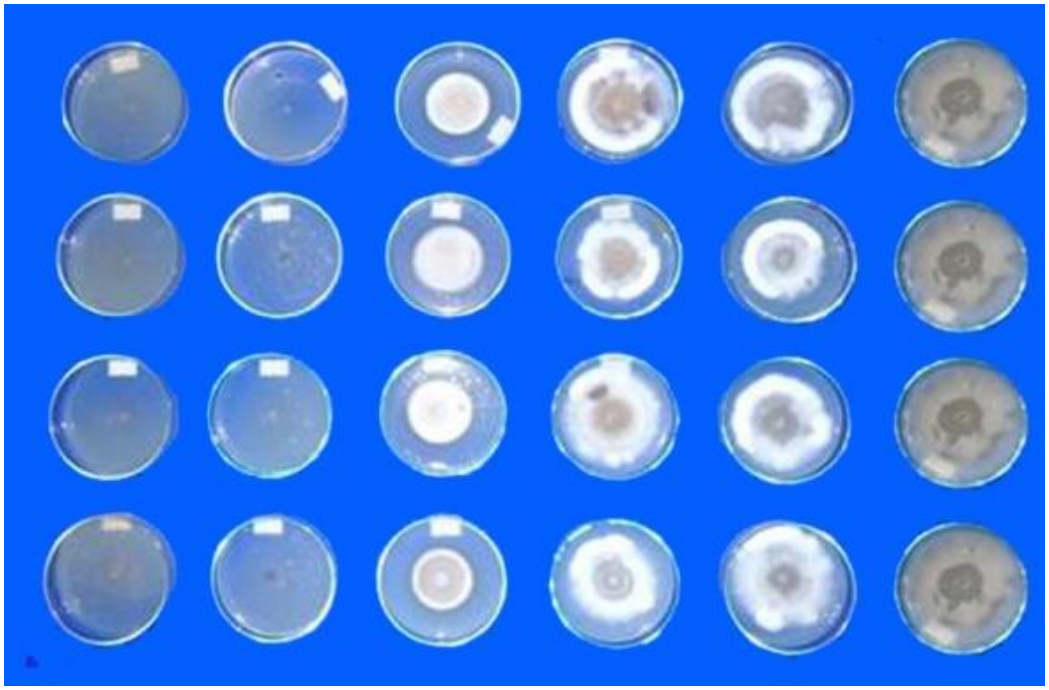
Eight different fungicides *viz.*, Kasugamycin, Tricylazole, Copper oxychloride, Isoprothiolane, Mancozeb, Carbendazim, Thiophanate methyl and Dicarboximide were evaluated against the mycelium growth of *P. oryzae* at five different concentrations. The inhibition percent at five concentrations of eight different fungicides are presented in Table 4.5. Among test concentrations of eight fungicides, the complete inhibition of mycelial growth was found at 800 ppm of Kasugamycin in Plate 4.5, 500 ppm of Tricylazole in Plate 4.3, 100 ppm of Isoprothiolane in Plate 4.6, 1000 ppm of Mancozeb in Plate 4.4, 10 ppm of Carbendazim in Plate 4.10, 7.5 ppm of Thiophanate methyl in Plate 4.9 and 10000 ppm of Dicarboximide in Plate 4.7. But 100 percent inhibition of mycelial growth was not resulted in Copper oxychloride at 20000 ppm in Plate 4.8 which was the highest concentration among tested concentrations of that fungicide. Inhibition percent of mycelium growth increased with increasing concentration of each fungicide.

Concentrations of fungicides inhibiting 50 % mycelium growth of *P. oryzae* (IC_{50}) values of eight fungicides were determined to compare the effectiveness of different fungicides. The IC_{50} of eight fungicides *in vitro* is shown in Table 4.6. The IC_{50} value ranged from the minimum of 2.03 ppm in Isoprothiolane to the maximum of 3384.60 ppm in Copper oxychloride. Mycelium growth of *P. oryzae* at five concentrations of eight fungicides treated plates and untreated plates are shown in Plate 4.3.

IC_{50} values of Isoprothiolane, Thiophanate methyl, Carbendazim, Mancozeb, Kasugamycin and Tricylazole were not significantly different with each other, however, these fungicides were significantly different with Copper oxychloride and Dicarboximide. Among the tested fungicides, the lowest IC_{50} value was resulted in Isoprothiolane and the maximum IC_{50} value was recorded from Copper oxychloride followed by Dicarboximide.

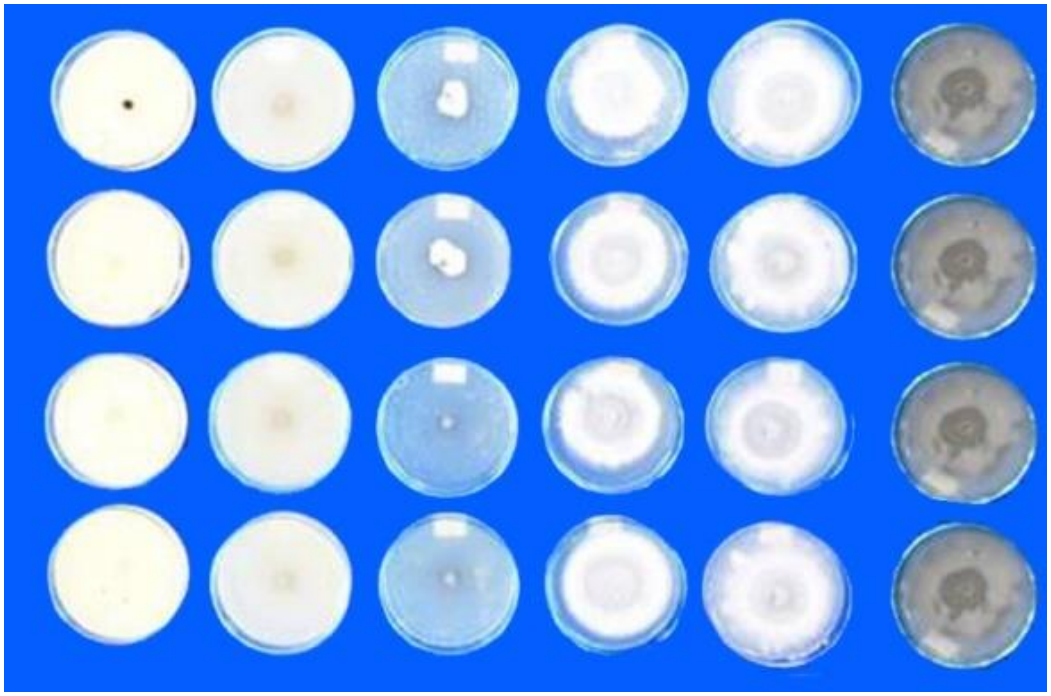
Table 4.5 Inhibition percent on mycelium growth of *P. oryzae* at five concentrations of eight fungicides

Fungicides	Concentrations (C) & Inhibition % (I)				
	C_1 & I	**C_2 & I**	**C_3 & I**	**C_4 & I**	**C_5 / I**
Tricyclazole	1.0 ppm 8.2 %	1.0 x10^{1} ppm 15.5 %	1.0 x10^{2} ppm 41.2 %	5.0 x10^{2} ppm 100 %	1 x10^{3} ppm 100 %
Mancozeb	1.0 ppm 12.3 %	1.0 x10^{1} ppm 20.1 %	1.0 x10^{2} ppm 86.9 %	1.0 x10^{3} ppm 100 %	1 x10^{4} ppm 100 %
Kasugamycin	2.4 ppm 6.2 %	1.2 x10^{1} ppm 9.3 %	6.0 x10^{1} ppm 39.5 %	3.0 x10^{2} ppm 75.5 %	8 x10^{2} ppm 100 %
Dicarboximide	1.0 ppm 6.0 %	1.0 x10^{1} ppm 7.9 %	1.0 x10^{2} ppm 23.5 %	1.0 x10^{3} ppm 74.5 %	1 x10^{4} ppm 100 %
Carbendazim	1.0 x10^{-2} ppm 0.8 %	1.0 x10^{-1} ppm 2.1 %	5.0 x10^{-1} ppm 6.4 %	1.0 ppm 61.9 %	1 x10^{1} ppm 100 %
Thiophanate methyl	1.0 x10^{-1} ppm 1.1 %	1.0 ppm 1.7 %	5.0 ppm 97.41 %	7.5 ppm 100 %	1 x10^{1} ppm 100 %
Copper oxychloride	2.0 ppm 3.5 %	2.0 x10^{1} ppm 12.5 %	2.0 x10^{2} ppm 17.1 %	2.0 x10^{3} ppm 59.0 %	2 x10^{4} ppm 61.5 %
Isoprothiolane	1.0 ppm 38.3 %	1.0 x10^{1} ppm 65.8 %	1.0 x10^{2} ppm 100 %	1.0 x10^{3} ppm 100 %	1 x10^{4} ppm 100 %



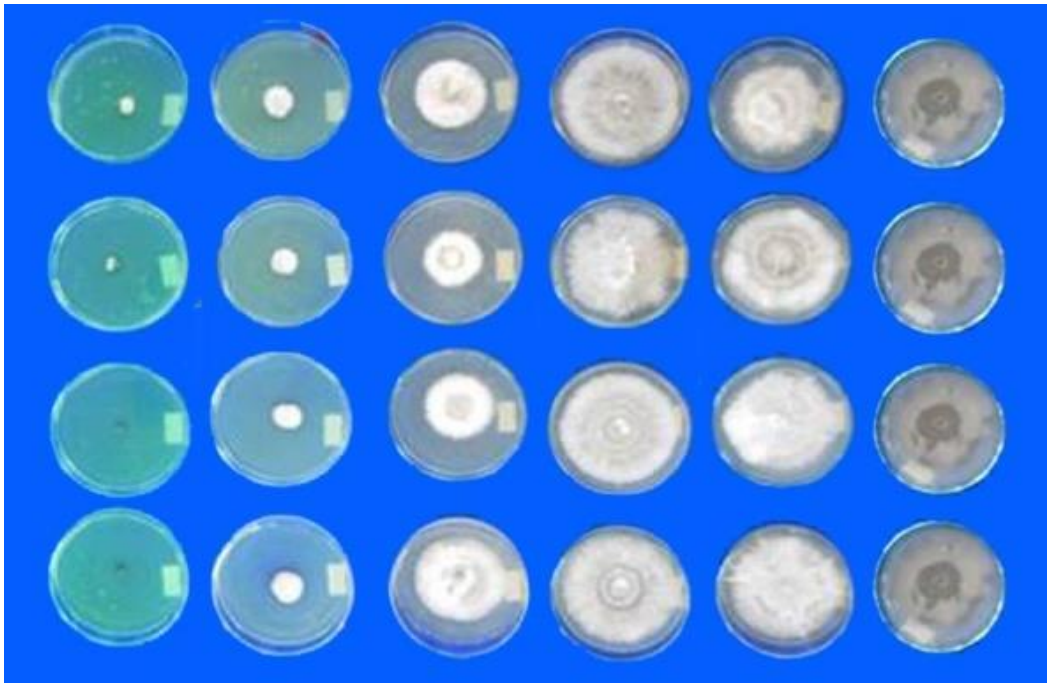
1000ppm 500ppm 100ppm 10ppm 1ppm Control

Plate 4.3 Inhibition effect of Tricyclazole on the mycelial growth of *P. oryzae* at five different concentrations



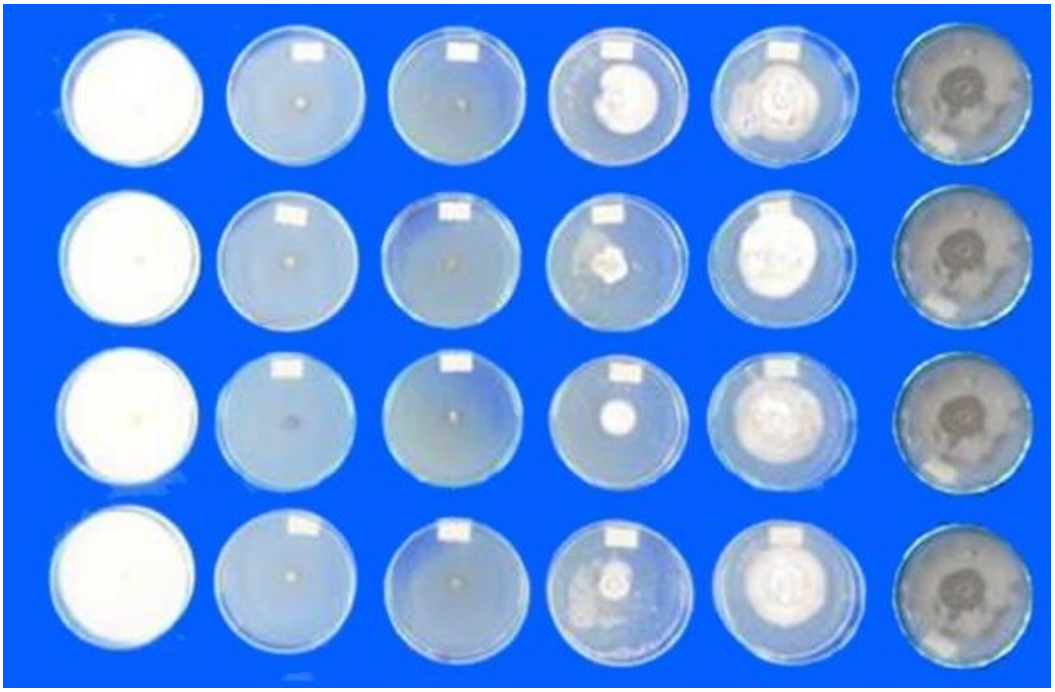
10000ppm 1000ppm 100ppm 10ppm 1ppm Control

Plate 4.4 Inhibition effect of Mancozeb on the mycelial growth of *P. oryzae* at five different concentrations



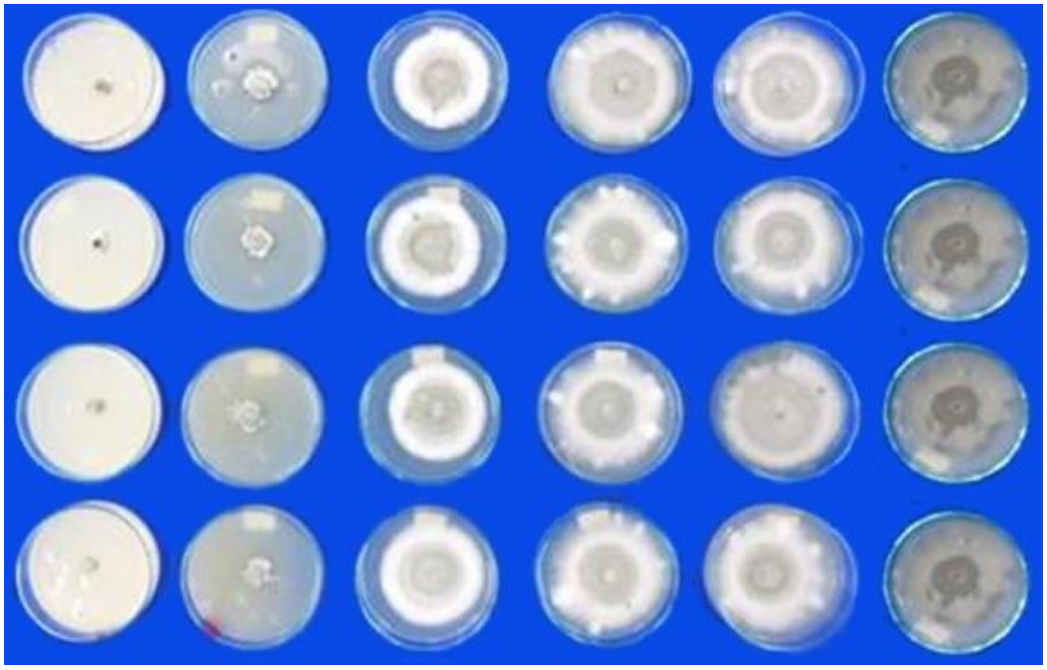
800ppm 300ppm 60ppm 12ppm 2.4ppm Control

Plate 4.5 Inhibition effect of Kasugamycin on the mycelial growth of *P. oryzae* at five different concentrations



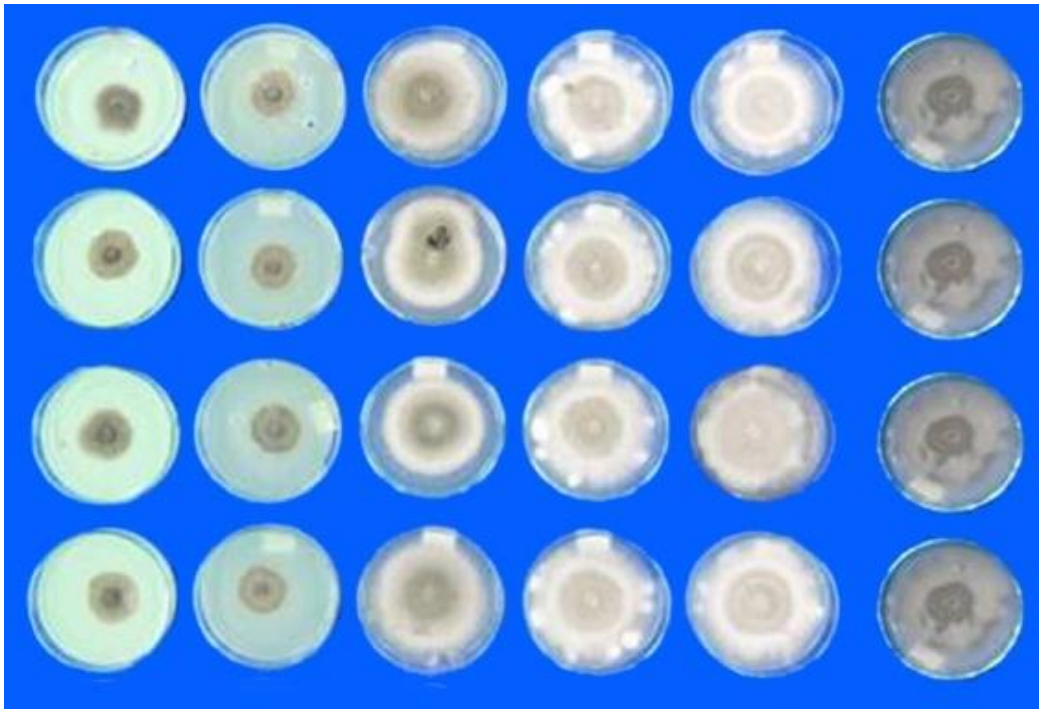
10000ppm 1000ppm 100ppm 10ppm 1ppm Control

Plate 4.6 Inhibition effect of Isoprothiolane on the mycelial growth of *P. oryzae* at five different concentrations



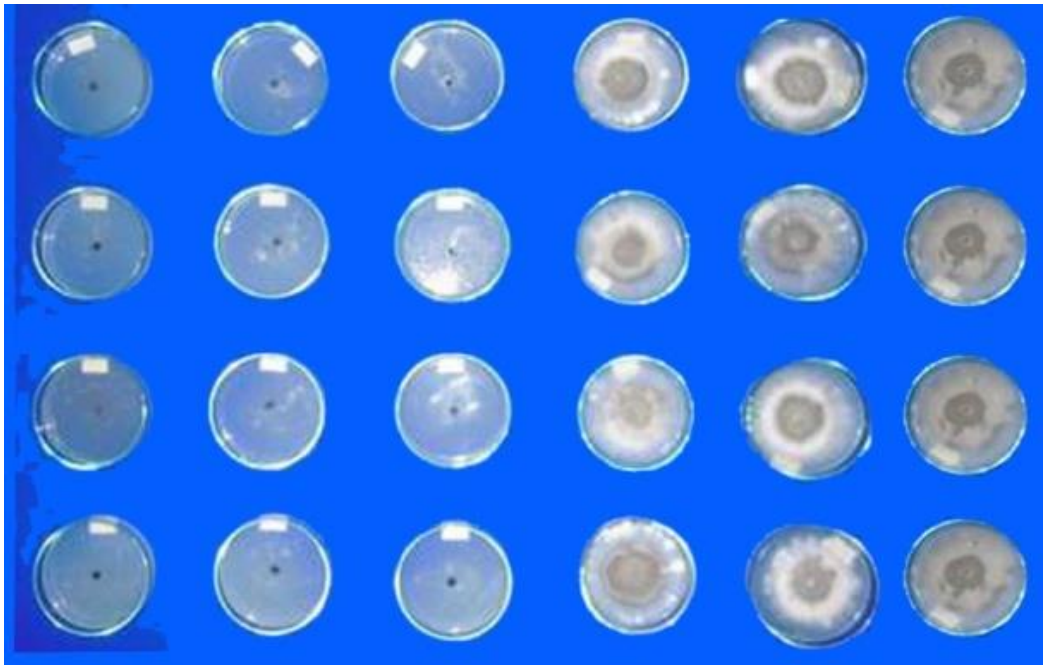
10000ppm 1000ppm 100ppm 10ppm 1ppm Control

Plate 4.7 Inhibition effect of Dicarboximide on the mycelial growth of *P. oryzae* at five different concentrations



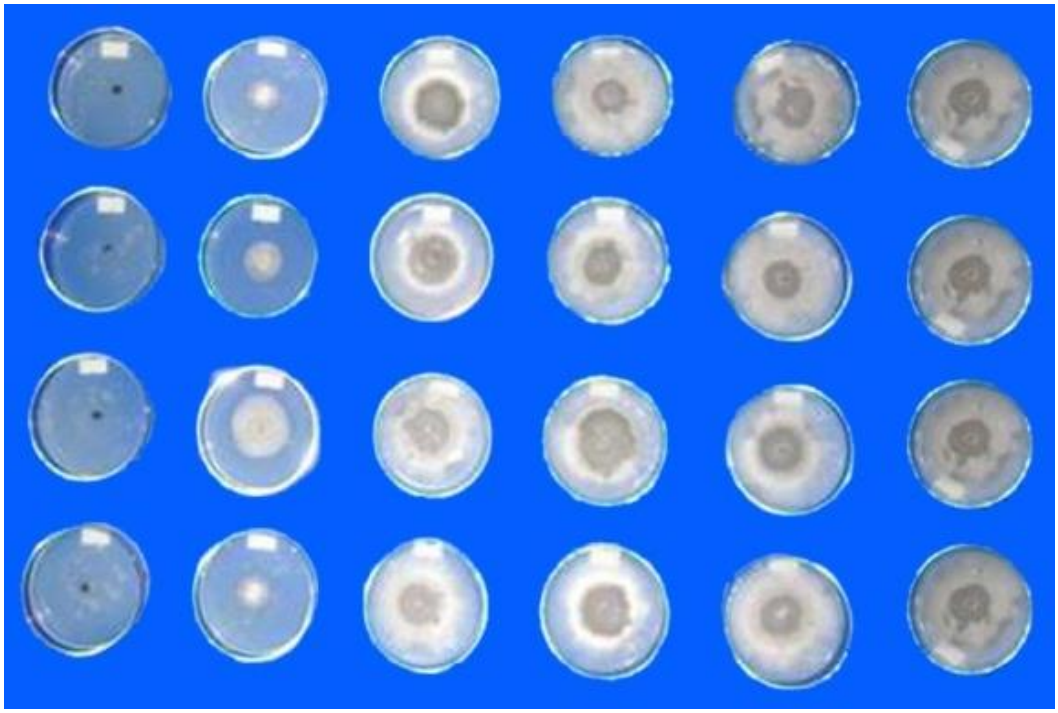
20000ppm 2000ppm 200ppm 20ppm 2ppm Control

Plate 4.8 Inhibition effect of Copper oxychloride on the mycelial growth of *P. oryzae* at five different concentrations



10ppm 7.5ppm 5ppm 1ppm 0.1ppm Control

Plate 4.9 Inhibition effect of Thiophanate methyl on the mycelial growth of *P. oryzae* at five different concentrations



10ppm 1ppm 0.5ppm 0.1ppm 0.01ppm Control

Plate 4.10 Inhibition effect of Carbendazim methyl on the mycelial growth of *P. oryzae* at five different concentrations

Table 4.6 Concentrations of fungicides inhibiting 50 % mycelium growth of *P. oryzae*

Fungicides	**IC_{50} [x] (ppm)**
Tricyclazole	126.90 c [y]
Mancozeb	105.76 c
Kasugamycin	125.48 c
Carbendazim	3.56 c
Dicarboximide	557.94 b
Thiophanate methyl	3.48 c
Copper oxychloride	3384.61 a
Isoprothiolane	2.03 c
$LSD_{0.05}$	230.41
Pr>F	< 0.001
CV %	29.31

[x] mean of four replications

[y] means followed by the same letter in the same column are not significantly different at 5 % level

Edgington, Khew and Barron (1971) classified the effectiveness of fungicides by the concentration inhibiting 50 % mycelium growth as highly effective fungicide when IC_{50} is less than 1 ppm, moderately effective fungicide when IC_{50} is between 1 ppm and 10 ppm, poorly effective fungicide when IC_{50} is between 10 ppm and 50 ppm, and ineffective fungicide when IC_{50} is greater than 50 ppm. According to the standard criteria of Edgington, Khew and Barron (1971), Isoprothiolane, Thiophanate methyl and Carbendazim were found to be moderately effective fungicides as the IC_{50} values of these three fungicides were between 1 ppm and 10 ppm. Mancozeb, Kasugamycin, Tricyclazole, Dicarboximide and Copper oxychloride were ineffective fungicides to inhibit the mycelium growth of *P. oryzae* due to their IC_{50} values greater than 50 ppm.

Among moderately effective fungicides Isoprothiolane, Thiophanate methyl and Carbendazim, the lowest IC_{50} value was resulted in Isoprothiolane. The lower the IC_{50}, the greater toxicity of the chemical. If IC_{50} value is lower, the fungicide will be more effective. The mode of action of Isoprothiolane is believed to be interference with transmethylation in the biosynthesis of phosphatidylcholine, a major membrane lipid in eukaryotic cells (Uesugi, 2001; Yoshida, Moriya & Uesugi, 1984). Therefore, Isoprothiolane was found as the more effective and more toxic fungicide to the mycelium growth of *P oryzae* in the *in vitro* study.

4.2.2 Effect of different fungicides on rice blast disease *in vivo*

Effect of minimun inhibition concentration of different fungicides on percent disease index (PDI) and area under disease progress curve (AUDPC) are presented in Table 4.7.

The data prevailing to percent disease index as affected with different fungicides is presented in Table 4.7 and Figure 4.1. Percent disease indexes ranged from 69.9 % to 76.4 %. The minimum percent disease index of 69.9 % was recorded in Mancozeb treated plants followed by 71.0 % in the Tricylazole treated plants. The maximum percent disease index of 76.4 % was resulted in Dicarboximide treated plants. But percent disease indexes of all fungicides treated plants were not significantly different with each other at 14 days after second fungicide application .

Efficacy of different fungicides on the area under disease progress of rice blast disease is presented in Table 4.7. The area under disease progress (AUDPC) values of all fungicides treated plants were significantly different with each other.

Table 4.7 Effect of minimun inhibition concentration of different fungicides on percent disease index (PDI) and area under disease progress curve (AUDPC)

Fungicides	Minimum inhibition concentration (ppm)	PDI	AUDPC [x]
Tricyclazole	849.3	71.00	74.54 d[y]
Mancozeb	938.2	69.96	75.75 cd
Kasugamycin	717.0	72.33	76.75 bcd
Carbendazim	92.4	72.09	77.35 abc
Dicarboximide	9525.6	76.40	79.10 ab
Thiophanate methyl	14.3	74.81	79.29 ab
Copper oxychloride	3620.6	75.10	79.51 a
Isoprothiolane	97.9	74.11	79.53 a
Untreated control	0.0	71.98	78.07 abc
$LSD_{0.05}$		5.20	2.7600
Pr>F		0.2395	< 0.0066
CV %		4.88	2.43

[x] mean of four replications

[y] means followed by the same letter in each column are not significantly different at 5% level

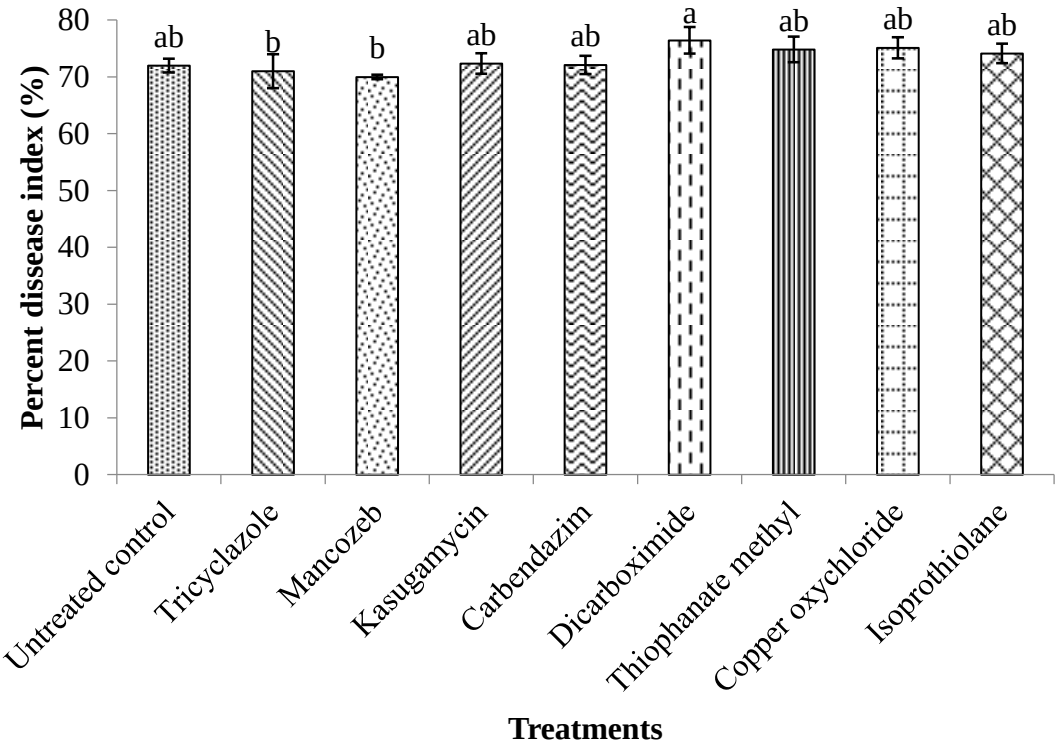


Figure 4.1 Effect of different fungicides on percent disease index of rice blast disease ($LSD_{0.05}$ = 5.20, CV% = 4.88)

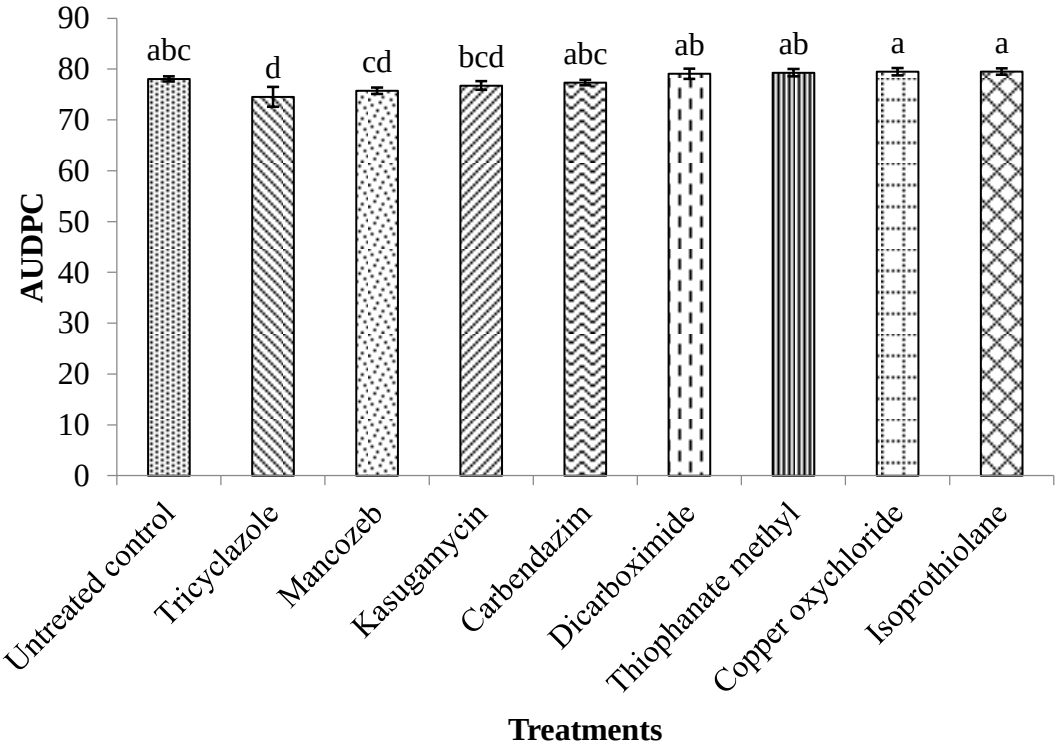


Figure 4.2 Effect of different fungicides on Area Under Disease Progress Curve (AUDPC) of rice blast disease ($LSD_{0.05}$ = 2.76, CV% = 2.43)

The lowest AUDPC value was recorded in Trycyclazole treated plants and was not significantly different from Mancozeb and Kasugamycin treated plants. The AUDPC value of Tricyclazole treated plants was significantly different as compared to the other fungicides treated plants and untreated plants (Figure 4.2). The effectiveness of Tricyclazole has been reported by several workers. Peterson (1990) stated that Tricyclazole is systemic in rice and protects plants from infection by *P. oryzae* by preventing penetration of the epidermis by the fungus. The compound acts by inhibiting melanization within the appressorium, thus causing a lack of rigidity in the appressorial wall. Chattopadhyay, Kushwaha, Chand and Srivastava (2013) reported that Tricyclazole affect the invasion and colonization of pathogen within plant tissue. 'Nonfungicidal' chemical Tricyclazole behave differentially as fungicidal molecule in plant system by restricting pathogen growth.

Effect of minimun inhibition concentration of different fungicides on infection rate (r) and reduction percent in plant height is presented in Table 4.8. The data regarding infection rate of rice blast disease as affected by different fungicides is presented in Figure 4.3. The effect of fungicides on infection rate of rice blast disease was significantly different. The mean of infection rate of Tricylazole, Mancozeb, Kasugamycin, Dicarboximide and Carbendazim treated plants were 4.2, 4.3, 4.3, 4.5 and 4.6, respectively, and were not significantly different with untreated plants but significantly lower as compared to the rest fungicides treated plants. The maximum infection rate was recorded in the Isoprothiolane treated plants followed by Copper oxychloride treated plants and Thiophanate methyl treated plants. The minimum infection rate was resulted in Tricyclazole treated plants. Lazarovits, Steel, Higgins and Stoesse (1989) stated that Tricyclazole inhibited the synthesis of polyketides by the pathogen. The high effectiveness of the Tricyclazole against the sporulation inhibition and secondary infection indicate a possible manner of additional action of this fungicide different from the melanin biosynthesis inhibition. Woloshuk, Sisler and Vigil (1983) suggested that the failure of appressoria to penetrate the cuticle was due to a loss of their rigidity because they were not permeated with melanin. Gouraminis (1996) reported that Tricyclazole systemic fungicide protects undamaged plant parts against blast pathogen but not those already damaged. This active ingredient does not affect the germination, growth or sporulation of blast pathogen, but acts by inhibiting the synthesis of a precursor of melanin, with consequent loss of pathogenic power.

Table 4.8 Effect of minimun inhibition concentration of different fungicides on infection rate (r) and reduction percent in plant height

Fungicides	Minimum inhibition concentration (ppm)	Infection rate (r) [x]	Reduction % in plant height [x]
Tricyclazole	849.3	4.2430 c[y]	27.72 d[y]
Mancozeb	938.2	4.3598 c	25.99 d
Kasugamycin	717.0	4.3613 c[y]	35.36 bc
Carbendazim	92.4	4.6010 c	30.46 cd
Dicarboximide	9525.6	4.5774 c	39.84 ab
Thiophanate methyl	14.3	5.0661 b	36.19 abc
Copper oxychloride	3620.6	5.3092 b	37.83 ab
Isoprothiolane	97.9	5.7412 a	42.93 a
Untreated control	0.0	4.5848 c	35.16 bc
$LSD_{0.05}$		0.397	7.34
Pr>F		< 0.001	< 0.001
CV %		5.72	16.26

[x] mean of four replications

[y] means followed by the same letter in each column are not significantly different at 5% level

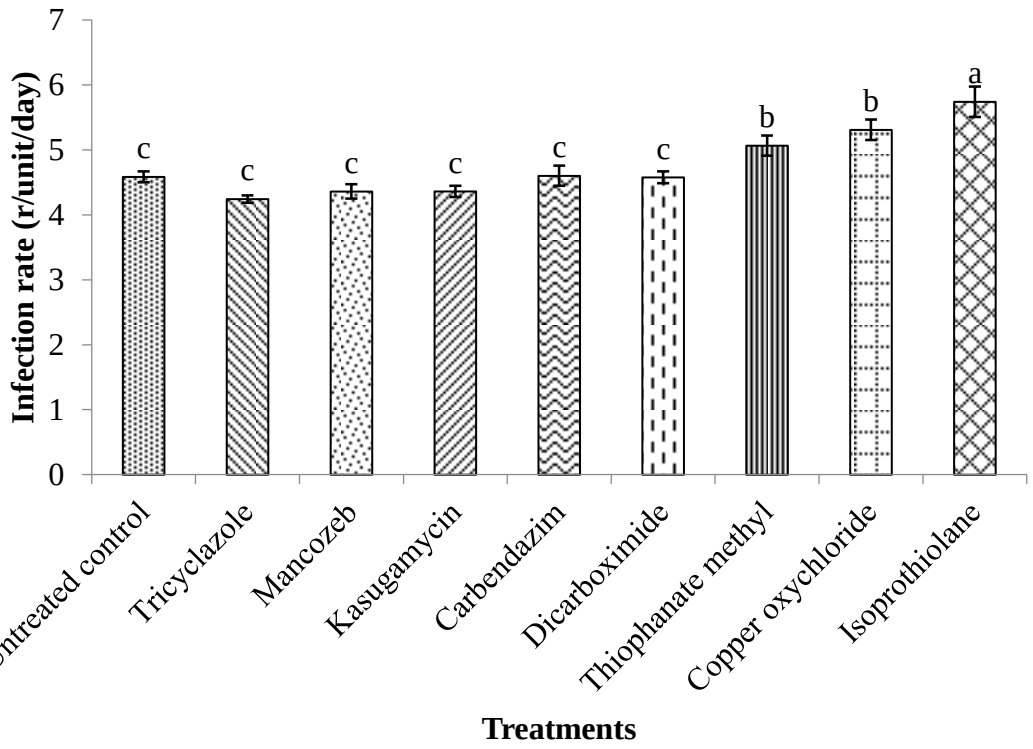


Figure 4.3 Effect of different fungicides on infection rate of rice blast disease ($LSD_{0.05}$ = 0.39, CV% = 5.72)

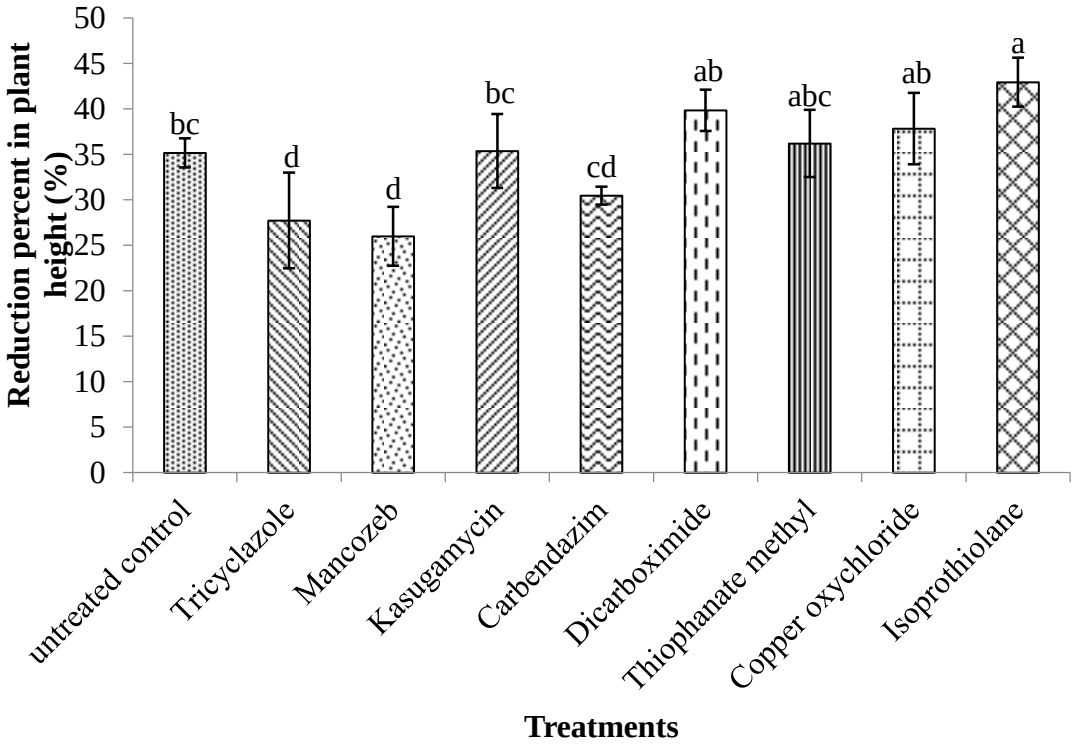


Figure 4.4 Effect of different fungicides on reduction percent in plant height due to rice blast disease ($LSD_{0.05}$ = 7.34, CV% = 16.26)

Efficacy of different fungicides on reduction percent in plant height by rice blast disease is presented in Table (4.8) and Figure (4.4). In this experiment, uninoculated plants showed higher in plant height compared to inoculated plants. Rice blast disease caused the reduction in plant height in this study. The result is in agreement with the statement given by Koutroubas, Katsantonis, Ntanos and Lupotto (2009), who reported that the inoculation affected the overall plant growth and resulted in a reduction in plant height. Reduction percent in plant height was calculated over plant height of uninoculated plant. The mean of reduction percent in plant height of Mancozeb and Tricyclazole treated plants were 25.9 % and 27.7 %, respectively, and were significantly lower than those of the other fungicides treated plants and untreated plants except that of Carbendazim treated plants.

However, the reduction percent in plant height of Carbendazim, Kasugamycin, Thiophanate methyl, Copper oxychloride and Dicarboximide were 30.4, 35.3, 36.1, 37.8 and 39.8 %, respectively and were not significantly different as compared to untreated plants. The maximum reduction in plant height was 42.9 % in Isoprothiolane treated plants. The magnitude of the reduction was dependent on the differences in the disease pressure between the effects of treatments to the disease (Koutroubas et al., 2009). Torres and Teng (1993) as cited in Koutroubas et al. (2009) stated a negative effect of blast disease on plant height proportional to disease level.

The finding is in line with many authors. Sood and Kapoor (as cited in Ghazanfar, Wakil, Sahi & Yasin, 2009) evaluated seven fungicides at recommended rate against leaf and neck blast of rice and reported that Tricyclazole was the most effective, reducing leaf and neck blast by 89.2 % and 97.5 % and increasing the yield 43.3 % as compared to untreated control. Neelakanth, Gowda, Chethana and Parasappa (2017) reported that lowest percent of blast was observed in Tricyclazole treated plot among four fungicides tested in field condition using recommended dosage. Pandey, (2016) observed that among 11 foliar fungicides at the same concentration, Tricyclazole was superior in controlling the leaf blast severity. Iqbal et al. (2014); Kumar and Veerabhadraswamy (2014) reported that Tricyclazole was the most effective in reducing the leaf blast severity.

Eight different fungicides were compared for their effectiveness by IC_{50} values and were evaluated their effectiveness at their respective minimum inhibitions *in vivo*. Copper oxychloride and Dicarboximide were found to be least effectiveness *in vitro* and *in vivo* as compared to other fungicides tested. The IC_{50} values of Isoprothiolane, Thiophanate methyl, Carbendazim, Mancozeb, Kasugamycin and Tricyclazole were statically not different, but they were different by the classification of effectiveness

such as moderately effective fungicides Isoprothiolane, Thiophanate methyl, Carbendazim and ineffective fungicides Mancozeb, Kasugamycin and Tricyclazole *in vitro*. Although Isoprothiolane was more effective and more toxic to the mycelium growth of *P. oryzae* as compared to the other fungicides tested *in vitro*, its effectiveness was found to be least as compared to other fungicides tested *in vivo*. But Tricyclazole showed the superior effect followed by Mancozeb as compared to untreated control *in vivo*. The reason might be due to different mode of actions of different fungicides tested *in vitro* and *in vivo*.

CHAPTER V
CONCLUSION

Seven isolates were isolated from Zeyarthiri Township of Nay Pyi Taw Union Territory and Myaungmya Township, Ayeyarwaddy Region. All isolates were virulent isolates on tested rice cultivar (Shwe Thwe Yin). *Po1* isolate from Aungzeya village of Zeyarthiri Township, Nay Pyi Taw Union Territory was found as the most virulent one among seven isolates collected.

In the screening of 33 local rice varieties and 32 YAU rice lines against rice blast disease, four local rice varieties namely Manaw Thu Kha, Paw San Hmwe, Ayer Padae Thar and Bu Toyl, and 13 YAU rice lines were found as moderately susceptible varieties. One local rice variety Naung Ta Moe Se and one YAU rice line YAU-1211-22-2-1 were found to be highly susceptible and they can be used as susceptible check in the future experiments. The rest tested local rice varieties and YAU rice lines were found as susceptible varieties. In assuming as quantitative resistant varieties, 15.2 % of tested local rice varieties, five local rice varieties namely Manaw Thu Kha, Paw San Hmwe, Ayeyar Padae Thar, Lone Phyu and Bu Toyl, and 50 % of tested YAU rice lines, 16 YAU rice lines, may have a good level of quantitative resistance. These quantitative resistant local rice varieties and YAU rice lines should be screened in future using different blast isolates to develop long lasting blast resistant variety. The present study provided the information of quantitative resistance in varieties that could be useful in low risk area of rice blast disease and will help in breeding program for rice blast disease resistant varieties.

In the *in vitro* study of eight fungicides on the mycelial growth of *P. oryzae*, tested fungicides were observed to have inhibition effect on the mycelial growth. Isoprothiolane was found as the most toxic chemical with the least IC_{50} value to the mycelial growth of *P. oryzae* as compared to the other fungicides tested. In the *in vivo* study of spraying with the minimum inhibition concentrations of eight fungicides on rice blast diseased plants, Tricyclazole was found to be the most effective to control rice blast disease as compared to other fungicides tested. Copper oxychloride and Dicarboximide were found to be not effective both of *in vitro* study and *in vivo* study. The effectiveness of Tricyclazole and Isoprothiolane fungicides should be tested at recommended low concentration to control rice blast disease under field condition.

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APPENDICES

Appendix 1 Regression equations, coefficient of determinations (R^2), 50% mycelial inhibitory concentrations (IC_{50}) of eight fungicides

No	Fungicides	Rep	Equation	R^2	IC_{50}
1	Tricyclazole	1	y=0.787x + 3.3247	0.93	134.49
		2	y= 0.7804x + 3.3663	0.97	123.99
		3	y= 0.7709x + 3.3688	0.96	130.58
		4	y= 0.7181X + 3.5107	0.92	118.54
2	Manceozeb	1	y= 0.503x + 3.994	0.79	100
		2	y = 0.539x + 3.894	0.78	112.69
		3	y = 0.556x + 3.836	0.78	124.02
		4	y= 0.501x + 4.03	0.80	86.31
3	Kasugamycin	1	y=0.887x + 3.2113	0.88	103.87
		2	y=1.1562x+ 2.5192	0.91	139.82
		3	y=1.0185x + 2.9766	0.92	96.96
		4	y=1.2311x + 2.2822	0.98	161.28
4	Dicarboximide	1	y = 0.625x + 3.304	0.89	517.13
		2	y = 0.65x + 3.21	0.91	567.28
		3	y = 0.71x + 3.034	0.90	587.48
		4	y = 0.671x + 3.156	0.92	559.88
5	Carbendazim	1	y=1.1112x + 4.6274	0.81	2.16
		2	y = 1.1084x + 4.5121	0.78	2.75
		3	y = 0.5607x + 4.526	0.34	6.91
		4	y = 1.0424x + 4.6017	0.73	2.40
6	Thiophanate methyl	1	y = 1.7721x + 3.9121	0.80	4.11
		2	y = 0.949x + 4.5592	0.42	2.91
		3	y = 1.6847x + 4.1636	0.75	3.13
		4	y = 1.6067x + 4.0762	0.80	3.75
7	Copper oxychloride	1	y= 0.516x + 3.2007	0.92	3069.02
		2	y= 0.536x + 3.1326	0.94	3047.19
		3	y= 0.618x + 2.774	0.92	3998.52
		4	y= 0.668x + 2.6389	0.92	3423.73
8	Isoprothiolane	1	y= 0.224x + 4.986	0.58	1.15
		2	y= 0.268x + 4.794	0.79	5.86
		3	y= 0.205x + 5.04	0.61	0.63
		4	y= 0.199x + 5.064	0.57	0.47

Appendix 2 Regression equations, coefficient of determinations (R^2), minimun inhibition concentrations of tested fungicides

No	Fungicides	equation	R^2	Minimum inhibition concentration (ppm)
1	Tricyclazole	y = 0.1014x + 13.877	0.89	849.3
2	Mancozeb	y = 0.0684x + 35.823	0.53	938.2
3	Kasugamycin	y = 0.1136x + 18.547	0.83	717.0
4	Carbendazim	y = 0.0518x + 95.211	0.32	92.4
5	Dicarboximide	y = 0.0079x + 24.747	0.66	9525.6
6	Thiophanate methyl	y = 6.7669x + 3.2331	1.00	14.3
7	Copper oxychloride	y = 0.0251x + 9.122	0.97	3620.6
8	Isoprothiolane	y = 0.524x + 48.682	0.86	97.9

Appendix 3 Disease scores of treated plants at five observations and plant height of 62 days old treated plants

No	Fungicides	Disease score					Plant height of 62 days old plant (cm)
		1st observation	2nd observation	3rd observation	4th observation	5th observation	
1	Kasugamycin	6.0	7.6	7.5	7.9	8.2	40.0
2	Tricyclazole	5.6	7.1	7.4	7.5	7.9	47.2
3	Copper oxychloride	7.7	7.8	8.2	8.3	8.4	38.0
4	Isoprothiolane	8.0	8.1	7.8	8.2	8.3	32.6
5	Mancozeb	6.0	7.1	7.4	7.9	7.9	49.0
6	Carbendazim	6.5	7.3	7.7	8.2	8.2	45.3
7	Thiophanate methyl	7.2	7.9	8.1	8.3	8.4	39.1
8	Dicarboximide	6.4	8.1	8.4	8.1	8.5	36.2
9	Untreated control	6.5	7.6	8.0	8.2	8.2	41.0
10	Uninoculated plant						61.1

Appendix 4 Preparation of inoculated plants

Inoculation with spore suspension

Incubation of inoculated plants

Inoculated plants at 6 days after inoculation

Appendix 5 Screening of local rice varieties and YAU rice lines against *P. oryzae*

Incubation of inoculated plasts

Inoculated plants at 6 days after inoculation

Appendix 6 Effect of eight fungicides on rice blast disease at 14 days after second time spraying

Untreated control

Tricyclazole

Mancozeb

Kasugamycin

Carbendazim

Dicarboximide

Thiophanate methyl

Copper oxychloride

Isoprothiolane

Uninoculated control