STATUS AND MANAGEMENT OF RICE BLAST DISEASE CAUSED BY *Pyricularia oryzae* Cav. IN UPLAND RICE IN SELECTED REGIONS IN TANZANIA

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EXTENDED ABSTRACT

This study aimed to enhance effective rice blast disease management by the establishment of the current status of the disease, its effect on yield of selected upland rice genotypes and the use of environmentally friendly methods such as bio-agents and hot water seed treatment. Two surveys were conducted in 2017/2018 and 2018/2019 rice growing seasons to investigate farmers' knowledge of rice blast disease and its management and to establish the incidence and severity of rice blast disease in farmers' rice fields in Morogoro and Tanga regions. Data were collected through face to face interviews using semi structured questionnaire. Rice blast disease assessment in farmers' fields was conducted by diagonal transect walk using 1.0 x 1.0 quadrant. Results indicated that about 46.3% of the farmers interviewed were not aware of the cause and means of spread of rice blast disease. The majority of farmers (92.3%) planted local upland rice varieties and about 54.0% did not apply any management method due to lack of knowledge, inability to afford the cost of buying fungicides and unavailability of effective blast disease control measures. Results from the surveys indicated that the highest rice blast disease incidence and severity were recorded in the 2017/2018 rice growing season. In this season, Mvomero and Korogwe districts had higher blast disease severity of 100% and 98.8%, respectively, than Morogoro Rural (88.1%) and Muheza (87.3%) districts. *In vitro* evaluation of microbial agents, indicated that *Trichoderma asperellum* and *Bacillus subtilis* had over 75% inhibition of radial growth of *P. oryzae* compared to fungicide Linkimil 72 WP (21 - 23%) and the control (0%). *In vivo* evaluation showed that rice blast disease incidence was reduced by 70% in plants treated with *T. asperellum* followed by 51.5% in *B. subtilis* treated plants and 26.5% in Linkimil 72 WP treated plants. There was a decrease in blast disease severity by 35.6% in rice plants treated with *T. asperellum* and 29.1% in *B. subtilis* treated rice plants, suggesting that *T. asperellum* and *B. subtilis* used in this study had high antagonistic capacity against *P. oryzae.* Completely randomized design (CRD) experiments in the laboratory and screen house were conducted to investigate the efficacy of *T. asperellum, B. subtilis* and hot water seed treatments on rice blast disease. Results indicated significant reduction ($P \geq 0.05$) of the percentage of infected rice seeds when *T. asperellum*, *B. subtilis* and hot water treatment were used. Rice blast disease incidence and severity were significantly reduced ($P \geq 0.05$) on rice seeds treated with *B. subtilis.* Therefore, the use of microbial agents has the potential for effective management of rice blast disease. Field experiments were conducted twice in three locations to determine the effect of rice blast disease on grain yield of upland rice genotypes. A randomized complete block design experiment (RCBD) was used in a paired block with *P. oryzae* naturally inoculated and fungicide sprayed blocks. Results showed that the effect of rice blast disease on grain yield of upland rice genotypes depended on disease pressure, which differed across rice genotypes, locations and rice growing seasons. In general, the disease caused 26.6 to 52.1% grain yield reduction in the two rice growing seasons. Improved rice genotypes such as NERICA 7 and WAB 450 were also found susceptible to rice blast disease in the study areas**.** In general, this study, gives highlights of the incidence and severity of rice blast disease, its management using bio - agents (*T. asperellum* and *B. subtilis*) and the effects of the disease on grain yield of selected upland rice genotypes grown under rain-fed conditions in Morogoro and Tanga regions. Such information is important in designing rice blast disease management options**.**

DECLARATION

I, **IBRAHIM HASHIM,** do hereby declare to the Senate of the Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This work is dedicated to my late grandmother, Halima Maghera for her endless love to me.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

<	Less than
≤	Less than or equal
ANOVA	Analysis of variance
CRD	Completely Randomized Design
FAO	Food and Agriculture Organization
h	Hour
ha	Hector
HSD	Honestly Significant Different
ICIPE	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
IRRI	International Rice Research Institute
NERICA	New Rice for Africa
PDA	Potato dextrose agar
PIRG	Percentage Inhibition of Radial Growth
RBDI	Rice blast disease incidence
RBDS	Rice blast disease severity
RCBD	Randomized Complete Block Design
SSA	Sub Saharan Africa
SUA	Sokoine University of Agriculture
t	Tons
TARI	Tanzania Agricultural Research Institute
TMA	Tanzania Meteorological Agency
USA	United States of America
USAID	United States Agency for International Development
WP	Wettable Powder

ORGANIZATION OF THE THESIS

This thesis is developed in publishable manuscripts format consisting of seven chapters. Chapter one is a general introduction and justification, chapter two, three, four, five and six consisted of manuscripts in the form of publishable papers. Chapter seven is the general conclusions and recommendations.

Parts of chapter two, four and five have been published as follows:

Paper I

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Paper II

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DOI: 10.5829/idosi.wjas.2018.108.117

Paper III

Hashim, I., Mamiro, D. P, Mabagala, R. B. and Tefera, T. (2019). Reduction of occurrence of rice blast (*Pyricularia oryzae*) inocula on rice seeds by microbial and hot water seed treatments. *Australian Journal of Crop Science* 13 (02): 309 - 314.

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CHAPTER ONE

1.0 General Introduction

Rice (*Oryza sativa* L.) is an important food crop for more than 50% of the world population (FAO, 2017). It is predominantly a food crop in many African and Asian countries. Although per capita consumption of rice in parts of Asia is declining, in Sub-Saharan Africa (SSA) the demand for rice has been increasing considerably in the last two decades (Mohanty, 2013). In Africa, rice production has been increasing in most countries with the highest production recorded in Madagascar followed by Benin and Tanzania (FAOSTAT, 2016). When comparing the increasing population in these countries, the production trends are not as impressive, forcing countries to be increasingly dependent on rice imports, driven by growing production-to-consumption gaps (Nasrin *et al.*, 2015).

In Tanzania rice ranks second in most widely cultivated and consumed staple crop after maize (*Zea mays* L.) and a source of cash in areas where it is grown (Mghase *et al.*, 2010). The importance of rice as a staple food crop has increased in the past four decades. Back in the 1960s, rice was regarded as a luxury food, but currently, it is consumed by more than 60% of the population mostly in the urban areas (Kanyeka *et al.*, 1994). Several factors such as an increase in total urban populations, Rural to urban movements and changes in eating habit of traditional foods in favour of rice contributed to increase in rice consumption (Kibanda and Luzi-Kihupi, 2007). Therefore, this need different interventions to increase rice productivity to fill the gap in rice demand caused by the increasing population. The possibilities of further growth in the rice sector will depend largely on farmers access to new sustainable farm technologies such as disease and insect pest management to enhance rice production to contribute to an improvement of food security (Nasrin *et al.*, 2015).

1.1 Rice Ecosystems in Tanzania

Rice can be grown in a wide range of different ecosystems. In Tanzania, rice ecosystems are categorized into two major categories namely upland and lowland rice ecosystems (Kanyeka *et al.*, 1994). Lowland rice ecosystem is further divided into sub-groups as follows below.

1.1.1 Lowland rice ecosystem

In the lowland rice ecosystem, rice is grown in fields which are continuously flooded, except for occasional drainage (Mtwaenzi, 2004). In Tanzania, most of the rice is cultivated under lowland rice ecosystem. It is made up of about 80% of the total area under rice cultivation (Kanyeka *et al.*, 1994). Rice productivity in lowland rice is variable due to major challenges such as difficult in water control (both drought and flood), diseases, weed management and low soil fertility (Wilson and Lewis, 2015).

Lowland rice ecosystem is divided into two sub-groups namely, rain fed lowland rice (deep flooded and shallow flooded) and irrigated lowland rice. Rain fed lowland rice is grown on around 65 million hectares, equivalent to about 74% of the total national rice area. Irrigated lowland rice is indirectly dependent on rain and sometimes rice can be grown twice per year due to the availability of water for irrigation. Irrigation of rice is practised on 5 million hectares, equivalent to 6% of the national rice area (Wilson and Lewis, 2015).

1.1.2 Upland rice ecosystem

Upland rice is grown on dry land under rain fed conditions. It accounts for about 20% of the total rice production land, which is equivalent to 17 million hectares (Kanyeka *et al.*, 1994; Wilson and Lewis, 2015). The areas growing upland rice have two major seasons also known as bimodal rainfall pattern. The short rain is known as “Vuli” start in the mid of October to December and long heavy rains known as “Masika” start from March to May. These areas experience high temperature ranging from 28°C and above from July to September, with a moderate to cool period ranging from 18°C to 22°C in October to June (Mghase *et al.*, 2010).

The soils composition ranges from sandy loam to clay soil with a pH range of 4.7 to 6.5 and annual rainfall ranging between 1062 and 2925 mm (Mghase *et al.*, 2010). Rice yield in these areas are low, ranging from 0.4 - 0.5 t/ha and the grain quality is also poor due to diseases, water stress, low soil fertility and acidity and other biotic stresses such as insect pests, weeds and birds (Wilson and Lewis, 2015).

1.2 Rice Production Constraints in Tanzania

Several abiotic factors such as drought, low soil fertility, improper agronomic practices, lack of access to input and credit and lack of knowledge and biotic factors such as diseases, insect pest, birds and weeds are the most important constraints to rice production (Hashim *et al.*, 2018b; January *et al.*, 2018). Currently, diseases such as rice blast disease, *Rice Yellow Mottle Virus*, rice brown spot disease and bacterial leaf blight are the major diseases of rice in Tanzania (Hubert *et al.*, 2014; Hashim *et al.*, 2018b). Among these diseases; Chuwa *et al.* (2015) reported that rice blast disease alone causes about 38% yield losses in Tanzania.

1.3 Rice Blast Disease, Occurrences and Distribution

Rice blast disease is a fungal disease caused by *Pyricularia oryzae* Cav. which is in the Kingdom Fungi, Division Ascomycota and the Genus *Pyricularia* (Rossman *et al.*, 1990). It is found everywhere in the world where rice is grown (Kato, 2001; Wang *et al.*, 2014).

The report by Ou *et al.* (1971) indicated that in 1637 rice blast disease was known as rice fever disease in China and then it was reported in Japan in 1704, Italy 1828, USA 1876 and India in 1913. Rice blast disease is currently found in approximately 85 countries all over the world (Figure 1.1); the disease is economically important in temperate, tropical, subtropical Asia, Latin America and Africa (Pooja and Katoch, 2014). In Tanzania, it is not known when rice blast disease entered the country. However, many authors (Chuwa *et al.*, 2015; Hubert *et al.*, 2015; Balimponya 2015; Hashim *et al.* 2018b) reported occurrence of rice blast disease in low land and upland rice in major rice-growing areas of Tanzania.

Figure 1.1: Worldwide distribution of rice blast disease. Red dots show the countries or regions where the disease has been reported. Source: (Wang *et al.*, 2014)

1.3.1 Biology of genus *Pyricularia* and its pathogenicity

The causal agent of rice blast disease is known and described as *Pyricularia oryzae* Cavara based on the restriction of its host range to the genus *Oryza* (Rossman *et al*., 1990). The genus *Pyricularia* is named after the pyriform (pear-shaped) shape of its conidia (Klaubauf *et al*., 2014). *Pyricularia* conidia are characterized by being solitary, pyriform to obclavate, narrowed toward tip, rounded at the base, 2-septa, hyaline to pale brown, with a distinct basal hilum, sometimes with marginal frill (Figure 1.2).

Pyricularia grisea is another specie of the genus *Pyricularia* from several grasses and non-grasses hosts (Rossman *et al*., 1990). Initially, *P. grisea* was considered morphologically distinct from *Pyricularia oryzae*. However, when strain from various hosts crossed; they were able to form sexual state which suggested that these taxa were genetically the same. The sexual state (teleomorph) of *P. grisea* was initially described to the genus *Magnaporthe* by Barr (1977). Later on, Yaegashi and Udagawa (1978) reported sexual state of *Pyricularia* isolates from crosses of several graminaceous hosts including *Oryza*. The teleomorph of *P. grisea* was placed in *Magnaporthe* as *Magnaporthe grisea* (Hebert) Barr.

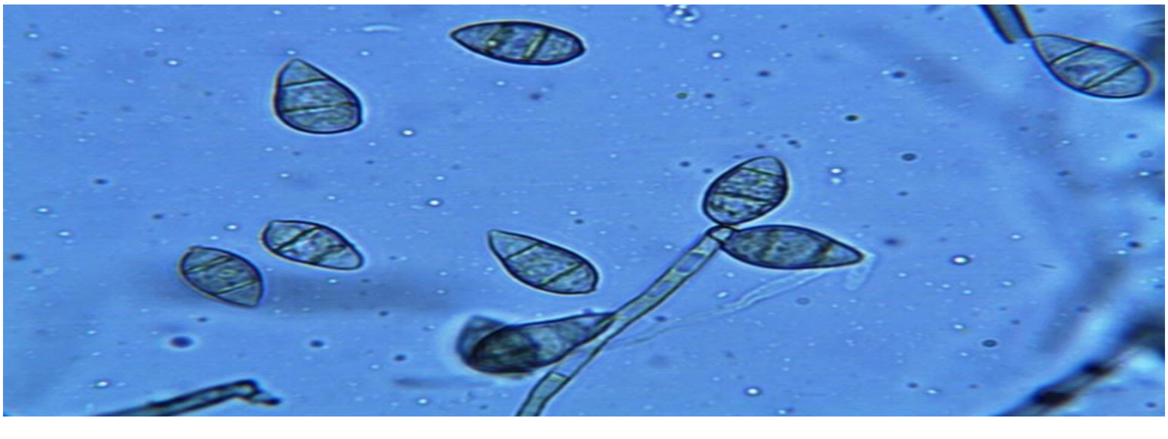

Figure 1.2: Pyriform conidia of the rice blast fungus (*Pyricularia oryzae*)

(Source: Groth, 2009)

The pathogenicity of the genus *Pyricularia* species differs on wide range of a monocot plants. *Pyricularia oryzae* isolates from rice are host specific to rice and other few plants such as barley and *Lolium* (Hajime *et al*., 2000; Couch *et al*., 2005). *Pyricularia oryzae* isolates from *Eleusine*, *Setaria* and *Triticum* are host-specific to respective plants, and are unable to infect rice (Hajime *et al*., 2000; Couch *et al*., 2005). *Pyricularia grisea* isolates from crabgrass are specific to crabgrass (*Digitaria*) and are unable to infect other hosts (Hajime *et al*., 2000).

1.3.2 Symptoms of rice blast disease

The pathogen infects all the above-ground parts of rice plants including the leaf, collar, nodes, internodes base or neck and other parts of the panicle at all growth stages in the nursery and under field conditions (Pooja and Katoch, 2014). The initial symptoms of rice blast appear as white to grey-green lesions or spots, with dark green borders. On the leaves, rice blast lesions are elliptical or spindle-shaped with whitish to grey centres and red to brownish or necrotic borders (Webster, 2000; IRRI, 2016). Mature lesions appear cottony in the centre with a dark bluish surface due to the production of conidia (Figure 1.2 A).

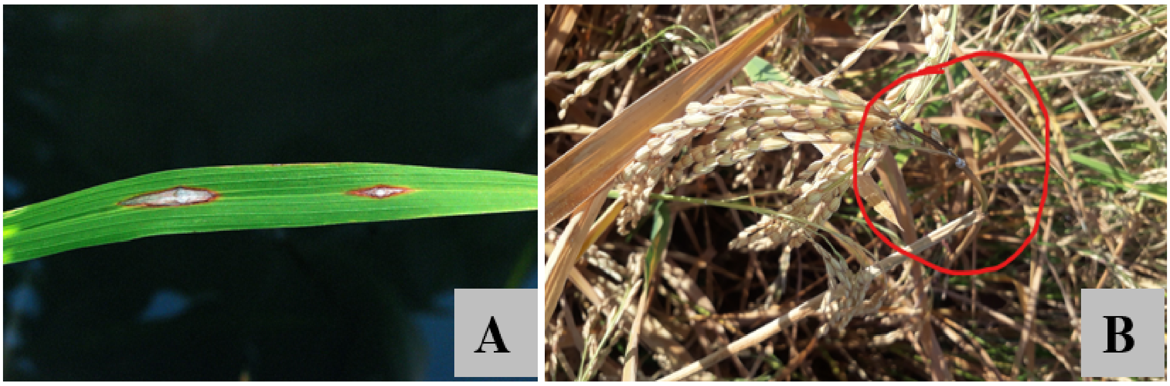

Figure 1.3: Spindle-shaped rice blast disease lesion with whitish to grey centre on rice leaf (A) and lesion on the neck (B) (Source: Ibrahim Hashim 2018)

The size of the blast lesion is commonly 1-1.5 cm long and 0.3-0.5 cm wide. Under favourable conditions, such as temperature (22°C - 27°C), relative humidity (89%) and extended leaf wetness, lesions can coalesce and kill the entire leaf (Webster, 2000; Kato, 2001). In collar areas, rice blast lesions are located at the junction of the leaf blade and leaf sheath and it can also kill the leaf (Bhatt and Singh, 1992).

Infection of the neck node by *P. oryzae* produces triangular purplish lesions, followed by lesion elongation on both sides of the neck node; and such symptoms have a very serious effect on grain development (Bonman, 1992). In the infected young neck nodes, the panicles become white in colour or white head, which are sometimes mistaken as insect damage such as stem borers attack, which also results into white and dead panicle (Bonman, 1992). Infected panicles appear white and are partly or completely unfilled. Leaf blast disease is common during the vegetative stage of growth and neck blast disease occurs during the reproductive stage when the pathogen infects the neck nodes and panicles (Roumen *et al.*, 1992). Leaf blast lesions reduce the net photosynthetic area of individual leaves (Bastiaans, 1991). Zhu *et al.* (2005) reported that neck blast can occur without being preceded by severe leaf blast and it is the most destructive phase of the blast disease.

1.3.3 Rice blast disease development and infection process

Rice blast is a polycyclic disease proliferated by asexual spores (conidia) that infect all aerial parts of the rice plant in the field (Raveloson *et al.*, 2018). Under artificial inoculation in controlled conditions, the pathogen can colonize the roots of the rice plant (Sesma and Osbourn, 2004). The sources of inocula for rice blast disease are mycelia and conidia from infected rice straws and seeds (Webster, 2000). The pathogen can produce 20,000 spores in one lesion on leaves and 60,000 spores on one spikelet in one night

(Zeigler *et al.*, 1997). It survives over seasons in infected piled rice straws, weeds and rice seeds (Du *et al.*, 1997; Webster, 2000). The fungus can sporulate on infected rice stem left on the soil surface for up to 18 months (Raveloson *et al.*, 2018). Under field conditions such as long periods of plant surface wetness, high humidity, little or no wind at night, night temperatures between 12 – 32°C and the presence of infected rice residues could initiate an epidemic of rice blast blast disease (Kato, 2001; Lu *et al.*, 2007; Kim *et al.*, 2009; Raveloson *et al.*, 2018).

The dispersal of the fungus in the field has been reported to be through airborne spores (Webster, 2000). The conidia produced from these sources are carried out by air currents to the secondary hosts. In the canopy of rice plants, newly developed leaves act as receptors for the spores. The incidence of rice blast disease and its damage is highly influenced by environmental conditions such as temperature (22°C - 27°C), relative humidity (89%) and extended leaf wetness (Webster, 2000; Kato, 2001; Prasad *et al.*, 2015). The optimum temperature for *P. oryzae* mycelial growth ranges from 25 to 30˚C (Arunkumar and Singh, 1995). The temperature range that causes the death of the pathogen is between 51 – 52°C (Yang *et al.*, 2011). The genetic variability of the rice blast fungus is an important factor affecting the severity of the disease (Yang *et al.*, 2011).

1.3.4 The host range for *Pyricularia oryzae*

The pathogen infects a broad range of grass species, including wheat, barley and millet and it is very successful due to survival on several alternative hosts such as *Rottboellia cochinchinensis* Lour., *Eleusine indica* L., *Panicum repens* L., *Digitaria marginata* Link., *D. sanguinalis* L., *Brachiaria mutica* Forssk., *Leersia hexandra* Sw., *Dinebra retroflexa* Vahl., *Echinochloa crusgalli* L., *Setaria intermedia* Roem and Schult., *S. viridis* L., *S. faberi* Herrm. and *Stenotaphrum secondatum* Walter. (Du *et al.*, 1997; Pooja and Katoch, 2014).

1.4 Rice Blast Disease Management

1.4.1 Chemical control of rice blast disease

In different areas of the world, rice growers have been applying fungicides as chemical control of rice blast disease (Magar *et al.*, 2015; Maji, 2015; Ghimire *et al.*, 2017). Several authors reported the effectiveness of fungicides to control rice blast disease (Ogoshi *et al.*, 2018). Studies by Ogoshi *et al.* (2018); Magar *et al.* (2015) and Ghimire *et al.* (2017) showed that Tricyclazole (Benzothiazole) is the most effective fungicide against rice blast disease based on increased grain yield. Other fungicides such as trifloxystrobin + tebuconazole, azoxystrobin and kresoxim-methyl have been reported to control blast disease and resulted in high grain yield (Chen *et al.*, 2015; Ogoshi *et al.*, 2018).

1.4.2 Biological control of rice blast disease using microbial agents

1.4.2.1 *Trichoderma* as biocontrol agent of rice blast disease

For many years, *Trichoderma* spp. has been used as an antagonistic biological control agent of many fungal plant diseases. This is due to their abilities to antagonize a wide range of phytopathogenic fungi, bacteria and oomycetes, through several mechanisms that are activated in *Trichoderma* by the pathogens. These mechanisms include competing for nutrients, space, by producing antibiotics as well as by inducing systemic resistance to plants. Also, *Trichoderma* sp. can stimulate plant growth and development by producing plant growth-promoting molecules (Awad, 2015). *Trichoderma* produces a wide range of lysing enzymes which degrade substrates and possess high resistance to microbial inhibitors (Strange, 1993).

Ecologically, the genus *Trichoderma* exists in many habitats, particularly in the soils as a natural habitat of the majority of microorganisms. The soil contains a rich source of several microorganisms from which *Trichoderma* as a saprophytic fungus, may obtain food and other chemical exudates to increase its effectiveness and sustain growth (Ali and Nadarajah, 2014).

The activity of *Trichoderma* spp. as antagonistic plant pathogens are highly influenced by weather conditions such as temperature, humidity and interactions with other microorganism communities (Ru and Di, 2012). Several *In vitro* and *In vivo* studies have indicated the inhibitory ability of *Trichoderma* against rice blast disease pathogen (Singh *et al.*, 2012; Ali and Nadarajah, 2014; Bhattacharjee and Dey, 2014; Krishna, 2016).

1.4.2.2 *Bacillus* as biocontrol agent of rice blast disease

Many species of *Bacillus* isolated from the rice phylloplane have shown an *in vitro* potential for rice blast disease control by inhibiting *P. oryzae* mycelia growth (de Oliveira Nascimento *et al.*, 2016). *Bacillus* spp. can suppress fungal disease by using several antagonistic mechanisms such as the production of antifungal and antibiotics like Iturina produced by *B. subtilis (*Araujo *et al.*, 2005; Velusamy and Gnanamanickan, 2008). Other mechanisms are hyper parasitism, predation and production of lytic enzymes, production of toxins, gene silencing, interference with the phenomenon of Quorum sensing, siderophore and hydrocyanic acid (Romeiro *et al.*, 2010). In dual inoculations, *B. subtilis* caused over 75% inhibition of mycelia radial growth of *P. oryzae* and foliar application under screen house conditions, *B. subtilis* was effective in reducing rice blast disease severity by 29% (Hashim *et al.*, 2018a).

1.4.3 Cultural methods for control of rice blast disease

The excessive use of nitrogen fertilizer promotes excessive vegetative crop growth, which increases the relative humidity and leaf wetness on the crop canopy that favours blast disease (Saifulla and Maharudrappa, 1992). Report by Lu *et al.* (2011) showed that rice blast disease intensity has been decreasing by split applications of nitrogen fertilizer based on recommended crop requirements. Field drainage or shallow water for extended periods is a favourable condition for rice blast disease development as it allows the formation of nitrate which may cause drought stress hence increase the susceptibility of rice plants to blast disease (Pooja and Katoch, 2014). Continuous flooding has been recommended to limit rice blast disease development.

Planting time has been reported to affect rice blast disease development within the crop. In upland conditions, rice sown early during the rainy season has a higher chance of escaping blast infection than later-sown crops, which are often affected by inocula produced on neighbouring farms (Prabhu and Morais, 1986). Diseased rice straws and stubble are one of the rice blast inocula sources; burning or composting them can be one of the ways to avoid *P. oryzae* inocula from the previous season (Webster, 2000).

When rice seeds from previously infected panicles are used for planting, infected seeds act as the primary source of inocula resulting in poor germination and abnormal seedlings (Imolehin, 1983; Webster, 2000; Long *et al.*, 2001; Faivre-Rampant *et al.*, 2013). Rice blast disease resulting from infested and infected rice seeds can be avoided by seed treatment using chemical fungicides, hot water and microbial agents (Jensen *et al.*, 2000; Nega *et al.*, 2003; Koch and Roberts, 2014).

1.4.4 Genetic resistance to rice blast disease

In any pathosystem, resistance is defined as the ability of the host to hinder the growth and/or development of the pathogen (Robinson, 1969). Rice blast resistance has been reported to be of two types; complete resistance which involves total prevention of multiplication of the pathogen in the host and partial resistance in which the pathogen has reduced rate of multiplication and slow rate of expression of disease symptoms (Mukherjee *et al.*, 2018).

The effective management of rice blast disease includes the use of resistant cultivars, which are cost-effective and environmentally safe (Sharma *et al.*, 2012). However, rice blast resistance was reported to be broken down in three to five years after its release (Ogoshi *et al.*, 2018). The loss of resistance is mainly due to the high degree of pathogenic variation exhibited by *P. oryzae* and occurrence of a selection of new virulent races that overcome the resistance (Zeigler *et al.*, 1997; Zhou *et al.*, 2007). This calls for a search of other blast disease management options that are effective and sustainable. In Tanzania, most rice varieties which are grown by farmers have different levels of susceptibility to rice blast disease depending on their genetic makeup (Chuwa *et al.*, 2015).

1.5 Problem statement

The average rice yield (2.1 to 3.4 t/ha) in Tanzania is low compared to the potential yield (4 to 5 t/ha) (Lwezaura *et al.*, 2011). Several abiotic and biotic factors have been reported to reduce rice yield in Tanzania. Rice blast disease is among the most important biotic factors reducing rice yield (Chuwa *et al.*, 2015; Hubert *et al.*, 2015). In the absence of control measures and where susceptible cultivars are grown, the disease cause yield losses ranging from 60 to 100% (Aravindan *et al.*, 2016). Globally, it is estimated to cause about 30% yield losses (Nalley *et al.*, 2016). In Kenya, losses of about 60% (Kihoro *et al.*, 2013) and in Tanzania, 38% have been reported (Chuwa *et al.*, 2015). With such losses, in Tanzania, the average yield of 2.1 t/ha in the farmer’s field may be reduced to 0.8 t/ha.

Considering the increasing importance of rice as a staple food and cash crop, such losses have a significant effect on food security.

Although rice blast disease occurs in Tanzania, information on farmers' knowledge of rice blast disease and management options used by farmers is scant. Currently, it is not known whether farmers know the cause of rice blast disease and how it spreads. Previous reports show that rice blast disease can be controlled by the use of resistant varieties, application of fungicides and cultural practices (Ali and Nadarajah, 2014; Suprapta *et al.*, 2014; Faruq *et al.*, 2015). However, the longevity of resistance of many resistant genotypes is shortened by the appearance of *P. oryzae* new races (Ali and Nadarajah, 2014), whereby within 2 to 3 years the genotype must be changed (Agrios, 2005). Fungicides have been reported as effective control measures of rice blast when applied as sprays and seed treatments (Agrios, 2005; Faruq *et al.*, 2015). However, frequent use and miss-use of fungicides result into harmful effects on the environment, human health and the pathogen may develop resistance (Ali and Nadarajah, 2014; Faruq *et al.*, 2015). The use of combinations of resistant genotypes and environmentally friendly strategies such as microbial agents are adequate and effective ways of rice blast disease management.

Several studies have been conducted in lowland rice and reports show that most of the lowland rice varieties grown by farmers in Tanzania are susceptible to rice blast disease (Chuwa *et al.*, 2015; Hubert *et al.*, 2015). However, rice blast disease incidence, severity and its effect on upland rice genotypes growing in Tanzania have never been established.

1.6 Justification

There is a need to investigate farmers' knowledge of rice blast disease and management options used by farmers. Such information is important in designing appropriate control options that meet farmers' need. Bio-agents such as *Trichoderma harzianum, T. viride* and *Bacillus subtilis* have been reported to reduce rice blast disease incidence by 70% in India

(Jayaraj *et al.*, 2004; Singh *et al.*, 2012). In Kenya and Tanzania, *T. asperellum* and *B. subtilis* are used to control soil-borne and foliar diseases of ornamental plants and vegetables (Mwangi *et al.*, 2011; Kipngeno *et al.*, 2015). Currently, the use of *T. asperellum* and *B. subtilis* for controlling rice blast disease in East Africa has not yet been reported. The two bioagents may be used in combination with resistant varieties to reduce the excessive use of synthetic fungicides. Hence, there is a need to investigate the efficacy of *T. asperellum* and *B. subtilis* against *P. oryzae.*

Morogoro and Tanga regions have suitable areas for upland rice production (Mghase *et al.*, 2010). In these areas, the weather condition is favourable for outbreak and development of rice blast disease. Furthermore, farmers have been reusing their own local upland rice varieties for a long time. The establishment of rice blast disease incidence and severity and its effect on upland rice genotypes grown in Tanzania is thus needed to help farmers set disease management priorities.

Therefore, this study addressed the farmers' knowledge and management of rice blast disease, its incidence, severity and effect on yield of upland rice genotypes grown in Tanzania as a base for future rice blast disease management.

1.7 Research Questions

i. Is rice blast disease present in upland rice ecosystem and at what magnitude?
ii. Do farmers know what is the cause of rice blast disease, how it spreads and how it is controlled?
iii. Could *Trichoderma asperellum* and *Bacillus subtilis* be used to control rice blast disease in Tanzania?
iv. Could *Trichoderma asperellum*, *Bacillus subtilis* and hot water seed treatment be used to reduce inocula of rice blast disease on rice seeds?

v. Does rice blast disease cause reduction of grain yield on upland rice genotypes growing in Tanzania?

1.8 Hypotheses

i. Rice blast disease does not occur in the upland rice ecosystem in Tanzania.

ii. Farmers do not know what causes rice blast disease, how it spreads and how it is controlled.

iii. There are no significant differences in the effect of *Trichoderma asperellum*, *Bacillus subtilis*, Linkimil 72WP and no spray on rice blast disease control.

iv. There are no significant differences in the efficacy of *Trichoderma asperellum*, *Bacillus subtilis*, hot water and Apron star® seed treatments on the reduction of inocula of rice blast disease on rice seeds.

v. Rice blast disease does not reduce grain yield of upland rice genotypes growing in Tanzania.

1.9 Overall Objective

The overall objective of this study was to increase rice productivity through effective rice blast disease management in Tanzania.

1.9.1 Specific objectives

The specific objectives of this study were to:

i. Investigate farmers' knowledge and management practices for rice blast disease.

ii. Establish the incidence and severity of rice blast disease in upland rice ecosystem

iii. Evaluate the effect of microbial agents (*Trichoderma asperellum* and *Bacillus subtilis*) on the management of rice blast disease.

iv. Evaluate the efficacy of selected seed treatment methods on rice blast disease.

v. Investigate the effect of rice blast disease incidence and severity on the yield of upland rice genotypes grown in Tanzania.

References

Agrios, G. N. (2005). Introduction to plant pathology. *Elsevier Academic Press Publication* 952 pp.

Ali, H. and Nadarajah, K. (2014). Evaluating the efficacy of *Trichoderma* spp and *Bacillus substilis* as biocontrol agents against *Magnaporthe grise*a in rice. *Australian Journal of Crop Science* 8 (9): 1324 - 1335.

Araujo, F. F., Henning, A.A. and Hungria, M. (2005). Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and soybean root development. *World Journal of Microbiology and Biotechnology* 21 (8-9): 1639 - 1645.

Aravindan, S., Yadav, M. K. and Sharma, P. (2016). Biological control of rice blast disease with *Trichoderma* spp. under upland rice system. *ORYZA-An International Journal on Rice* 53 (2): 167-173.

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.

Awad, H., M., Hamed, E. R., Ghazi, E. A., El-Gamal, N. G. and Shehata, H. S. (2015). *Trichoderma asperellum* isolated from salinity soil using rice straw waste as a biocontrol agent for cowpea plant pathogens. *Journal of Applied Pharmaceutical Science* 5 (2): 91 – 98.

Balimponya, E. (2015). Application of genomic selection and association mapping to breeding for resistance to rice blast and bacterial blight of rice (*Oryza sativa* L.), Dissertation for Award of Master of Science at Ohio State University, p. 122.

Bastiaans, L. (1991). The ratio between virtual and visual lesion size as a measure to describe a reduction in leaf photosynthesis of rice due to leaf blast. *Phytopathology* 81 (6): 611- 615.

Barr, M. E. (1977). *Magnaporthe*, *Telimenella*, and *Hyponectri*a (Physosporellaceae). *Mycologia* 69: 952 - 966.

Bhatt, N. and Singh, R. (1992). Cobweb disease of *Agariucus bisporus*: incidence, losses and effective management. *Indian Journal of Mycology and Plant Pathology* 22 (2): 178 -181.

Bhattacharjee, R. and Dey, U. (2014). An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. *African Journal of Microbiology Research* 8 (17): 1749 -1762.

Bonman, J. (1992). Durable resistance to rice blast disease environmental influences, breeding for disease resistance. *Springer* 63 (1 - 2): 115 -123.

Chen, Y., Yang, X., Yuan, S.K., Li, Y. F., Zhang, A. F., Yao, J. and Gao, T. C. (2015). Effect of azoxystrobin and kresoxim-methyl on rice blast and rice grain yield in China. *Annals of Applied Biology* 166 (3): 434 - 443.

Chuwa, C., Mabagala, R. and Reuben, M. S. O. W. (2015). Assessment of grain yield losses caused by rice blast disease in major rice-growing areas in Tanzania. *International Journal of Science and Research* 4 (10): 2211- 2218.

de Oliveira N. I., Rodrigues, A. A. C., Moraes, F. H., de Sousa, F., Arruda, A., Corsi, M. C. F. and de Moraes Catarino, A. (2016). Isolation, identification and *In vitro* evaluation of *Bacillus* spp. in control of *Magnaporthe oryzae* comparing evaluation methods. *African Journal of Agricultural Research* 11 (19): 1743 -1749.

Du, X. F., Sun, S.Y., Tao, R. X., Sun, G. C, Zhang, Z. M. and Zheng, Z. (1997). Effects of weed-hosts of *Pyricularia* on the incidence of rice blast diseases. *Acta Phytopathologica Sinica* 27 (4): 327 – 332.

Faivre-Rampant, O., Genies, L., Piffaneli, P. and Thareau, D. (2013). Transmission of rice blast from seeds to adult plants in a non-systemic way. *Plant Pathology* 62: 879 – 887.

FAO (2017). Rice market monitor. Food and Agriculture Organization of the United Nation, 39 p.

FAOSTAT. (2016). Food and Agriculture Organization of the United Nations. Accessed on 01/03/ 2018.

Faruq, A., Amin, M., Islam, M., Islam, M. and Alam, M. (2015). Evaluation of some selected seed treatments against leaf blast, brown spot and narrow brown leaf spot diseases of hybrid rice. *Advance in Agriculture and Biology* 4 (1): 8 - 15.

Ghimire, P., Gopal, K. C., Shrestha, S.M. and Parajuli, G. (2017). Evaluation of fungicides and bio-agent against neck blast disease of rice. *Journal of Plant Pathology and Microbiology* 8 (12): 1 - 2.

Groth, D. (2009). Spores of the rice blast fungus (*Pyricularia oryzae*). https://species. wikimedia.org/ wiki/*Pyricularia oryzae*. Accessed on 08/04/2020

Hajime, K.A., Yamamoto, M., Yamaguchi-Ozaki, T., Kadouchi, H., Iwamoto, Y., Nakayashiki, H., and Naoki, M. O. I. (2000). Pathogenicity, mating ability and DNA restriction fragment length polymorphisms of *Pyricularia* populations isolated from Gramineae, Bambusideae and Zingiberaceae plants. *Journal of General Plant Pathology* 66 (1): 30 - 47.

Hashim, I., Mamiro, D. P, Mabagala, R. B. and Tefera, T. (2018a). *In vitro* and *In vivo* evaluation of microbial agents for the management of rice blast disease in Tanzania. *World Journal of Agricultural Sciences* 14 (4): 108 - 117.

Hashim, I., Mamiro, D. P., Mabagala, R. B. and Tefera, T. (2018b). Smallholder farmers' knowledge, perception and management of rice blast disease in upland rice production in Tanzania. *Journal of Agricultural Science* 10 (7):137 - 145.

Hubert, J., Luzi-Kihupi, A. L., Hébrard, E. and Lyimo, H. J. F. (2014). Farmers' knowledge and perception of *Rice Yellow Mottle Virus* in selected rice growing areas in Tanzania. *International Journal of Science and Research* 5: 549 - 559.

Hubert, J., Mabagala, R. B. and Mamiro, D. P. (2015). Efficacy of selected plant extracts against *Pyricularia grisea*, the causal agent of rice blast disease. *American Journal of Plant Sciences* 6: 602 - 611.

Imolehin, E. (1983). Rice seed-borne fungi and their effect on seed germination. *Plant Disease* 67 (12): 1334 -1336.

IRRI. (2016). *Magnaporthe oryzae: Rice Blast*. International Rice Research Institute www.irri.org. Accessed on 10/06/ 2016.

January, B., Rwegasira, G. M. and Tefera, T. (2018). Farmers' perceptions of rice production constraints and stem borers management practices in Tanzania. *Journal of Agricultural Science* 10 (6): 57 - 71.

Jayaraj, J., Yi, H., Liang, G., Muthukrishnan, S. and Velazhahan, R. (2004). Foliar application of *Bacillus subtilis* AUBS1 reduces sheath blight and triggers defence mechanisms in rice. *Journal of Plant Diseases and Protection* 111 (2): 115-125.

Jensen, B., Knudsen, I. M. and Jensen, D. F. (2000). Biological seed treatment of cereals with fresh and long-term stored formulations of *Clonostachys rosea*: biocontrol efficacy against *Fusarium culmorum*. *European Journal of Plant Pathology* 106: 233 – 242.

Kanyeka, Z., Msomba, S., Kihupi, A. and Penza, M. (1994). Rice ecosystems in Tanzania: characterization and classification. *Tanzania Agricultural Research and Training Newsletter* 9 (1-2): 13 -15.

Kato, H. (2001). Rice blast disease. *Pesticide Outlook* 12 (1): 23 - 25.

Kato, H. and Yamaguchi, T. (1982). The perfect state of *Pyricularia oryzae* Cav. from rice plants in culture. *Annals of the Phytopathological Society of Japan* 48: 607-612.

Kibanda, J. N. and Luzi-Kihupi, A. (2007). Influence of genetic and genotype x environment interaction on the quality of rice grain. *African Crop Science Journal* 15 (4): 173 - 182.

Kihoro, J., Bosco, N. J., Murage, H., Ateka, E. and Makihara, D. (2013). Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. *SpringerPlus* 2 (1): 308 - 318.

Kim, S., Park, S.-Y., Kim, K. S., Rho, H. S., Chi, M. H., Choi, J., Park, J., Kong, S., Park, J. and Goh, J. (2009). Homeobox transcription factors are required for conidiation and appressorium development in the rice blast fungus *Magnaporthe oryzae*. *PLoS genetics* 5 (12): e1000757.

Kipngeno, P., Losenge, T., Maina, N., Kahangi, E. and Juma, P. (2015). Efficacy of *Bacillus subtilis* and *Trichoderma asperellum* against *Pythium aphanidermatum* in tomatoes. *Biological Control* 90: 92 - 95.

Klaubauf, S., Tharreau, D., Fournier, E., Groenewald, J. Z., Crous, P. W., De Vries, R. P., and Lebrun, M. H. (2014). Resolving the polyphyletic nature of *Pyricularia* (Pyriculariaceae). *Studies in Mycology* 79: 85 - 120.

Koch, E. and Roberts, S. J. (2014). Non-chemical seed treatment in the control of seed-borne pathogens, Global perspectives on the health of seeds and plant propagation material. Springer, Dordrecht, pp. 105 -123.

Krishna, K.P. (2016). *In vitro* and *in vivo* studies on the effect of *Trichoderma* species, to control the rice sheath blight disease *Rhizoctonia solani*. *Journal of Environmental Science, Computer Science and Engineering and Technology* 5 (1): 52 – 62.

Long, D., Correll, J., Lee, F. and TeBeest, D. (2001). Rice blast epidemics initiated by infested rice grain on the soil surface. *Plant Disease* 85 (6): 612 - 616.

Lu, J.-P., Feng, X.-X., Liu, X.-H., Lu, Q., Wang, H.-K. and Lin, F.-C. (2007). Mnh6, a nonhistone protein, is required for fungal development and pathogenicity of *Magnaporthe grisea*. *Fungal Genetics and Biology* 44 (9): 819 - 829.

Lu, Xu, Yang, Z. X., Xu, Y. B., He, X. H. and Zhu., Y. Y. (2011). Effects of different nitrogen fertilizer use level on yield component and grain yield in Yuanyang terrace. *Journal of Yunnan Agricultural University* 26 (3): 376 – 381.

Lwezaura, D., Madulu, R., Ndunguru, A., Paul, C. and Chalamila, B. (2011). *Regional Rice Centre of Excellence: Baseline survey report*. MAFC, Dar es Salaam, Tanzania. 38 pp.

Magar, P. B., Acharya, B. and Pandey, B. (2015). Use of chemical fungicides for the management of rice blast (*Pyricularia grisea*) disease at Jyotinagar, Chitwan, Nepal. *International Journal of Applied Sciences and Biotechnology* 3 (3): 474 - 478.

Maji, E.A. and Imolehn, E. D. (2015). Use of some fungicide in the management of infection levels of blast on rice varieties planted at different dates on yield. *East African Journal of Science and Technology* 5 (1): 9 - 20.

Mghase, J. J., Shiwachi, H., Nakasone, K. and Takahashi, H. (2010). Agronomic and socio-economic constraints to a high yield of upland rice in Tanzania. *African Journal of Agricultural Research* 5 (2): 150 -158.

Mohanty, S. (2013). Trends in global rice consumption. *Rice Today* 12 (1): 44 - 45.

Mtwaenzi, H. H. (2004). Evaluation of tillage methods and herbicide for the control of wild rice (*Oryza punctata* and *Oryza longistaminata*) in low land rice ecosystem, Thesis for Award of PhD at the Sokoine University of Agriculture, Morogoro, Tanzania, 222 pp.

Mukherjee, A., Mohapatra, N. and Nayak, P. (2018). Assessment of partial resistance to rice blast disease. *ORYZA-An International Journal on Rice* 55 (3): 363-382.

Mwangi, M. W., Monda, E. O., Okoth, S. A. and Jefwa, J. M. (2011). Inoculation of tomato seedlings with *Trichoderma harzianum* and arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings. *Brazilian Journal of Microbiology* 42 (2): 508 - 513.

Nalley, L., Tsiboe, F., Durand-Morat, A., Shew, A. and Thoma, G. (2016). Economic and environmental impact of rice blast pathogen (*Magnaporthe oryzae*) Alleviation in the United States. *PLoS One* 11 (12): e0167295.

Nasrin, S., Lodin, J. B., Jirström, M., Holmquist, B., Djurfeldt, A. A. and Djurfeld, G. (2015). Drivers of rice production: evidence from five Sub-Saharan African countries. *Agriculture and Food Security* 4 (12): 1-19.

Nega, E., Ulrich, R., Werner, S. and Jahn, M. (2003). Hot water treatment of vegetable seed an alternative seed treatment method to control seed-borne pathogens in organic farming. *Journal of Plant Diseases and Protection* 110 (3): 220 - 234.

Ogoshi, C., Carlos, F. S., Ulguim, A. R., Zanon, A. J., Bittencourt, C. R. C. and Almeida, R. D. (2018). Effectiveness of fungicides for rice blast control in lowland rice cropped in Brazil. *Tropical and Subtropical Agroecosystems* 21: 505- 511.

Ou, S., Nuque, F., Ebron, T. and Awoderu, V. (1971). A type of stable resistance to blast disease of rice. *Phytopathology* 61 (6): 703 – 706.

Pooja, K. and Katoch, A. (2014). Past, present and future of rice blast management. *Plant Science Today* 1 (3): 165 -173.

Prabhu, A. and Morais, O. (Ed.) (1986). Blast disease management in upland rice in Brazil. Progress in Upland Rice Research: Proceedings of the 1985 Jarkata Conference. *International Rice Research Institute*, *Jarkata*, 25 November 1986.

Prasad, R., Sharma, A. and Sehgal, S. (2015). Influence of weather parameters on the occurrence of rice blast in mid-hills of Himachal Pradesh. *Himachal Journal of Agricultural Research* 41 (2): 132 -136.

Raveloson, H., Ratsimiala Ramonta, I., Tharreau, D. and Sester, M. (2018). Long term survival of blast pathogen in infected rice residues as a major source of primary inoculum in high altitude upland ecology. *Plant Pathology* 67 (3): 610 - 618.

Robinson, R. A. (1969). Disease resistance terminology. *Applied Mycology* 48: 593 - 606.

Romeiro, R. S., Filho, R. L., Macagnan, D., Garcia, F. A. O. and Silva, H. S. A. (2010). Evidence that the biocontrol agent *Bacillus cereus* synthesizes protein than can elicit increased resistance of tomato leaves to *Corynespora cassiicola*. *Tropical Plant Pathology* 35 (1): 215 - 235.

Rossman, A.Y., Howard, R. J. and Valent, B. (1990). *Pyricularia grisea*, the correct name for the rice blast disease fungus. *Mycologia* 82 (4): 509 -512.

Roumen, E., Bonman, J. and Parlevliet, J. (1992). Leaf age-related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measured by the number of sporulating lesions. *Phytopathology* 82 (12): 1414 -1417.

Ru, Z. and Di, W. (2012). *Trichoderma* spp. from rhizosphere soil and their antagonism against *Fusarium sambucinum*. *African Journal of Biotechnology* 11 (18): 4180 - 4186.

Saifulla, M. and Maharudrappa, K. (1992). Effect of nitrogen levels on the incidence of blast and yield of rice varieties. *Agricultural Science Digest Karnal* 12 (4): 226 – 228.

Sesma, A. and Osbourn, A. E. (2004). The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature* 431: 582 - 586.

Sharma, T. R., Rai, A. K. and Gupta, S. K. (2012). Rice blast management through host-plant resistance: retrospect and prospect. *Agricultural Research* 1 (1): 37 – 52.

Singh, P., Singh, A., Singh, H. and Dhakad, B. (2012). Biological control of rice blast disease with *Trichoderma harzianum* in direct-seeded rice under medium low land rainfed conditions. *Environmental Ecology* 30 (3B): 834 - 837.

Strange, R.N. (1993). Implications of parasite identity, epidemiology and disease measurement for control measures, Plant Disease Control. Springer, pp. 107-135.

Suprapta, D. N., Quintao, V. and Khalimi, K. (2014). Effectiveness of rhizobacteria to reduce rice blast disease intensity. *Journal of Biology, Agriculture and Healthcare* 4 (3): 35 - 41.

Velusamy, P. and Gnanamanickan, S. S. (2008). The effect of bacterial secondary metabolite on fungal pathogens of rice. *Soil Biology* 14:93- 106.

Wang, X., Lee, S., Wang, J., Ma, J., Bianco, T. and Jia, Y. (2014). Current advances on genetic resistance to rice blast disease, Rice-Germplasm, Genetics and Improvement. IntechOpen Limited, London, pp. 195-217.

Webster, R. K. 2000. *Rice blast disease identification guide*. University of Calfornia, Davis. https://www2.ipm.ucanr.edu/agriculture/rice/Rice-Blast/. Accessed on 05 /08/ 2017.

Wilson, T. R. and Lewis, I. (2015). The rice value chain: A report from Southern Highlands Food Systems Programme. *Food and Agriculture Organization of the United Nations*. Rome, Italy. 111 pp.

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

Yang, H., Yang, X. H., Lu, C. M. and Wang, Y. Y. (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.

Zeigler, R. S., Scott, R. P., Leung, H., Bordeos, A. A., Kumar, J. and Nelson, R. J. (1997). Evidence of parasexual exchange of DNA in rice blast fungus challenges its exclusive clonality. *Phytopathology* 87: 284 – 294.

Zhou, E., Jia, Y., Singh, P., Correll, J. C. and Lee, F. N. (2007). Instability of the *Magnaporthe oryzae* avirulence gene AVR-Pita alters virulence. *Fungal Genetics and Biology* 44: 1024 –1034.

Zhu, Y.Y., Fang, H., Wang, Y.Y., Fan, J. X., Yang, S. S., Mew, T. W. and Mundt, C. C. (2005). Panicle blast and canopy moisture in rice cultivar mixtures. *Phytopathology* 95 (4): 433 - 438.

CHAPTER TWO

2.0 SMALLHOLDER FARMERS' KNOWLEDGE, PERCEPTION AND MANAGEMENT OF RICE BLAST DISEASE IN UPLAND RICE PRODUCTION IN TANZANIA

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Abstract

The objective of this paper was to investigate farmers' knowledge and management of rice blast disease in Tanzania. Farmers' household survey was conducted in five districts namely Mvomero, Morogoro Rural, Ulanga, Korogwe and Muheza in April and May 2017. Data were collected through face-to-face interviews using a semi-structured questionnaire and observations made through transect walks across selected villages. Farmers observed symptoms of rice blast disease for the first time in the past 3 to 10 years, with higher severity of blast disease in April to May each year. About 46.3% of the respondents were not aware of the cause and spread of rice blast disease. About 39.9% of the respondents associated rice blast disease with drought, high rainfall and temperature (8.7%) and soil fertility problems (5.1%). About 18.7% of the farmers reported burning of crop residues, 17.0% use of ash, 4.0% use of nitrogen fertilizer and 6.3% application of fungicide for management of rice blast disease. The majority of farmers (54.0%) did not apply any management method. Most farmers planted local upland rice varieties, with only 7.7% using improved varieties. About 69.6% of the respondents shared information on disease management among themselves. Lack of knowledge, ability to afford and unavailability of effective blast disease control methods were reported to affect the management of the disease. This study indicated that rice blast disease remains as the main constraint to rice production in the study area. Strengthening the capacity of farmers to identify the disease and proper management practices will sustainably solve the management problems of rice blast disease in upland rice production.

Keywords: farmers’ knowledge, *Oryza sativa*, *Pyricularia oryzae*, rice blast, Tanzania

2.1 Introduction

Rice (*Oryza sativa* L.) is the second most widely cultivated and consumed staple crop and cash grain after maize (*Zea mays* L.) (Mghase *et al.*, 2010). In Tanzania, the average yield production is 2.4 t/ha and 3.0 tons/ha for local and improved varieties, respectively (Lwezaura *et al.*, 2011). The rice yield in Tanzania is lower than that from other countries in Africa (4.4 tons/ha and 3.4 tons/ha from Madagascar and Benin), respectively, (FAOSTAT, 2016). Rice diseases, use of improper agronomic practices, drought, low yielding varieties, soil infertility and lack of knowledge on good agronomic practices by farmers have been reported to contribute to low grain yield (Lwezaura *et al.*, 2011; Chuwa *et al.*, 2015). Among these constraints, rice blast disease caused by *Pyricularia oryzae*

Cav. is an important disease that causes yield loss of 10 to 100% (Chuwa *et al.*, 2015; Velusamy, 2008; Hai *et al.*, 2007).

Persistence of the disease is attributed to lack of knowledge on how the pathogens are transmitted, their infection cycle and farmers perception on synthetic pesticides as the only option to control disease (Schreinemachers *et al.*, 2015). The majority of smallholder farmers do not adopt recommended disease management practices such as cultural and chemical methods due to the high cost of implementation or ineffectiveness of the methods (Schreinemachers *et al.*, 2015). Furthermore, it is common that during the development of technology, farmers' knowledge and perception are neglected (Roling and Fliert, 1994). The great success of farmers' involvement in the development of technologies has been reported (Adesina *et al.*, 1994; Roling and Fliert, 1994; Traoré *et al.*, 2015). Traoré *et al.* (2015) reported the role of integrating farmers' knowledge with modern technologies in disease management.

Despite the importance of farmers' knowledge on disease management in rice, there is scanty information of this knowledge in Tanzania. Assessment of rice production constraints, farmers' perceptions of rice blast disease and farm management practices is essential in designing appropriate control options that meet farmers' needs. An important component in achieving these objectives is an insight into farmers' knowledge of the disease and farm practices influencing the development of the disease. This study, reports on farmers' perceptions of rice production constraints, with reference to rice blast and farm management practices affecting the disease in upland rice in Tanzania.

2.2 Materials and Methods

2.2.1 Description of the study sites

The study was conducted in Morogoro and Tanga regions in Tanzania. These regions represent areas where upland rice production is constrained by rice blast disease (Figure 2.1). Mvomero, Morogoro Rural and Ulanga districts represented Morogoro region. These districts are located at 6°49′15″S and 37°39′40″E, 6°14′8.22″S and 38°41′37.49″E, and 9°00′00″S and 36°40′00″E, respectively, with an altitude of 500-1500 m above sea level. The sites are dominated by the soil of various types and characteristics due to variation in topography and ecological zones. In these areas, Oxisols dominated in the mountainous and hilly, and alluvial soil in the valley and low lands. Sandy and clay soil dominated in the woodlands and grassland areas. The area experienced a bi-modal rainfall (seasons) with long rains in March to May and short rains in November to January. Average annual rainfall is 800 to 1600 mm with a mean temperature ranging from 18°C in June to 26°C in October.

Tanga region was represented by Korogwe and Muheza districts. The districts are located at 5°00′00″S and 38°25′00″E (Korogwe) and 5°00′00″S and 38°55′00″E (Muheza) with an altitude of 200-1200 m above sea level. The area experiences warm weather with average temperatures of 24 - 28°C in May to October and 28 - 30°C in December to March. The area experiences two rain seasons, the long season from February to May and the short rains in October to December each year. Soil characteristics varied from low fertility with medium water holding capacity to medium fertility with high water holding capacity.

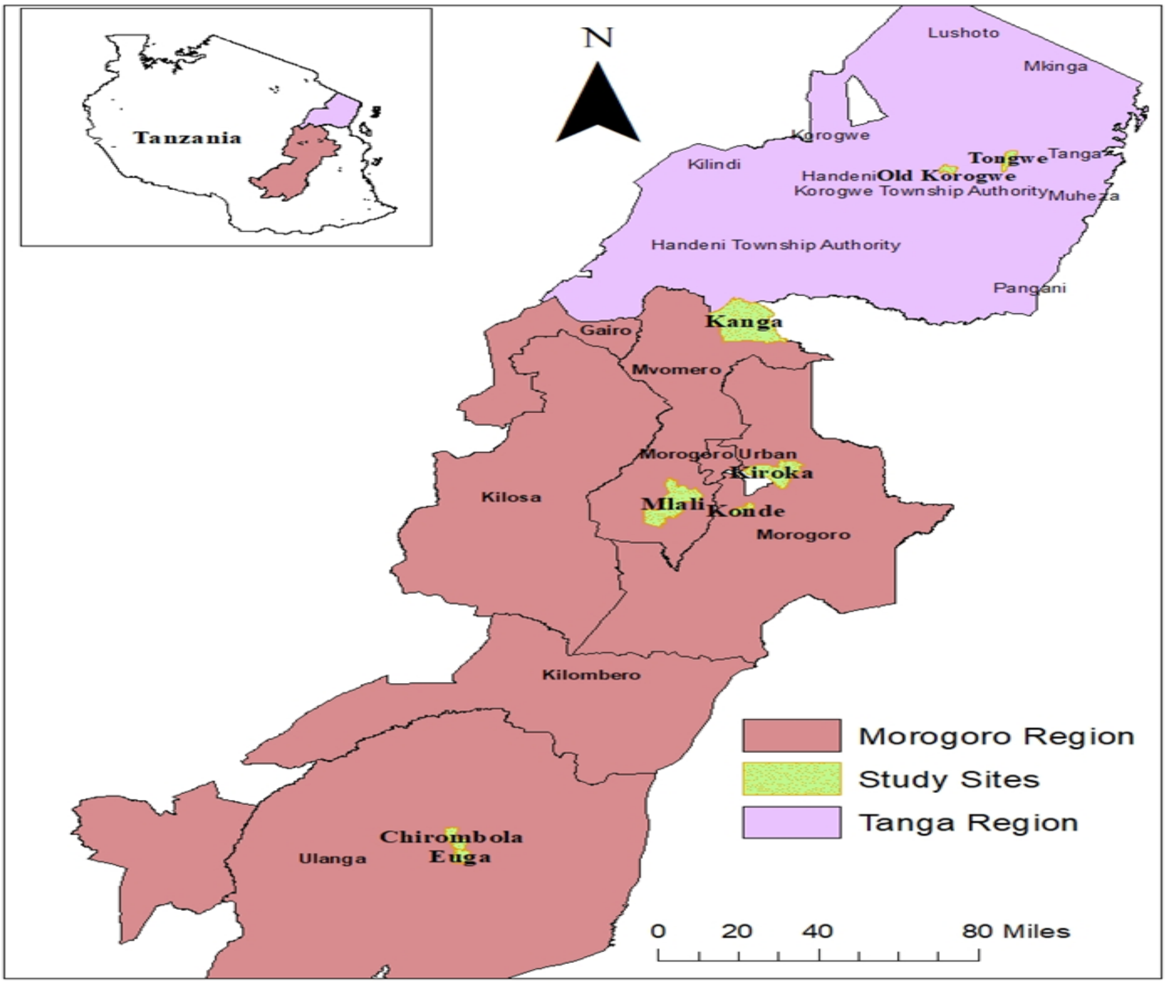

Figure 2.1: Map of Morogoro and Tanga regions showing the study areas (Source: Ibrahim Hashim, 2017)

2.2.2 Sample selection

Interviewed farmers were selected using a multi-stage random sampling procedure (Schreinemachers *et al.*, 2015). In Morogoro region, three districts (Mvomero, Ulanga and Morogoro Rural) and Tanga region two districts (Muheza and Korogwe) were selected. These districts were selected based on their long history of upland rice production. The district's administration was contacted to select villages where upland rice was widely grown. In each district, two villages were purposively selected, namely Kanga and Mlali (Mvomero), Diovuva and Rusiwa (Morogoro Rural), Chirombola and Euga (Ulanga), Old Korogwe and Lwengela (Korogwe) and Tongwe and Masimba (Muheza). The sample size (n = number of farmers to be interviewed) was determined using the formula suggested by Wonnacott and Wonnacott (1990) (as cited in Mghase *et al.*, 2010).

$$n = \frac{Z^2 p(1-p)}{Q^2} \quad \text{...} \quad \text{(i)}$$

Where, n = required sample size, Z = confidence level at 95% (standard value of 1.96), p = estimated proportion of an attribute (per cent of farmers in population), estimated at 90% and the Q = margin of error at 5% (standard value of 0.05). Therefore, the number of farmers interviewed was determined using the formula shown below:

$$n = \frac{Z^2 p(1-p)}{Q^2} = \frac{1.96^2(0.9)(1-0.9)}{(0.05)^2} = 138.29 = 138 \quad \text{..} \quad \text{(ii)}$$

About one hundred thirty-eight (138) farmers were selected from ten villages. Fifteen farmers per village were chosen in Mvomero, Morogoro Rural and Ulanga districts and 12 farmers per village in Korogwe and Muheza districts.

2.2.3 Data collection

Data were collected through face-to-face interviews and observations made through transect walks across selected villages. The semi-structured questionnaire (Appendix 1) was prepared based on factors related to farmers' preferences in rice production, production constraints, rice blast disease and control practices. To assess farmers' perception of rice blast disease, respondents were shown a series of coloured photographs of rice plants with rice blast disease symptoms (Schreinemachers *et al.*, 2015). Coloured photographs of rice plants with symptoms of rice brown spot and *Rice Yellow Mottle Virus* diseases were included in the list to avoid confusion in that these diseases have similar symptoms as rice blast disease. The data collected included farmers' socioeconomic profiles (*e.g.* age, gender and education), farm characteristics, knowledge and perceptions of the blast disease and their management practices.

2.2.4 Data analysis

Quantitative and qualitative data collected through the questionnaire were coded and subjected to statistical analyses using the Statistical Package for Social Sciences software (IBM SPSS Statistics version 21). Cross-tabulation tables were constructed and descriptive statistics were calculated to summarize data from the questionnaires. To make statistical inferences, contingency chi-square tests were computed at $P \leq 0.05$ levels of significance, to analyse the relationships between variables. This allowed empirical analyses and description of associations between the collected parameters across the three study districts.

2.3 Results

2.3.1 Description of households and their demographic characteristics

Among the respondent interviewed, about 58.5% were male and 41.5% female. Their ages ranged from 20 to 69 years. Significant differences ($\chi^2 = 26.301$; $P = 0.01$) were observed among respondents on the level of education. About 89.5% of the respondents completed primary education; however, 3.3% had secondary education (Table 2.1). The majority of the interviewed farmers have worked on rice production for 3 to 10 years; however, their experience of rice farming did not differ significantly ($\chi^2 = 9.51$; $P = 0.656$). Their average land unit devoted to rice production ranged from 1 to 2 ha (Table 2.1).

Significant differences ($\chi^2 = 6.301$; $P = 0.178$) were not detected among farmers concerning rice varieties they cultivated, however, the majority (92.3%) planted local upland rice varieties. Only 7.7% planted New Rice for Africa (NERICA) an introduced improved variety. The response of interviewed farmers on the sources of advice on rice production activities showed that 69.6% shared information among themselves, 17.4% received information from agriculture extension officers and 13.0% attended various training. However, the use of these sources of information across the districts did not differ significantly (χ^2 = 9.643; P = 0.291).

Table 2.1: Demographic characteristics of farmers from five rice-growing districts used in this study

Characteristics	Percentage of respondents					Mean	df	χ^2	P-value
	Mvomero (n = 30)	Morogoro Rural (n = 30)	Ulanga (n= 30)	Korogwe (n = 24)	Muheza (n = 24)				
Age of respondent (years)									
20-39	23.3	30.0	43.3	41.7	37.5	35.2			
40-59	70.0	46.7	53.3	29.2	20.8	44.0			
60-69	6.7	23.3	3.3	29.2	41.7	20.8			
Sex of the respondent									
Male	76.7	73.3	46.7	62.5	33.3	58.5	4	15	0.005
Female	23.3	26.7	53.3	37.5	66.7	41.5			
Education level									
None	0.0	10.0	0.0	0.0	12.5	4.5	12	26.3	0.01
Adult education	6.7	6.7	0.0	0.0	0.0	2.7			
Primary	93.3	83.3	100.0	95.8	75.0	89.5			
Secondary	0.0	0.0	0.0	4.2	12.5	3.3			
Size of the rice farm (acres)									
0.5	23.3	26.7	40.0	4.2	29.2	24.8			
0.75	0.0	0.0	20.0	0.0	20.8	8.1			
1	26.7	50.0	36.7	41.7	41.7	39.3			
2	33.3	23.3	3.3	37.5	4.2	20.3			
3	13.3	0.0	0.0	16.7	4.2	6.8			
> 5	3.3	0.0	0.0	0.0	0.0	0.7			
Experience in rice farming (years)									
1-3	13.3	13.3	3.3	25.0	25.0	16.0	12	9.51	0.656
4-7	16.7	13.3	16.7	12.5	8.3	13.5			
8-10	16.7	13.3	13.3	12.5	4.2	12.0			
More than 10	53.3	60.0	66.7	50.0	62.5	58.5			
Type of rice varieties									
Local	93.3	100.0	83.3	100.0	85.0	92.3	4	6.301	0.178
Improved	6.7	0.0	16.7	0.0	15	7.7			
Source of advice on rice production									
Extension staff	13.3	10.0	26.7	8.3	29.2	17.4	8	9.643	0.291
Training on upland rice	13.3	10.0	16.7	8.3	16.7	13.0			
Own, fellow farmer /friend	73.3	80.0	56.7	83.3	54.2	69.6			

Note. df = degree of freedom, χ^2 = Chi-Square test, $P \leq 0.05$ showed significant differences

2.3.2 Upland rice production constraints

The rank of farmers' production constraints is summarized in Table 2.2. Rice blast disease was ranked the first by 48.0% of the respondents, followed by insect pests (19.9%), drought (14.9%), lack of knowledge (9.5%) and lack of access to input (7.7%) (Table 2.2).

The majority of the interviewed farmers have observed the rice blast disease for the first time in the past 3 to 10 years, both in their neighbours' and in their own rice fields. There were no significant differences ($\chi^2 = 5.621$; $P = 0.229$) and ($\chi^2 = 2.579$; $P = 0.630$) among farmers who have observed rice blast disease in their own rice fields and their neighbours' fields, respectively. The majority of the respondents (86.2%) reported the occurrence of high blast disease severity in April and May each year (Table 2.2). Other diseases reported were *Rice Yellow Mottle Virus* (26.1%), rice brown spot disease (8.7%) and bacterial leaf blight (0.7%) (Figure 2.2).

Table 2.2: Rice production constraints and the history of rice blast disease in the study areas

Constraints and rice blast disease history	Percentage of respondents					Mean	df	χ^2	P-value
	Mvomero (n = 30)	Morogoro Rural (n = 30)	Ulanga (n = 30)	Korogwe (n = 24)	Muheza (n = 24)				
Rice production constraints									
Rice blast disease	56.7	40	33.3	45	65	48.0	28	55.89	0.01
Drought	16.7	6.7	18.3	21.3	11.7	14.9			
Insects (stem borer, leaf rollers, Army wormy)	23.3	21.3	0.0	4.2	0.0	19.9			
Lack of knowledge	6.7	8.9	12.7	8	11.3	9.5			
Lack of access to inputs	3.3	6.3	6.7	17	5	7.7			
Rice blast disease in the farmer's field									
Observed	90.0	83.3	100.0	83.3	87.5	89.1	4	5.621	0.229
Not observed	10.0	16.7	0.0	16.7	12.5	10.9			
Rice blast disease in neighbour's field									
Observed	96.7	93.0	96.7	100.0	100.0	97.3	4	2.579	0.630
Not observed	3.3	7.0	3.3	0.0	0.0	2.7			
The first time rice blast disease observed in the field									
10 years ago	0.0	20.0	16.7	12.5	29.2	15.2	16	34.36	0.05
5-9 years ago	6.7	6.7	3.3	12.5	20.8	9.4			
3-4 years ago	46.7	43.3	33.3	16.7	16.7	32.6			
1-2 years	43.3	16.7	46.7	54.2	20.8	36.2			
Don't remember	3.3	13.3	0.0	4.2	12.5	6.5			
Time of the year with high rice blast disease severity									
April and May	76.7	96.7	86.7	91.7	79.2	86.2	8	14.283	0.075
June	3.3	0.0	10.0	0.0	12.5	5.1			
Do not know	20.0	3.3	3.3	8.3	8.3	8.7			

Note. df = degree of freedom, χ^2 = Chi-Square test, $P \leq 0.05$ shows there was a significant difference.

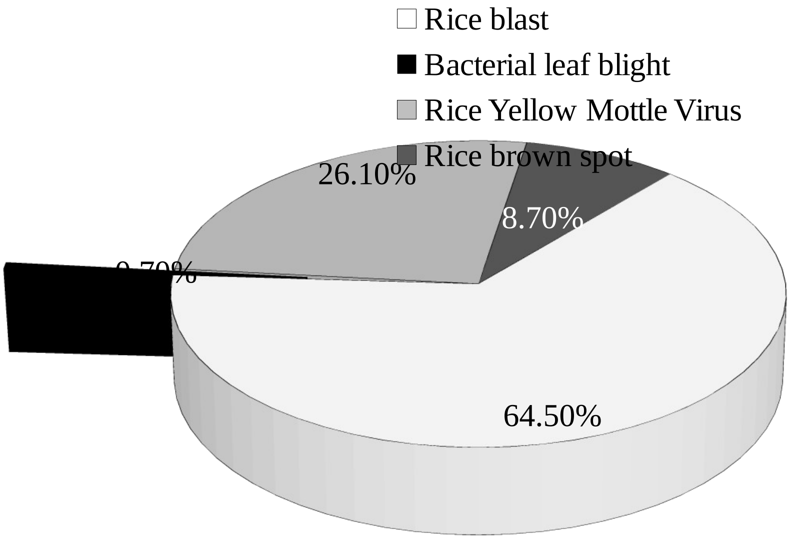

Figure 2.2: Rice diseases reported by farmers in the study areas

2.3.3 Farmers knowledge of rice blast disease

Significant differences (χ^2 = 37.142; P = 0.000) were observed among farmers in the perception of the rice blast disease. About 46.3% of the respondents were not aware of the cause and spread of rice blast disease (Figure 2.3). When they were asked about the association of the disease with other environmental factors, they reported that rice blast disease was associated with drought (39.9%), high rainfall and temperature (8.7%) and soil fertility problems (5.1%).

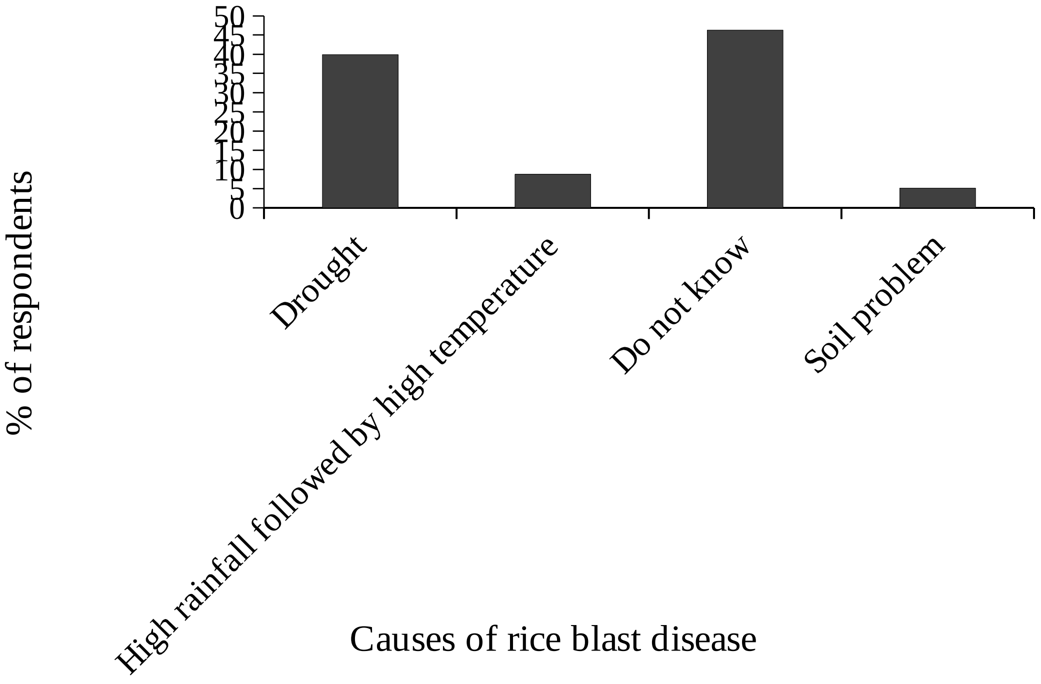

Figure 2.3: Farmers' perception of the possible major causes of rice blast disease (χ^2 = 37.142; P = 0.000)

2.3.4 Farmers' management of rice blast disease

The majority (54.0%) of farmers did not apply any control measures on rice blast disease (Figure 2.4). The possible reasons were lack of knowledge (70.3%), high cost and unavailability of effective pesticides (16.0%) and low disease incidence (13.7%) (Table 2.3). However, few farmers reported using several management practices of rice blast disease. About 18.7% of farmers reported burning of crop residue, 17.0% use of ash, 4.0% use of nitrogen fertilizer and 6.3% application of fungicide. None of the respondents knew the names and handling procedures of pesticides used. Management practices were significantly different (χ^2 = 36.142; P = 0.003) across the districts (Figure 2.4). In addition, respondents in Mvomero and Morogoro Rural district reported the use of rice straws for mulching in vegetable production.

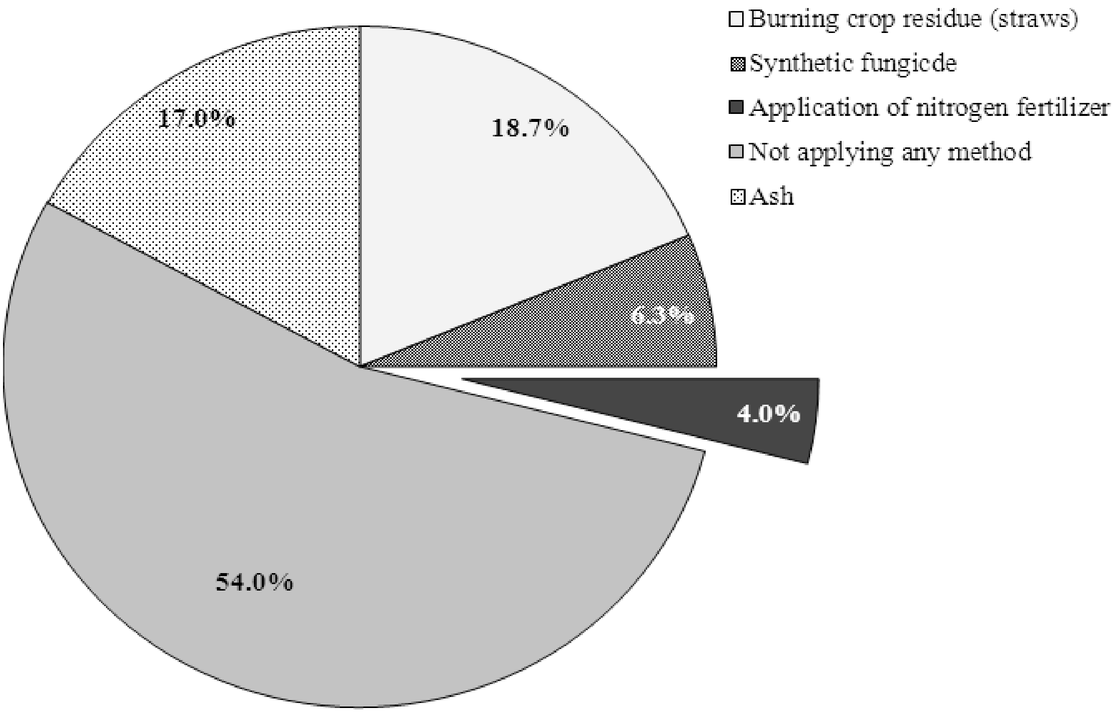

Figure 2.4: Management methods for rice blast disease used by farmers in the study area (χ^2 = 36.142; P = 0.003)

Table 2.3: The possible reasons for farmers not applying any management methods for rice blast disease in the study area

	Percentage of the respondents								
Reasons	**= 30)Mvomero**	**30)Morogoro Rural**	**(n = 30)Ulanga**	**= 24)Korogwe**	**n = 24)Muheza**	**Mean**	**df**	**χ^2**	***P*-value**
Lack of knowledge	60.0	56.7	70.0	95.0	70.0	70.3	8	16.48	0.036
Low blast disease incidence	16.7	16.7	26.7	5.0	15.0	16.0			
Cost and unavailability of effective pesticides	23.3	26.7	3.3	0.0	15.0	13.7			

2.4 Discussion

In this study, the majority of the respondents had seen rice blast disease for the first time in the past 2 to 10 years, both in neighbours' and in their own rice fields, with high severity occurring in April to June each year. This indicates that rice blast disease remains one of the main threats to rice production in the study area. The recent introduction of new rice varieties by farmers from other rice -growing areas and expansion of rice fields may have also contributed to the abundance of the rice blast disease.

Most farmers used their own saved rice seeds; only 7.7% of them used improved rice varieties. These improved rice varieties included New Rice for Africa (NERICA); five of them were officially released in Tanzania (Lwezaura *et al.*, 2011). The low adoption of these rice varieties may be due to unavailability in terms of source, time and the inability of farmers to afford the costs of buying seeds. Factors such as preference, availability in terms of quantity and market prices also forced farmers to use their own saved rice seeds (Hubert *et al.*, 2014). Many farmers preferred local rice varieties due to their good milling qualities, drought tolerance, early maturity and cooking qualities like good aroma and taste (Hubert *et al.*, 2014). High yield and marketability were reported to increase the preference of rice varieties (Traore *et al.*, 2015). However, some local varieties had good milling and cooking qualities were susceptible to rice blast disease and cultivated for consumption to increase farmers' food security.

Rice farmers were sourcing advises on rice production and disease management from the agriculture extension workers and fellow farmers or friends. Most of the farmers were using advice from their fellow farmers or friends followed by agricultural extension officers. This implies that farmer-to-farmer interactions were the main source of advice and such a method can be used in sharing knowledge on rice disease management. Farmers' knowledge has been acquired through long experience of rice farming regarding different challenges encountered in rice production (Traore *et al.*, 2015). To improve farmers' understanding of the management options for the rice blast disease, there is a need for creating awareness to farmers on the possible control measures.

Training has been reported to improve knowledge and change the farmers' attitude in crop pest management, which leads to the use of proper and safe crop disease management methods (Gautam *et al.*, 2017). Lack of awareness on the course and spreading of rice blast disease reported by the majority of the respondents has also been reported by Adam *et al.* (2015). In his studies, farmers were able to identify unhealthy sweet potato plants, but unable to tell the specific type of disease infecting the plants both from direct and photographic observations. Rice blast disease was observed in parts of the rice fields which were prone to drought and high disease severity was observed after a period of rainfall. These factors may be the reason for farmers to associate the disease with drought condition, high rainfall and temperature and soil problems. Consistent prevalence of the disease from April to June, each year, indicated a season of the year with conditions that favour disease outbreak. This study showed that the severity of rice blast disease has been increasing year after year for the last 3 to 10 years. However, most of the farmers hardly adopted management methods of blast disease on their farm. The consistent increase in disease abundance may be attributed to lack of information, knowledge of blast disease and the high cost and unavailability of effective fungicides. The use of rice straws for mulching on vegetable production reported by most of the respondents in Mvomero and Morogoro Rural may also be one of the reasons that contributed to the increase of the disease incidence reported. Crop residues (mulch) act as a source of inocula for the next rice growing season. The sources of inocula for rice blast disease are mycelia and conidia from infected rice straws and seeds (Webster, 2000). The pathogen can over season in piles of rice straws and seeds during unfavourable conditions (Webster, 2000).

Ash was used as a traditional method for rice blast disease management in Mvomero, Morogoro Rural and Muheza districts. The method was reported to be cheap and easy to use but less effective on rice blast disease management. Burning of crop residues was used to manage rice blast disease; however, some farmers believed that burning rice crop residues discouraged grazing of livestock on harvested rice farms. Farmers who associated rice blast disease with a soil fertility problem applied nitrogen fertilizer such as urea to manage rice blast disease. This was due to a lack of knowledge on proper identification of rice diseases from nitrogen deficiency, especially when the plants were infected with blast disease and exposed to nitrogen deficiency.

The use of pesticides to control rice blast disease was reported by very few respondents in Mvomero (13.33%), Morogoro Rural (10%) and Ulanga (3.33%). The awareness of using pesticides to manage blast disease was attributed to the experience of pesticide use on vegetable diseases. However, pesticide names and proper handling practices were not known. Mendesil *et al.* (2016) reported similar information that a survey conducted in Ethiopia showed that most farmers did not know the name of the pesticide applied to pea weevil in storage. Furthermore, the pesticide used by farmers was not effective in controlling rice blast disease. The use of non-recommended pesticides, improper application of pesticides, counterfeit and expired pesticides were among the reasons reported for the persistence of the crop diseases (Ngowi *et al.*, 2007; Nonga *et al.*, 2011; Lahr *et al.*, 2015; Mendesil *et al.*, 2016). Training of farmers enhances the adoption of Integrated Pest Management practices (IPM), reduce the quantity of pesticide use, frequency of spraying and the habit of mixing different pesticides (Gautam *et al.*, 2017).

2.5 Conclusion and Recommendation

This study showed that rice blast disease has been present in the study area for more than 10 years. This indicates that the disease remains as one of the main constraints to rice production in the area. Generally, lack of information, knowledge, and ability to afford the cost of buying fungicides and unavailability of effective control methods were the main reasons limiting the effective management of rice blast disease in the study area. The use of susceptible rice varieties and improver agronomic practices were additional constraints to the management of rice blast disease. The interactions of the farmer to farmer and farmer to agricultural extension staff were the main source of information on disease management. Therefore, to improve rice yield in the area covered, there should be an integrated rice blast disease research approach by various discipline and organisations (research and extension). Also, there is a need for strengthening the capacity of farmers in identifying and controlling rice blast disease.

2.6 Acknowledgments

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References

Adam, R. I., Sindi, K. and Badstue, K. (2015). Farmers' knowledge, perceptions and management of diseases affecting sweet potatoes in the Lake Victoria Zone region, Tanzania. *Crop Protection* 72 (74): 97-107.

Adesina, A. A., Johnsonand, D. E. and Heinrich, E. A. (1994). Rice pests in the Ivory Coast, West Africa: Farmers' perceptions and management strategies. *International Journal of Pest Management* 40 (4): 293 - 299.

Chuwa, C. J., Mabagala, R. B. and Reuben, M. S. (2015). Assessment of grain yield losses caused by rice blast disease in major rice-growing areas in Tanzania. *International Journal of Science and Research* 4: 2211 - 2218.

FAOSTAT (2016). Rice production data. http://www.fao.org/faostat/en/#data Accessed on 29/03/2018.

Gautam, S., Schreinemachers, P., Uddin, N. and Srinivasan, R. (2017). Impact of training vegetable farmers in Bangladesh in integrated pest management (IPM). *Crop Protection* 102: 161-169.

Hai, L. H., Kim, P. V., Du, P. V., Thuy, T. T. and Thanh, D. N. (2007). Grain yield and grain-milling quality as affected by rice blast disease (*Pyricularia oryzae*), at my Thanh Nam, Cai Lay, Tien Giang. *Omonrice* 15: 102 – 107.

Hubert, J., Luzi-Kihupi, A. L., Hébrard, E., and Lyimo, H. J. F. (2014). Farmers' knowledge and perception of Rice *Yellow Mottle Virus* in selected rice growing areas in Tanzania. *International Journal of Science and Research* 5: 549 - 559.

Lahr, J., Buij, R., Katagira, F. and van der Valk, H. (2016). *Pesticides in the Southern Agricultural Growth Corridor of Tanzania*. Wageningen Environmental Research. https://library.wur.nl/WebQuery/wurpubs/509323. Accessed on 20/08/2017.

Lwezaura, D., Madulu, R., Ndunguru, A., Paul, C. and Chalamila, B. (2011). *Regional Rice Centre of Excellence*. *Baseline survey report*. MAFC, Dar-es- Salaam, Tanzania. 38 pp.

Mendesil, E., Shumeta, Z., Anderson, P. and Ramert, B. (2016). Smallholder farmers' knowledge, perception and management of pea weevil in North-Western Ethiopia. *Crop Protection* 81: 30 - 37.

Mghase, J. J., Shiwachi, H., Nakasone, K. and Takahashi, H. (2010). Agronomic and socio-economic constraints to a high yield of upland rice in Tanzania. *African Journal of Agricultural Research* 5 (2): 150 -158.

Ngowi, A. V., Mbise, T., Ijani, A. S., London, L. and Ajayi, O. C. (2007). Pesticides use by smallholder farmers in vegetable production in Northern Tanzania. *Crop Protection* 26 (11): 1617-1624.

Nonga, H. E., Mdegela, R. H., Lie, E., Sandvik, M. and Skaare, J. U. (2011). Assessment of farming practices and uses of agrochemicals in Lake Manyara basin, Tanzania. *African Journal of Agricultural Research 6*: 2216 - 2230.

Roling, N. and Van Der Fliert, E. (1994). Transforming extension for sustainable agriculture: The case of integrated pest management in rice in Indonesia. *Agriculture and Human Values* 11 (2-3): 96 -108.

Schreinemachers, P., Balasubramaniam, S., Boopathi, N. M., Viet Ha, C., Kenyon, L., Praneetvatakul, S. and Srinivasan, R. M. (2015). Farmers’ perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. *Crop Protection* 75: 115 - 123.

Traoré, V. S., Néya, B. J., Camara, M., Vernon Gracen, V., Offei, S. K. and Traoré, O. (2015). Farmers’ perception and impact of rice yellow mottle disease on rice yields in Burkina Faso. *Agricultural Sciences* 6 (9): 943 - 952.

Velusamy, P. and Gnanamanickan, S. S. (2008). The effect of bacterial secondary metabolite on fungal pathogens of rice. *Soil Biology* 14: 93 - 106.

Webster, R. K. 2000. *Rice blast disease identification guide*. University of Calfornia, Davis. https://www2.ipm.ucanr.edu/agriculture/rice/Rice-Blast/. Accessed on 08/08/ 2017.

Wonnacott, T. H., and Wonnacott, R. J. (1990). Introductory statistics for business and economics. Wiley International 832 pp.

CHAPTER THREE

3.0 INCIDENCE AND SEVERITY OF RICE BLAST DISEASE CAUSED BY *Pyricularia oryzae* Cav. ON UPLAND RICE IN SELECTED AREAS IN TANZANIA

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Abstract

The upland rice growing areas in Tanzania have weather conditions favourable for rice blast disease development. However, to date information regarding the incidence and severity of the disease in upland rice growing areas is very scant. The present study was undertaken for two successive rice growing seasons, 2017/2018 and 2018/2019 to determine the incidence and severity of rice blast disease in upland rice through field surveys, isolation and morphological characterization of rice blast pathogen. The results of this study revealed that rice blast disease was found in all the surveyed areas in both 2017/2018 and 2018/2019 rice growing seasons. The incidence and severity of the disease varied between seasons with high disease incidence and severity in the 2017/2018. In 2017/2018, the highest rice blast disease incidence was 97.3% and the lowest was 80.3%, while in 2018/2019, the highest incidence was 41.9% and the lowest was 33.4%. The highest rice blast disease severity (100% and 98.8%) was recorded in Mvomero and Korogwe districts, respectively. There is a possibility that continuous use of local rice genotype for a long time in these areas might have played an important role in rice blast disease occurrence. This suggests the need for effective blast disease management option such as the use of improved resistant genotype. The current study provides basic information which will be used for rice blast disease management on upland rice in Tanzania.

Key words: Incidence, *Pyricularia oryzae,* rice blast, severity, upland rice

3.1 Introduction

Rice blast disease caused by an Ascomycetes fungus *Pyricularia oryzae* Cav. is an economically important disease of rice worldwide. The disease was first identified in China in 1637 and spread to other parts of the world; wherein 1913 it was reported in India (Ou, 1971). The occurrence of rice blast disease in Africa was reported for the first time in 1922 (Ou,1985). Since then, rice blast disease has been spreading to all rice-growing areas of East and Central Africa. It is one of the most important fungal diseases of rice in countries such as Tanzania, Kenya and Uganda (Kihoro *et al.*, 2013; Mgonja *et al.*, 2016). Given the increasing importance of rice as a staple and cash crop in these countries, the outbreak of rice blast disease threatens food security and livelihood of many people.

The blast disease pathogen causes necrotic lesions on both leaves and panicles. Leaf blast results in the reduced area for photosynthesis (Bastiaans, 1993a). Panicle blast is more directly related to yield loss as it occurs in the reproductive stage of rice and severe panicle blast sometimes can occur without being preceded by leaf blast (Zhu *et al.*, 2005). Fungal disease establishment, development, and severity are greatly affected by weather conditions such as temperature, relative humidity and rainfall (Nwanosike and Mabagala, 2017). Growth and sporulation of *P. oryzae* increase with temperature ranging from 22 to 27°C and decline with a temperature above 32°C (Rajput *et al.*, 2017). The infection process of *P. oryzae* involving spore germination, germ tube development and appressoria formation is optimum at a relative humidity of 95% and an average temperature of 26 to 27°C (Muñoz, 2008). The combination of night temperature ≤ 20°C, rainfall lower than 250 mm and cloud cover of about 25 h is important for the appearance and development of rice blast disease symptoms (Prasad *et al.*, 2015).

Previous studies by Balimponya (2015), Chuwa *et al.* (2015) and Hubert *et al.* (2015) showed that rice blast disease is present in all low land rice grown regions of Tanzania. Nevertheless, to date the information on rice blast disease incidence and severity in upland rice is scant. Upland rice contributes about 20% of the total rice production in the country (Wilson and Lewis, 2015). Studies by Mghase *et al.* (2010) indicated that Morogoro and Tanga regions represent the major upland rice-growing areas in Tanzania. These regions have favourable weather conditions for rice blast disease proliferation and upland rice has been grown in these areas for a long time. Hence, quantification of rice blast disease incidence and severity in upland rice was needed to enable rice farmers to set priorities in rice blast disease management. This study reports on the incidence and severity of rice blast disease in major upland rice growing areas of Tanzania.

3.2 Materials and Methods

3.2.1 Location of the study area

Two localities, Morogoro (S 06° 53’27.6”, E 037° 36’44.5”, 516 m.a.s.l) and Tanga (S 06° 47’12.3”, E 37° 39’01.7”, 501 m.a.s.l) regions were used for this study as described by Hashim *et al.* (2018b). The surveys were conducted in farmers’ fields within Mvomero and Morogoro Rural districts in Morogoro region and Muheza and Korogwe districts in Tanga region. These areas were purposively selected due to the long history of upland rice cultivation and easy accessibility. The rainfall patterns in both regions were bimodal with a long rain season from mid-March to May and a short rain season from November to December.

In 2017/2018 rice growing season, the highest amount of rainfall (77.3 mm and 38 mm) per week was recorded in March and April, respectively (Figure 3.1). The relative humidity ranged from 65 to 98% while maximum temperature ranged from 23°C to 35°C and minimum temperature ranged 20.6 °C to 35.8°C (Figure 3.1). In both locations, weather conditions were on the range favourable for rice blast disease establishment and development.

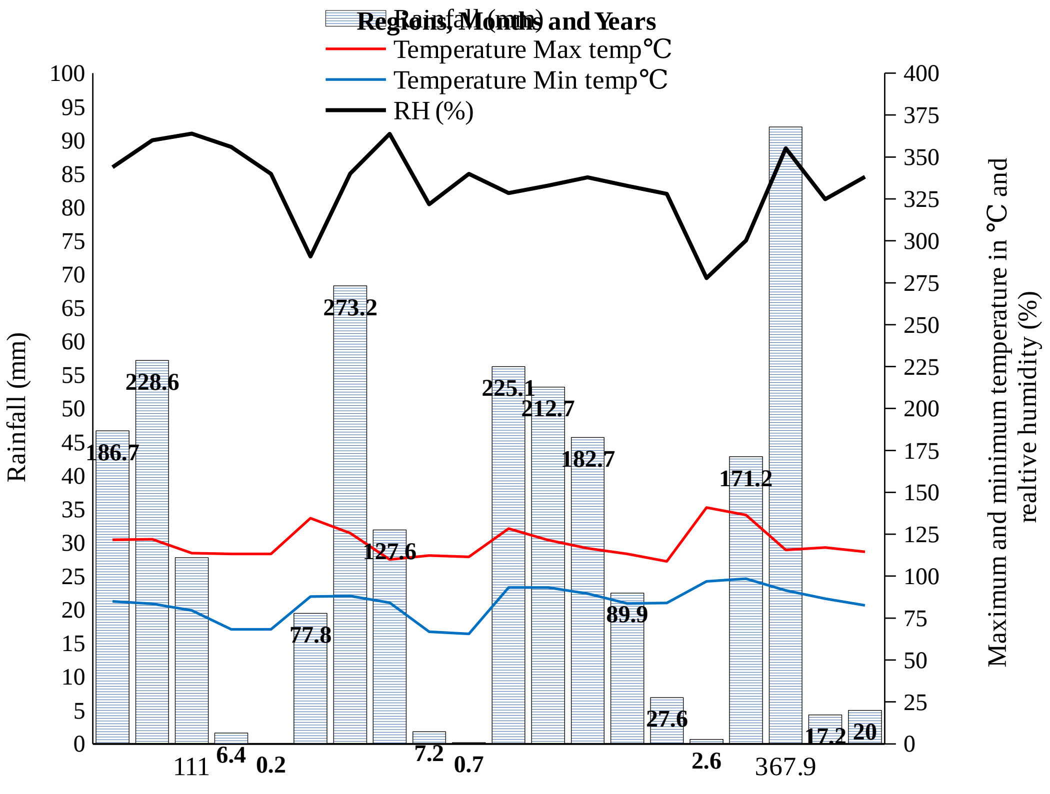

Figure 3.1: Monthly temperature, relative humidity and rainfall in Tanga and Morogoro during the 2017/2018 and 2018/2019 rice growing seasons (source: TMA 2018/2019).

3.2.2 Rice blast disease assessment and collection of infected samples

Field inspection and laboratory studies of suspected rice blast disease samples were used to determine the presence of rice blast disease in upland rice. The survey was done twice from April to June in 2017/2018 and 2018/2019 seasons when rice plants were at the flowering to milking stage. Leaves, panicles, neck and nodes showing rice blast symptoms were collected and transported for the analysis to the African Seed Health Centre laboratory located in the Sokoine University of Agriculture, Morogoro, Tanzania.

During the surveys, farmers' fields were randomly selected based on rice growing history for the past three years with the help of agricultural extension staff. Rice blast disease assessment was conducted by diagonal transect walk using 1.0 m x 1.0 m quadrant as described by Lin *et al.* (1979). The quadrant was thrown randomly on the two diagonals in 'X' pattern. In each field, 5 quadrants were inspected and the total number of plants and number of plants infected by rice blast disease were counted. A total of 120 same farmers' fields was surveyed each season. The percentage disease incidence in the field was calculated using the formula below (i). Rice blast disease severity was scored as the percentage of leaf area with symptoms using a scale of 0 – 9 developed by IRRI (2002).

Rice blast lesions per leaf from 12 rice genotypes were counted and the size was measured using a 30 cm ruler. Rice blast disease severity scores were converted into percentage disease by using the formula as described by Magar *et al.* (2015) (ii).

$$\text{Disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of inspected plants}} \text{ x } 100\,\% \quad \text{..............................} \quad \text{(i)}$$

$$\%\,\text{Disease severity} = \frac{\text{Sum of scores x } 100\,\%}{\text{Total number of observations x highest number on the rating scale}} \ldots\text{(ii)}$$

A geographical positioning system (GPS Gamin) was used to record the coordinates which were incorporated to create a map showing the magnitude of rice blast disease using a GIS software Arc GIS 10. Daily weather data on temperature, relative humidity and rainfall for each site were collected from the Tanzania Meteorological Agency (TMA), Morogoro, Tanzania.

3.2.3 Isolation and identification of *Pyricularia oryzae*

Collected rice samples were cut into small pieces, placed on 3 layers of moist filter paper in 90 mm glass Petri dishes and incubated at 26 to 28°C for 2 days (Figure 3.1). The dissecting and compound microscopes were used for the identification of *P. oryzae* based on the production of characteristic conidia and their morphology (Mathur and Kongsdal, 2003).

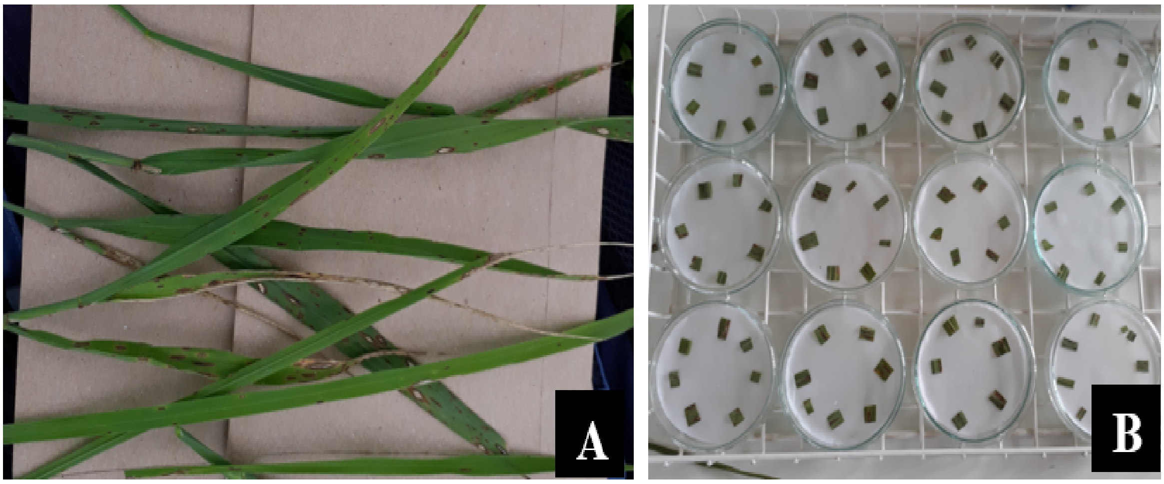

Figure 3.2: Sample of rice leaves with symptoms of rice blast disease (A) and pieces of rice leaves placed on moist filter paper in 90 mm Petri dishes ready for incubation at 26 to 28°C for 2 days (B).

3.2.4 Statistical analysis

The percentage data were subjected to Arcsine transformation to fit the normal distribution (Gomez and Gomez, 1984). Analysis of variance on disease incidence and severity across the different locations, genotype and rice growing seasons was performed. Where statistically significant different means were detected, the mean separation tests were done using the Tukey's HSD. All statistics were computed using the R Software, version 3.5.2 (R Development Core Team, 2015).

3.3 Results

3.3.1 Incidence and severity of rice blast disease in selected upland rice-growing areas

The present study established the incidence and severity of rice blast disease on upland rice in different areas of Morogoro and Tanga regions. Rice blast disease distribution map indicates that the disease was present in all the surveyed districts of Morogoro and Tanga regions. The disease incidence ranged from 39.8 to 100% in Mvomero and Morogoro Rural districts and 91.1 to 100% and 78.9 to 100% in Korogwe and Muheza districts, respectively (Figure 3.2).

Symptoms of leaf and panicle blast were observed in all surveyed rice fields. The symptoms such as elongated brown lesions with grey centres on leaves and panicles were obvious in most of the surveyed rice fields (Figure 3.3A). Identification and confirmation of the disease-causing pathogen were done using the characteristic growth of *P. oryzae* observed on potato dextrose agar (PDA) and oatmeal agar media (Figures 3.3B and 3.3C).

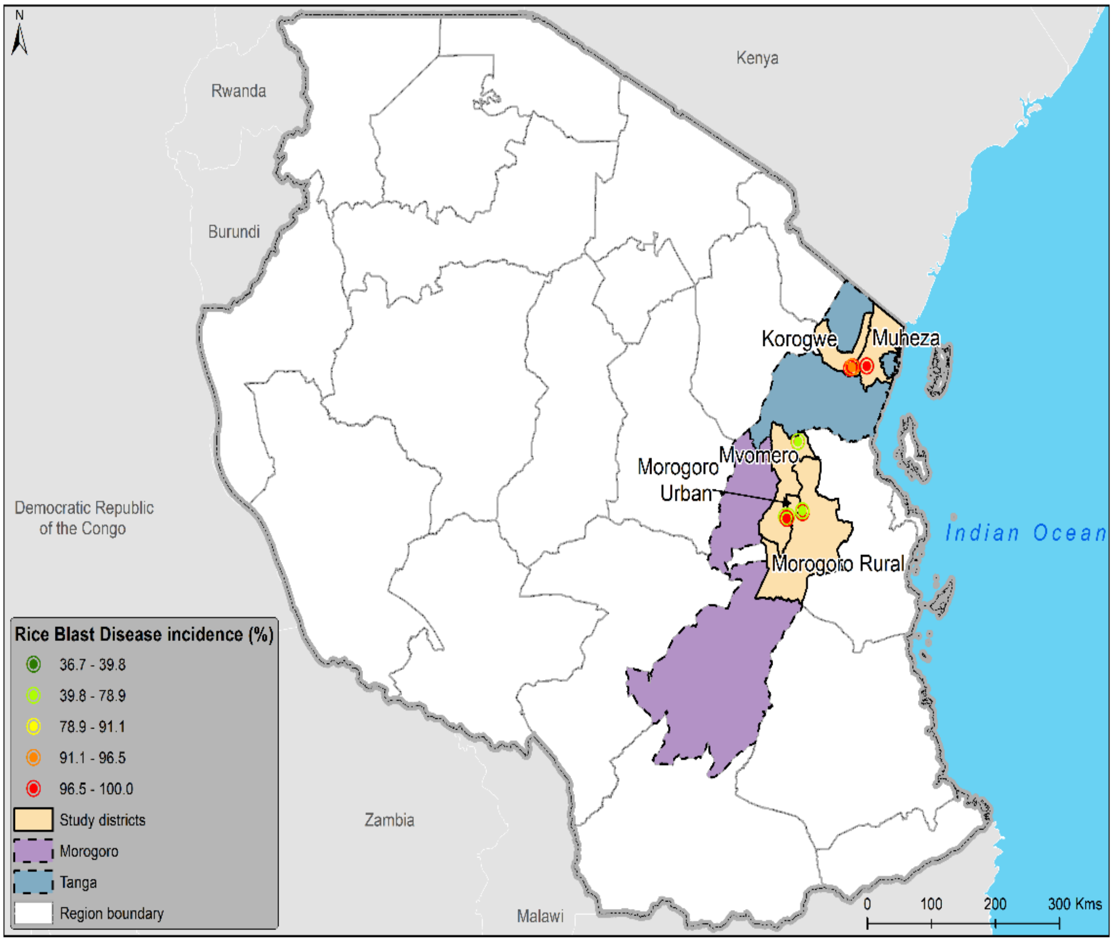

Figure 3.3: A map of Morogoro and Tanga regions showing the relative distribution based on incidence of rice blast disease in upland rice in four selected districts. (Source: Ibrahim Hashim, 2019).

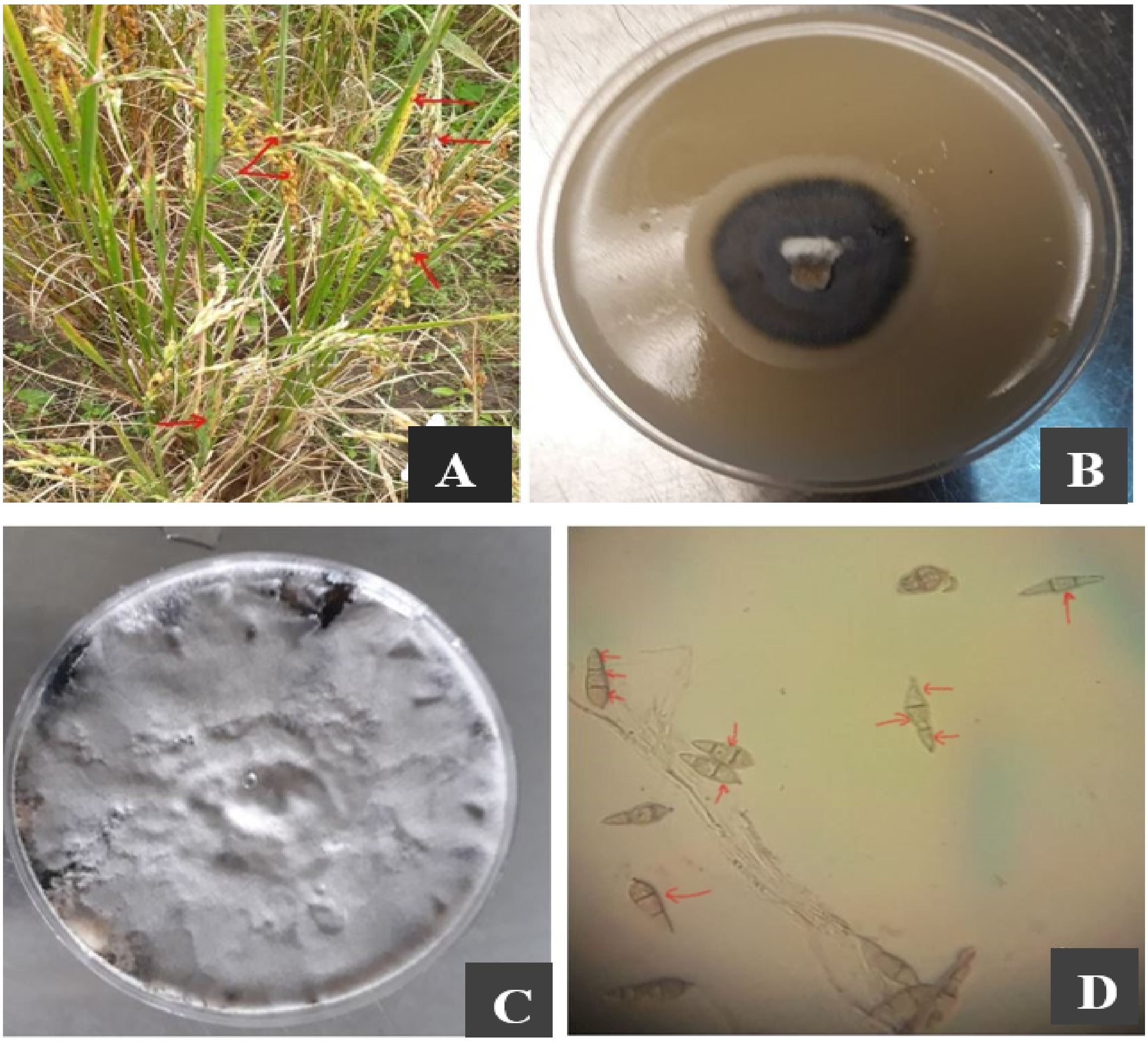

Figure 3.4: Rice plants showing symptoms of blast disease on leaves and panicles (A), a five days old culture of *Pyricularia oryzae* on potato dextrose agar media (B), a 14 days old culture of *Pyricularia oryzae* on oatmeal agar media (C), conidia of *P. oryzae* showing 3 septa (red arrow) (D).

3.3.2 Blast disease incidence and severity in upland rice

The analysis of variance indicated that rice blast disease incidence was significantly different ($P \leq 0.001$) across different locations, rice genotypes and growing seasons (Table 3.1). The same trend was observed on rice blast disease severity and lesion size except that significant differences ($P \leq 0.05$) were not observed on different rice genotypes. The results from the 2017/2018 rice growing season showed significant differences ($P<0.01$) on percentage blast disease severity across different surveyed districts (Table 3.2).

However, during the 2018/2019 rice growing season, the percentage of rice blast disease incidence and severity did not differ significantly at $P \leq 0.05$. Furthermore, results showed that, in 2017/2018 rice growing season, the highest blast disease incidence was recorded in Korogwe (97.3%), and the highest blast disease severity was in Mvomero (100.0%) followed by Korogwe (98.8%), Morogoro Rural (88.1%) and Muheza district (87.3%). Similar trend was observed in the 2018/2019 rice growing season (Table 3.2).

Table 3.1: Analysis of variance of the effect of location, genotype and season on rice blast disease incidence, severity and lesion size

Source	Df	Incidence		Severity		Lesion size (mm)	
		F value	Pr (>F)	F value	Pr (>F)	F value	Pr (>F)
Location	3	5.123	0.00304**	4.244	0.0084*	9.194	0.0003***
Genotype	11	3.775	0.00048***	1.119	0.3623	0.626	0.786
Season	1	460.528	0.00016***	1155.365	0.0002***	4.689	0.034*
Residue	65						

* Significant at $P < 0.01$; ** = significant at $P < 0.001$, *** = significant at $P < 0.0001$

Table 3.2: Percentage of rice blast disease incidence and severity in four surveyed districts in Morogoro and Tanga regions during the 2017/2018 and 2018/2019 rice growing seasons

Locations	Season 2017/2018		Season 2018/2019		Mean Incidence (%)	Mean Severity (%)
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)		
Muheza	80.4 a	87.3 a	33.4 a	17.8 a	56.9	50.8
Morogoro Rural	85.8 a	88.1 ab	36.9 a	24.4 a	61.4	56.3
Mvomero	90.7 a	100.0 b	39 a	22.2 a	64.9	61.1
Korogwe	97.3 a	98.8 ab	41.9 a	24.4 a	69.6	61.6
$LSD_{0.05}$	14.2	9.5	7.5	7.1		
CV%	17.7	11.2	21.8	35		
F pr.	0.118 ns	0.0114 *	0.153 ns	0.198 ns		

* Significant at $P < 0.01$; ns = non-significant at $P \leq 0.05$ and different means in the same column followed by the same letters are not significantly different according to Tukey's HSD test at $P < 0.05$

3.3.3 Rice blast disease incidence and severity on different commonly grown upland rice genotypes

Results in Table 3.3 showed significant differences ($P \leq 0.01$) for rice blast disease incidence among different rice genotypes for the 2017/2018 growing season. In the same season, rice blast disease severity did not differ significantly ($P \leq 0.05$) among genotypes. In the 2018/2019 growing season, no significant differences ($P \leq 0.05$) were observed for the disease incidence and severity on different rice genotypes (Table 3.3). The highest rice blast disease incidences in 2017/2018 and 2018/2019 growing seasons (100% and 43%) were recorded in Karafuu, Masantula and Mbawambili genotypes. In the 2017/2018 growing season rice blast disease severity was higher (100%) for Supa Mbeya, Supa Serena and Karafuu while in 2018/2019 growing season, the highest rice blast disease severity (33.3%) and the lowest disease severity (11.1%) were recorded on Masantula and Kalamata (Table 3.3).

Table 3.3: Percentage of rice blast disease incidence and severity on different rice genotypes commonly grown in the study area during 2017/2018 and 2018/2019 crop season

Genotype	Season 2017/2018		Season 2018/2019	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Fayamsitu	39.8 a	81.8 ab	17.1 a	22.2 a
Kalamata	69.3 ab	81.8 ab	29.8 a	11.1 a
Wahi	73.1 ab	85.7 ab	29.1 a	14.8 a
Supa Mbeya	88.4 ab	100.0 ab	38.0 a	22.2 a
Tule na bwana	89.2 ab	69.2 a	38.4 a	22.2 a
Supa	90.1 b	96.8 b	38.7 a	23.5 a
Line 80	91.4 b	89.8 ab	39.9 a	22.2 a
Kihogo	94.9 b	98.5 b	40.8 a	22.2 a
Supa Serena	98.7 b	100.0 b	42.4 a	28.9 a
Karafuu	100.0 b	100.0 ab	43.0 a	22.2 a
Masantula	100.0 b	77.6 ab	43.0	33.3 a
Mbawambili	100.0 b	98.7 ab	43.0	22.2 a
LSD 0.05	14.2	23.4	16.6	16.9
CV%	15.0	11.3	19.9	34.6
F pr.	0.0136 *	0.101ns	0.072 ns	0.304 ns

* Significant at $P < 0.01$; ns = non-significant at $P < 0.05$ and means in the same column followed by the same letters are not significantly different at $P < 0.05$ according to Tukey HSD test.

Results in Table 3.4 show that in the 2017/2018 rice growing season, there was a significant (P < 0.05) positive correlation between rice blast disease severity and lesion size. However, during the 2018/2019 growing season, there was a negative non-significant (P < 0.05) correlation between rice blast disease severity and lesion size. The increase in rice blast disease severity resulted in a decrease in lesion size across different locations (Table 3.4).

Table 3.4: Relationship between rice blast disease severity and lesion size in 2017/2018 and 2018/2019 rice growing seasons

Locations	2017/2018		2018/2019	
	Severity (%)	**Lesion size (mm)**	**Severity (%)**	**Lesion size (mm)**
Muheza	87.3	5.4	17.8	4.3
Morogoro Rural	88.1	5.7	24.4	5.9
Mvomero	100.2	34.5	22.2	17.2
Korogwe	98.8	17.8	24.4	8.4
Pearson's correlation		0.32		0.03
P-value		0.046*		0.8401ns

* Significant at P < 0.01; ns = non-significant at P < 0.05

3.4 Discussion

This study established the status of rice blast disease in upland rice in Mvomero, Morogoro Rural, Muheza and Korogwe districts in Tanzania. In this study rice blast disease incidence and severity varied in different areas and seasons. In the 2017/2018 rice growing season, the highest blast disease incidence and severity were greater than that of 2018/2019 growing season by about 43% and 24%, respectively. The variations of disease incidence and severity were mostly associated with variations in weather conditions, continuous use of different susceptible local rice genotypes (Table 3.3, Figure 3.4) and poor field and seed sanitation (Hashim *et al.*, 2018b). Previous studies conducted by Titone *et al.* (2015), Onaga and Asea (2016) and Rasool *et al.* (2016), showed that year to year weather variability resulted into changes of the magnitudes of leaf and panicle rice blast disease development.

During the current survey, 12 different local rice genotypes were found commonly grown by farmers in the study area. In all the surveyed farmers' fields, rice blast disease symptoms were found on all rice genotypes, indicating that such rice genotypes were all susceptible to the disease. The disease incidence and severity varied within genotypes and rice growing seasons (Table 3.2), indicating that these genotypes had different levels of susceptibility to rice blast disease. This agrees with previous studies by Bregaglio *et al.* (2017) that changes in growing seasons and rice genotypes are important factors for rice blast disease development. In Tanzania, similar to other African countries, improved upland rice genotypes such as NERICA (New Rice for Africa) were introduced as disease resistant and high yielding material. However, they were poorly adopted by farmers due to low market demand (Yokouchi and Saito, 2017). To enhance adoption and sustainable reduction of the effect of rice blast disease, there is a need to improve the farmers preferred rice genotype by adding the disease-resistant traits in rice breeding programs.

Onaga and Asea (2016) associated high rice blast disease severity with improper cultural practices. In this study, it was found that upland rice farmers were neither applying fertilizer nor proper plant spacing. The broadcasting method of planting rice was commonly practised by farmers for the reason that it is easy and saved time. However, it was also observed to increase plant density. The combination of high plant population and changes in the micro-environmental conditions in the crop canopy such as changes in relative humidity, leaf wetness and precipitation has been reported to enhance proliferation and development of the rice blast disease pathogen (Greer and Webster, 2001). In the current study, it was also noted that in the 2017/2018 rice growing season, there was a positive correlation between rice blast disease severity and lesion size, indicating that an increase in blast disease severity resulted in an increase in lesion size (Table 3.4). The variations of rice blast disease severity and lesion size have been associated with variations of virulence of the pathogen and susceptibility of the rice genotype to rice blast (TeBeest *et al.*, 2012).

3.5 Conclusions and Recommendations

Rice blast disease was widely distributed in the upland rice growing areas of Morogoro and Tanga regions with different magnitudes. The highest rice blast disease incidence and severity were recorded in the 2017/2018 rice growing season. This study showed that Mvomero and Korogwe districts are the hot spot areas for this disease, while Morogoro Rural and Muheza districts were the low rice blast disease severity areas. The results of this study provide bases for policy and research prioritization of rice blast disease management. The average disease incidence of 56.9 to 69.6% in most surveyed areas indicates the need for the efforts to develop and use more effective and sustainable management tools such as disease-resistant varieties and environmentally friendly methods (eg. bio-agents). Furthermore, regular rice blast disease surveillance is important to guide farmers the right time for the application of appropriate disease control measures and to reduce the use of chemicals where possible.

3.6 Acknowledgements

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References

Balimponya, E. (2015). Application of genomic selection and association mapping to breeding for resistance to rice blast and bacterial blight of rice (*Oryza sativa* L.), Dissertation for Award of Master of Science at Ohio State University, 122p.

Bastiaans, L. (1993a). Effects of leaf blast on photosynthesis of rice. *Netheland Journal of Plant Pathology* 99: 197-203.

Bregaglio, S., Titone, P., Hossard, L., Mongiano, G., Savoini, G., Piatti, F. M., Paleari, L., Masseroli, A. and Tamborini, L. (2017). Effects of agro-pedo-meteorological conditions on dynamics of temperate rice blast epidemics and associated yield and milling losses. *Field Crops Research* 212: 11-22.

Chuwa, C., Mabagala, R. and Reuben, M. S. O. W. (2015). Assessment of grain yield losses caused by rice blast disease in major rice growing areas in Tanzania. *International Journal of Science and Research* 4 (10): 2211-2218.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research. John Wiley and Sons Inc. London 680 pp.

Greer, C. and Webster, R. (2001). Occurrence, distribution, epidemiology, cultivar reaction, and management of rice blast disease in California. *Plant Disease* 85 (10): 1096 -1102.

Hashim, I., Mamiro, D. P., Mabagala, R. B. and Tefera, T. (2018b). Smallholder farmers' knowledge, perception and management of rice blast disease in upland rice production in Tanzania. *Journal of Agricultural Science* 10 (7): 137- 145.

Hubert, J., Mabagala, R. B. and Mamiro, D. P. (2015). Efficacy of selected plant extracts against *Pyricularia grisea*, causal agent of rice blast disease. *American Journal of Plant Sciences* 6: 602 - 611.

IRRI (2002). Standard Evaluation System. *International Rice Research Institute,* Manila, Philippines, 11-30.

Kihoro, J., Bosco, N. J., Murage, H., Ateka, E. and Makihara, D. (2013). Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. *SpringerPlus* 2 (1): 308 - 318.

Lin, C. S., Poushinsky, G. and Mauer, M. (1979). An examination of five sampling methods under random and clustered disease distributions using simulation. *Canadian Journal of Plant Science* 59 (1): 121-130.

Magar, P. B., Acharya, B. and Pandey, B. (2015). Use of chemical fungicides for the management of rice blast (*Pyricularia grisea*) disease at Jyotinagar, Chitwan, Nepal. *International Journal of Applied Sciences and Biotechnology* 3 (3): 474 - 478.

Mathur, S. B. and Kongsdal, O. (2003). Common laboratory seed health testing methods for detecting fungi. Bassersdorf, Switzerland 89 pp.

Mghase, J. J., Shiwachi, H., Nakasone, K. and Takahashi, H. (2010). Agronomic and socio-economic constraints to high yield of upland rice in Tanzania. *African Journal of Agricultural Research* 5 (2): 150 -158.

Mgonja, E. M., Balimponya, E. G., Kang, H., Bellizzi, M., Park, C. H., Li, Y., Mabagala, R., Sneller, C., Correll, J. and Opiyo, S. (2016). Genome-wide association mapping of rice resistance genes against *Magnaporthe oryzae* isolates from four African countries. *Phytopathology* 106 (11): 1359 -1365.

Muñoz, M. C. (2008). The effect of temperature and relative humidity on the airbone concentration of *Pyricularia oryzae* spores and the development of rice blast in Southern Spain. *Spanish journal of agricultural research* (1): 61-69.

Nwanosike, M. R. O. and Mabagala, R. B. (2017). Influence of metrological parameters on the development of *Exserohilum turcicum* (Pass.) Leonard and Suggs on maize in Tanzania. *International Journal of Agricultural and Food Research* 6 (3): 1-9.

Onaga, G. and Asea, G. (2016). Occurrence of rice blast (*Magnaporthe oryzae*) and identification of potential resistance sources in Uganda. *Crop Protection* 80: 65 -72.

Ou, S. H. (1985). Rice Diseases. *Commonwealth Mycological Institute, Kew, Surrey, UK* 380 pp.

Ou, S., Nuque, F., Ebron, T. and Awoderu, V. (1971). A type of stable resistance to blast disease of rice. *Phytopathology* 61 (6): 703 – 706.

Prasad, R., Sharma, A. and Sehgal, S. (2015). Influence of weather parameters on occurrence of rice blast in mid hills of Himachal Pradesh. *Himachal Journal of Agricultural Research* 41 (2): 132-136.

Rajput, L. S., Sharma, T., Madhusudhan, P. and Sinha, P. (2017). Effect of temperature on growth and sporulation of rice leaf blast pathogen *Magnaporthe oryzae*. *International Journal of Current Microbiology and Applied Sciences* 6 (3): 394 - 401.

Rasool, F., Ahmed, M. and Sahni, S. (2016). Survey and Status of Rice Blast Caused by *Magnaporthe oryzae* BC Couch in Commercial Rice Growing Areas of Kashmir. *Vegetos-An International Journal of Plant Research* 29 (3): 28-31.

R Development Core Team. (2015). *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing*. http://www.rproject.org/ Accessed on 10/9/2019.

TeBeest, D. O., Guerber, C. and Ditmore, M. 2012. Rice blast. *https://www. Cabdirect. org/cabdirect/abstract*/. Accessed on 18/07/2019.

Titone, P., Mongiano, G. and Tamborini, L. (2015). Resistance to neck blast caused by *Pyricularia oryzae* in Italian rice cultivars. *European Journal of Plant Pathology* 142 (1): 49 - 59.

Wilson, T. R. and Lewis, I. (2015). The rice value chain: A report from Southern Highlands Food Systems Programme. *Food and Agriculture Organization of the United Nations*. Rome, Italy. 111 pp.

Yokouchi, T. and Saito, K. (2017). Why did farmers stop cultivating NERICA upland rice varieties in central Benin? *International Journal of Agricultural Sustainability* 15 (6): 724 -734.

Zhu, Y. Y., Fang, H., Wang, Y. Y., Fan, J. X., Yang, S. S., Mew, T. W. and Mundt, C. C. (2005). Panicle blast and canopy moisture in rice cultivar mixtures. *Phytopathology* 95 (4): 433 - 438.

CHAPTER FOUR

4.0 *IN VITRO* AND *IN VIVO* EVALUATION OF MICROBIAL AGENTS FOR MANAGEMENT OF RICE BLAST DISEASE IN TANZANIA

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Abstract

Evaluation of two microbial agents, *Trichoderma asperellum* and *Bacillus subtilis* and the fungicide Linkimil 72 WP against rice blast disease caused by *Pyricularia oryzae* were done in a laboratory and pot experiments. Dual inoculation of *T. asperellum B. subtilis* and Linkimil 72 WP caused significant inhibition of radial growth of *P. oryzae*. Both *T. asperellum* and *B. subtilis* showed over 75% inhibition of radial growth (PIRG) compared to Linkimil 72 WP with PIRG range of 21 - 23 % and control with 0 %. In a pot experiment, 70 % reduction in disease incidence was in plants treated with *T. asperellum* followed by *B. subtilis* (51.5 %) and Linkimil 72 WP (26.5 %). There was a 44.5 % decrease in disease severity in plants treated with Linkimil 72 WP compared to plants treated with *T. asperellum* (35.6 %) and *B. subtilis* (29.1 %). The number of lesions per leaf was low on rice plants treated with *T. asperellum* followed by Linkimil 72 WP and *B. subtilis*. *Trichoderma asperellum* and *B. subtilis* used in this study showed high antagonistic capacity against *P. oryzae* and these microbes can be recommended as part of an integrated management strategy of rice blast disease.

Keywords: *Bacillus subtilis*, Microbial agent, *Pyricularia oryzae,* Rice blast disease, *Trichoderma asperellum*

4.1 Introduction

Rice blast disease is a fungal disease caused by *Pyricularia oryzae* Cavara, a devastating disease of rice reducing yields worldwide (Faivre-Rampant *et al.*, 2013; Hubert *et al.*, 2015). The disease is found all over in the world where rice is grown (Kato, 2001). The pathogen belongs to the Kingdom Fungi, Phylum Ascomycota and the genus *Pyricularia* and infects all growth stages of rice plants (Pooja and Katoch, 2014). It survives on infected rice crop residues, weeds and rice seeds (Sun *et al.*, 1997; Webster, 2000; Faivre-Rampant *et al.*, 2013). Reports show that in the field the fungus disperses through airborne spores, infected crop residues and infected rice seeds (Webster, 2000; Faivre-Rampant *et al.*, 2013; Raveloson *et al.*, 2018).

Initial symptoms of rice blast disease appear as white to grey-green lesions or spots, with dark green borders. Mature lesions appear cottony in the centre with a dark bluish surface due to the production of conidia. On leaves, rice blast lesions are elliptical or spindle-shaped with whitish to grey centres and red to brownish or necrotic borders (Webster, 2000; IRRI, 2016).

Several studies (Faruq *et al*., 2015; Suprapta *et al.*, 2014; Ali and Nadarajah, 2014) indicate that management of rice blast disease is by resistant genotypes, fungicides and appropriate cultural practices. However, none of these methods can permanently control rice blast disease. The longevity of resistance of many resistant genotypes is shortened by the high pathogenic variability of *P. oryzae* (Ali and Nadarajah, 2014). Fungicide, such as Linkimil 72 WP, has been reported to have a negative effect on environmental and human health. Sustainable and effective rice blast control can be achieved through a combination of resistant genotypes and the use of environmentally friendly control strategies such as microbial agents.

In recent years, there has been an increasing number of studies on antagonistic microbial agents for control of rice blast disease (Zarandi *et al.*, 2009; Singh *et al.*, 2012; Ali and Nadarajah, 2014; Faruq *et al.*, 2015). *Trichoderma harzianum*, *T. viride* and *B. subtilis* have been reported to reduce rice blast disease incidence by 70% in India (Jayaraj *et al.*, 2004; Singh *et al.*, 2012). In some parts of Africa, *T. asperellum* and *B. subtilis* are used for controlling soil-borne and foliar diseases of ornamental plants and vegetables (Mwangi *et al.*, 2011; Kipngeno *et al.*, 2015). However, there is no report on the use of *T. asperellum* and *B. subtilis* for control of rice blast disease in Africa. The effectiveness of bio agent for control of plant diseases is highly influenced by the interactions of microbial communities found in the area and changes in weather conditions such as temperature and relative humidity (Ru and Di, 2012). Weather conditions and microorganism populations in Tanzania might be different from those in India, where the effectiveness of the microbial agent for controlling rice blast disease has been reported (Singh *et al.*, 2012). Therefore, there is a need to determine the efficacy of *T. asperellum* and *B. subtilis* against *P. oryzae* in Tanzania. The current study reports on the efficacy of using *T. asperellum* and *B. subtilis* in inhibiting the growth of *P. oryzae in vivo* and reducing the incidence and severity of rice blast disease.

4.2 Materials and Methods

4.2.1 Source of microbial agents, Linkimil 72 WP and rice seeds

Microbial agents: Commercial *Trichoderma asperellum* and *Bacillus subtilis* were obtained from Real IPM, Nairobi, Kenya. Linkimil 72 WP (Mancozeb 64% + Metalaxyl 8%) was purchased in Morogoro town. Rice genotypes Kihogo and Lunyuki were collected from farmers in Morogoro district while Supa and Usiguse genotypes were from the International Rice Research Institute (IRRI) germplasm collection at Dakawa Agricultural Research Institute, Morogoro, Tanzania.

4.2.1 Inocula collection and preparation

Leaf samples showing symptoms of rice blast disease were collected from rice fields in Morogoro and Tanga region, Tanzania. Samples were packed in paper envelopes and transported to the African Seed Health Centre Laboratories, Sokoine University of Agriculture (SUA), Tanzania, for isolation of *P. oryzae*. Small sections (1 – 1.5 cm) were taken from infected parts of leaves and placed on three layers of wet filter papers lined in (90 mm) Petri dishes. The Petri dishes were incubated at 25°C. After 24 – 48 hours of incubation, the lesions were examined under a dissecting microscope to check for sporulation as described by Jia (2009). A sterilized inoculating loop with a small piece of potato dextrose agar (PDA) was used to pick conidia that emerged and were transferred to Petri dishes containing PDA (Jia, 2009). Then the Petri dishes were sealed using sealing tape and incubated upside down for five days at 25°C. The pure cultures of the fungal isolates were grown on oatmeal agar for 10 - 14 days to induce sporulation.

4.2.2 Pathogenicity test

Different isolates were inoculated on rice seedlings (variety Supa as a susceptible check) planted in 4-litre plastic pots. Inoculation was done following procedures described by (Akagi *et al.*, 2015). The conidia of *P. oryzae* were suspended in two drops of Tween 20 adjusted at 1 x 10^5 spores/ml and sprayed on 14-day-old rice seedlings. Inoculated seedlings were covered using translucent plastic sheets and placed under screen house conditions (26°C - 28°C) for 24 h. After 7 to 10 days of infection, assessment of rice blast disease severity was done using a 0 – 9 scale (IRRI, 1996). Pathogenicity of the suspected isolates of *P. oryzae* was confirmed by re-isolation and re-inoculation to the rice plants. The fungal isolates with disease severity score value more than 4 were selected for further tests.

4.2.3 *In vitro* test of *Trichoderma asperellum* and *Bacillus subtilis* against the growth of *Pyricularia oryzae*

The dual culturing technique was used to test the antagonistic effect of *T. asperellum* and *B. subtilis* against *P. oryzae* (Krishna, 2016). The inoculum was prepared as described by Hubert *et al.* (2015). A sterile cork borer (5 mm in diameter) was used to make holes diametrically opposite to a 5 mm disc of the test pathogen. Three concentrations (0.5, 1.0 and 2.0 ml/L) of *T. asperellum B. subtilis* and Linkimil 72 WP were placed singly into the media holes. Petri plates inoculated with *P. oryzae* alone were used as negative controls and Petri plates inoculated with *P. oryzae* and Linkimil 72 WP were used as positive controls. The inoculated Petri dishes were sealed using sealing tape and incubated at 20˚C, 25˚C and 28˚C in alternating cycles of 12-hour light and darkness.

The layout of the experiment was 4 x 3 x 3 factorial in a completely randomized design (CRD) with four replications where; factor (i) was *P. oryzae* inhibition treatments: (i – 1) = *T. asperellum* (i – 2) *B. subtilis,* (i – 3) = Linkimil 72 WP and (i – 4) = negative control (sterile distilled water). Factor (ii) consisted of concentrations of *T. asperellum, B. subtilis* and Linkimil 72 WP: (ii – 1) = 0.5 ml/L (ii – 2) = 1.0 ml/L and (ii – 3) = 2.0 ml/L. Temperature was used as the third factor (iii) where: (iii – 1) = 20˚C, (iii – 2) = 25˚C and (iii – 3) = 28˚C. Data on colony diameter (ø) growth of *P. oryzae* was recorded for each plate after every 28 hours for up to 14 days, following procedures of Hubert *et al*. (2015) with modifications. Fungal colony radii towards the antagonistic colony were measured using a 300 mm ruler. Percentage growth inhibition of *P. oryzae* by microbial agents was calculated using the formula (i) described by Ru and Di (2012) as shown below:

$$N = \frac{(Lc\ - Lp)}{Lc} \times 100\,\% \quad \text{...} \quad \text{(i)}$$

Where N is the percentage inhibition, Lc is the radius of *P. oryzae* in the negative control and the Lp is the radius of *P. oryzae* in treated dishes. Determination of antagonistic activity was done using a scale developed by Soytong, (1988) as cited by Sharfuddin and Mohanka (2012). The percentage inhibition radial growth (PIRG) was described as; > 75 % = very high antagonistic activity, 61 – 75 % = high antagonistic activity, 51 – 60 % = moderate antagonistic activity, < 50 % = low antagonistic activity and 0 = no antagonistic activity.

4.2.4 ***In vivo* evaluation of *Trichoderma asperellum* and *Bacillus subtilis* against *Pyricularia oryzae***

Rice seeds of four upland rice genotypes were planted in 4 - litre plastic pots filled with heat sterilized soil (4 seeds/pot). After germination, seedlings were thinned to two plants per pot at 10 days after emergence. Inoculation with *P. oryzae* was performed when rice seedlings were at the age of 14 days after emergence (Akagi *et al.*, 2015). At seven and fourteen days after inoculation with *P. oryzae*, 1 ml/L of a water suspension formulation of *T. asperellum, B. subtilis* and Linkimil 72 WP (a broad - spectrum protectant and curative fungicide) were sprayed on rice seedlings. Non - sprayed rice plants were treated as a negative control, while positive control plants were sprayed with Linkimil 72 WP (4 g/L). The experiment was laid out in a 4 x 4 factorial in a CRD where; factor (i) was blast disease control treatments: (i - 1) = *T. asperellum* (i - 2) = *B. subtilis,* (i - 3) = Linkimil 72 WP and (I - 4) = No spray. Factor (ii) was rice genotypes where: (ii - 1) = Supa, (ii - 2) = Kihogo, (ii - 3) = Usiguse and (ii - 4) = Lunyuki. The assessment of rice blast disease incidence and severity was done at 2 and 3 weeks after inoculation for leaf blast disease, and at 3 weeks after heading for the neck and panicle blast disease (Gana *et al.*, 2014). Plants were visually evaluated by using a scale of 0 - 9 developed by (IRRI, 1996). Disease incidence was calculated using the formula (ii) and rice blast disease severity scores were converted into per cent disease using the formula (iii) described by Magar *et al.* (2015).

$$\text{Disease incidence} = \frac{\text{Number of diseased leaves}}{\text{Total number of inspected leaves}} \text{ x } 100\,\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

$$\%\ \text{Disease severity} = \frac{\text{Sum of scores x } 100\,\%}{\text{Total number of observations x highest number on the rating scale}} \ldots \text{(iii)}$$

4.2.5 Data analysis

Data on rice blast disease incidence and percentage disease severity were ArcSine transformed before analysis. Before the transformation, all 0% values were replaced by (1/4n) and all 100% values by (100 – 1/4n), where n is the number of units upon which the percentage data were based (Gomez and Gomez, 1984). Logarithmic transformation was performed on the number of lesions per leaf, number of tillers per plant, and number of panicles per plant. Log (X+1) was used for all values instead of Log X, where X represented the original data (Gomez and Gomez, 1984). All data were subjected to analysis of variance and the means were compared using Tukey's test at P≤ 0.05. All statistics were performed using the Genstat Computer Software, 15th Edition (2012).

4.3 Results

4.3.1 *In vitro* evaluation of *Trichoderma asperellum* and *Bacillus subtilis* against the growth of *Pyricularia oryzae*

The effect of various treatments used in the study on the inhibition of mycelia radial growth is shown in Table 4.1. Significant differences (P ≤ 0.05) were observed between *P. oryzae* growth inhibition treatments and temperature (Table 4.1, Figures 4.1 and 4.2, Plate 4.1). *Trichoderma asperellum* and *Bacillus subtilis* showed similar growth inhibition of *P. oryzae* (Figure 4.1). The interaction between growth inhibition treatments and the temperature was significantly different (P≤ 0.05) (Table 4.1). Different concentrations of

T. asperellum and *B. subtilis* showed similar inhibition of growth of *P. oryzae* (Plate 4.2 and Figure 4.3).

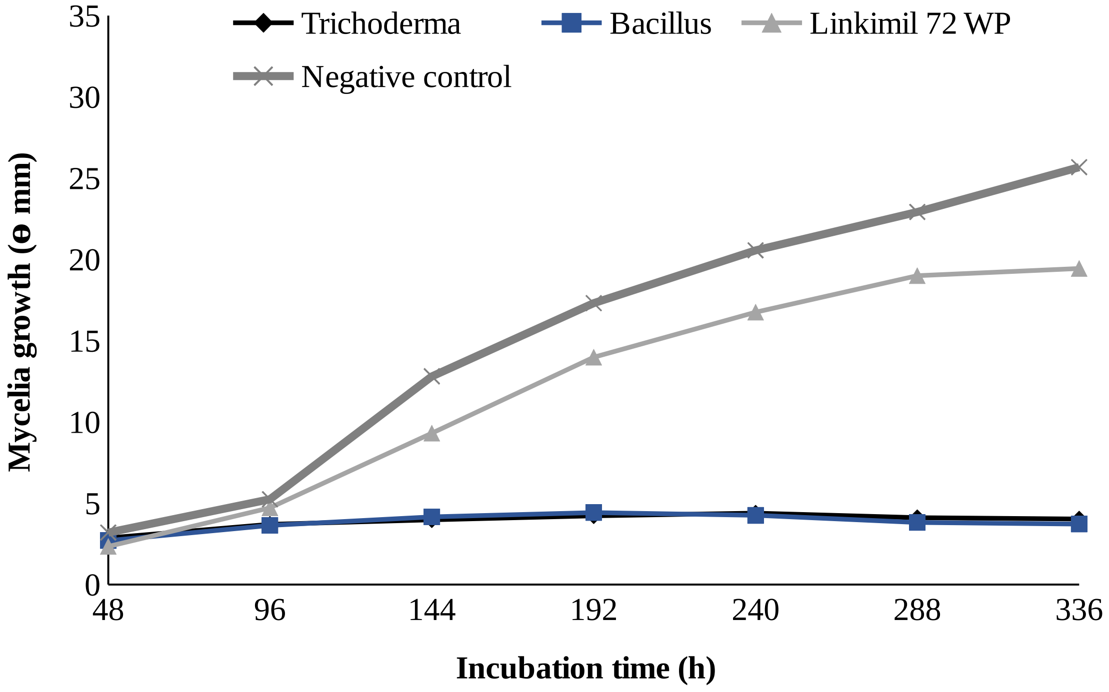

Figure 4.1: The effect of *Trichoderma asperellum*, *Bacillus subtilis*, Linkimil 72WP and negative control on radial growth of *Pyricularia oryzae* after incubation for 14 days. Bar values are means ± standard error of means.

Table 4.1: Analysis of variance of the effect of different treatments, concentrations and temperature on mycelia radial growth inhibition of *Pyricularia oryzae*

Treatments	**df**	**Mycelia radial growth of *Pyricularia oryzae***											
		96 h		**144 h**		**192 h**		**240 h**		**288h**		**336 h**	
		F	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
M	3	17.3	<.001	199.9	<.001	526.3	<.001	596	<.001	464.99	<.001	650.64	<.001
C	2	3.97	0.022	0.01	0.994	2.22	0.11	2.03	0.137	2.5	0.087	0.84	0.437
T	2	102	<.001	69.37	<.001	85.99	<.001	87.37	<.001	64.68	<.001	73.31	<.001
M x C	6	2.88	0.012	1.03	0.408	2.9	0.01	2.07	0.062	2.35	0.036	1.03	0.407
M x T	6	10	<.001	6.82	<.001	6.74	<.001	8.97	<.001	10.66	<.001	10.85	<.001
C x T	4	6.25	<.001	1.17	0.327	1.1	0.36	0.48	0.748	0.34	0.849	1.31	0.271
M x C x T	12	2.19	0.017	1.17	0.313	2.05	0.03	2.19	0.017	1.26	0.251	1.47	0.148

M= Growth inhibition treatments, C= Concentration, T= Temperature, F= F, value, P= Probability

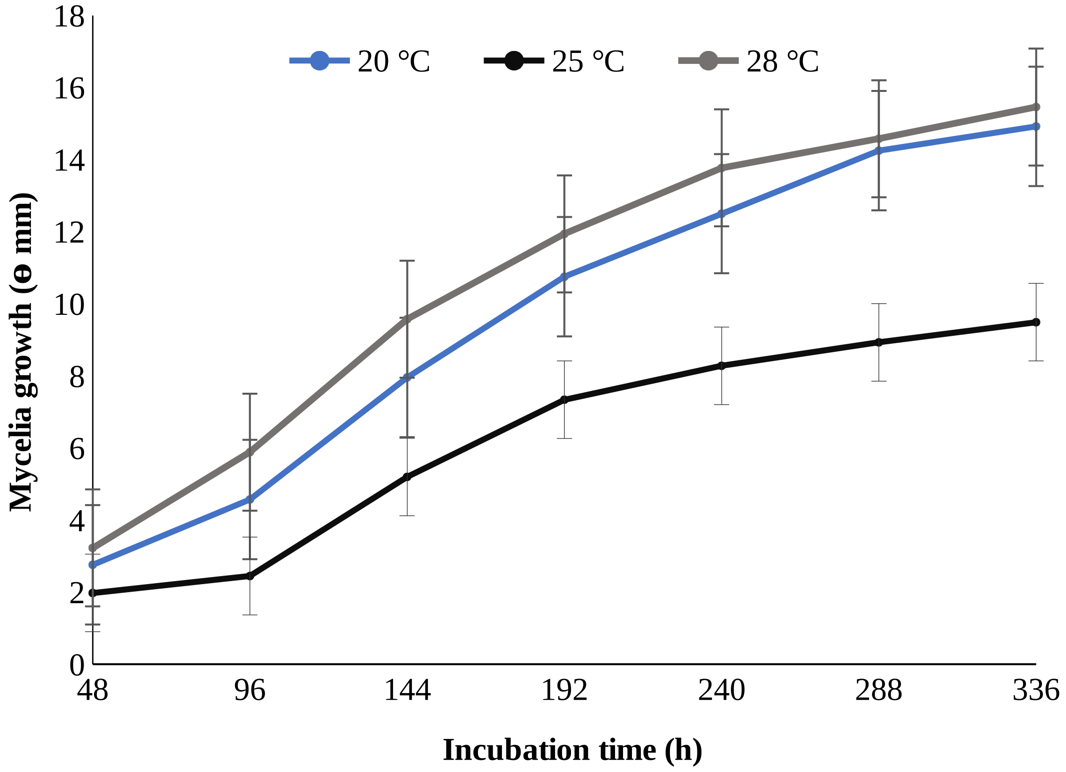

Figure 4.2: The effect of different exposure temperature on radial growth of *Pyricularia oryzae* after incubation for 14 days. Bar values are means ± standard error of means.

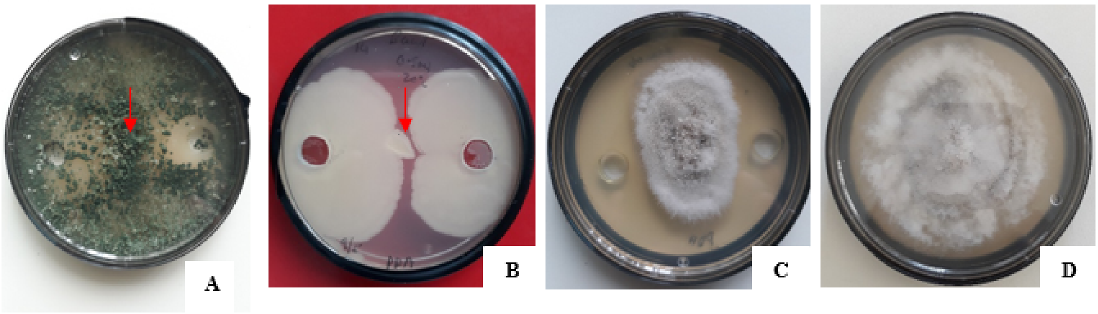

Plate 4. 1: Effect of A = *Trichoderma asperellum*, B = *Bacillus subtilis*, C = Linkimil 72WP and D = negative control) on inhibition of growth of *Pyricularia oryzae* after incubation for 14 days

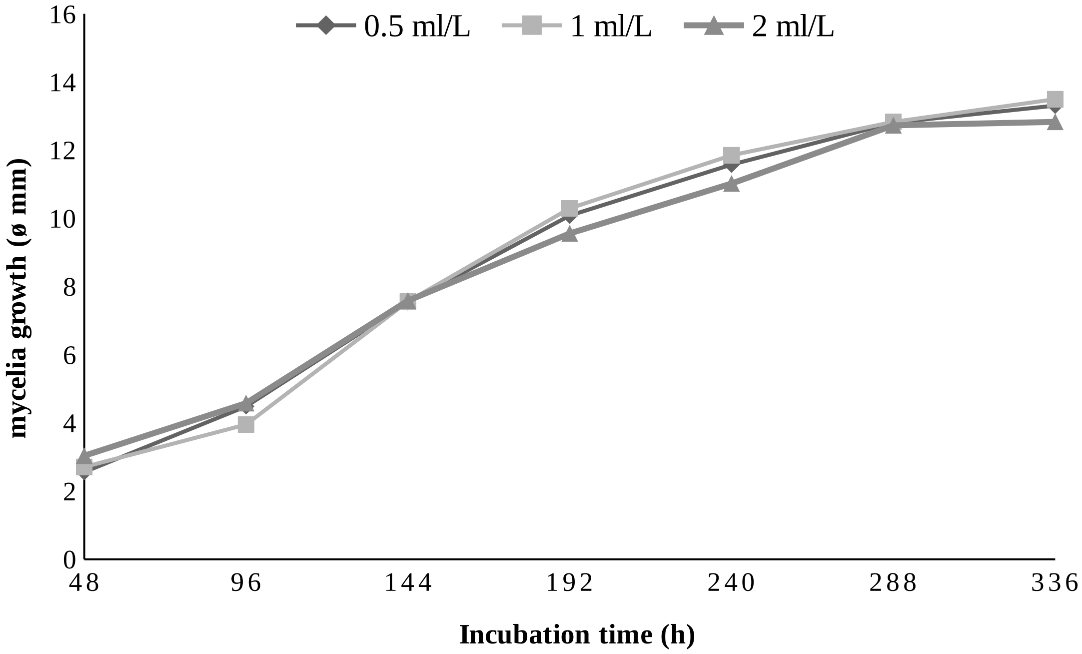

Figure 4.3: The effect of different concentrations of *Trichoderma asperellum, Bacillus subtilis* and Linkimil 72 WP on radial growth of *Pyricularia oryzae* after incubation for 14 days. Each line indicates the average values for three concentrations. Bar values are means ± standard error of means.

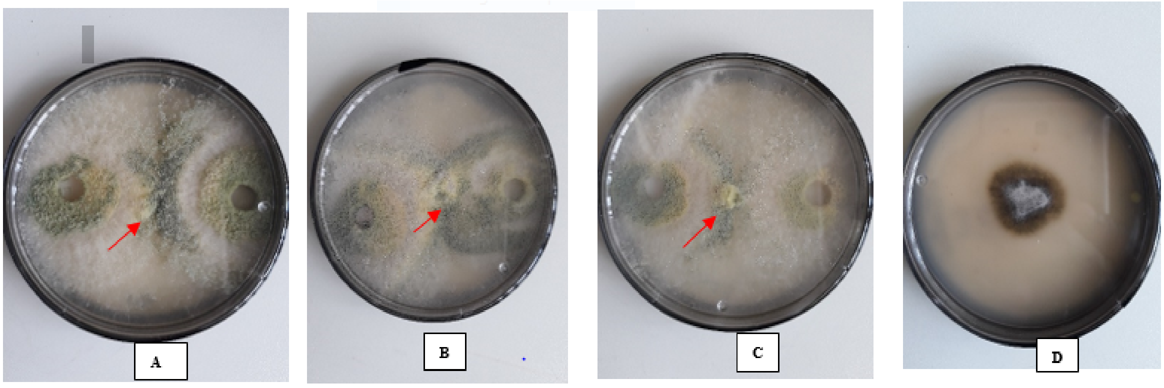

Plate 4.2: The effect of different concentrations of *Trichoderma asperellum* (A = 2.0 ml/L, B = 1.0 ml/L, C = 0.5 ml/L and D = negative control) on growth inhibition of *Pyricularia oryzae* (at red arrow)

4.3.1.1 Effect of different treatments and temperature on inhibition of mycelia radial growth of *Pyricularia oryzae*

Trichoderma asperellum and *B. subtilis* at 20˚C, 25˚C and 28˚C showed a similar percentage radial growth inhibition (PIRG) of *P. oryzae* ranging from 82 to 88% (Figure 4.5). The highest PIRG was 88.1% by *B. subtilis* at 20˚C and 86% by *T. asperellum* at 25˚C. Results also showed that Linkimil 72 WP had significantly lower ($P \leq 0.05$) PIRG than *B. subtilis* and *T. asperellum* at 20˚C, 25˚C and 28˚C.

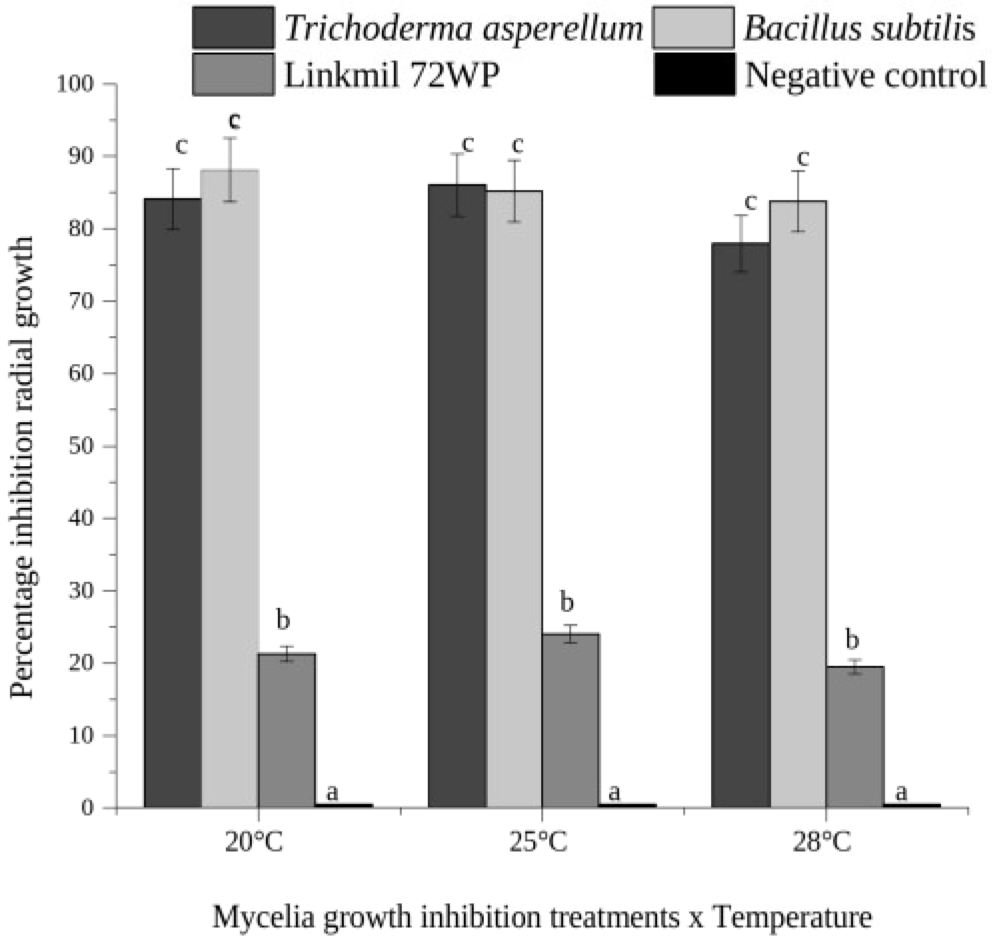

Figure 4.4: Percentage inhibition of radial growth of *Pyricularia oryzae* by *Trichoderma asperellum, Bacillus subtilis* and Linkimil 72 WP in dual cultures. Bar values are means ± standard error of means.

4.3.2 *In vivo* evaluation of microbial agents for control of rice blast disease

4.3.2.1 Rice blast disease incidence and severity

Results indicate that there were significant differences ($P \leq 0.05$) on the incidence of rice blast disease between microbial agents and Linkimil 72 WP (Figure 4.6). Rice blast disease incidence was low on rice plants treated with *T. asperellum* (18.70 %) followed by *B. subtilis* (37.3 %) and Linkimil 72 WP (62.3%) compared to the control (No spray) (88.8 %). Results also indicated significant differences between blast management methods ($P \leq 0.05$) on disease severity (Figure 4.7). Rice blast disease severity on rice plants treated with *T. asperellum* (24 %) and *B. subtilis* (30.5 %) did not differ significantly ($P \leq 0.05$).

Significantly low disease severity ($P \leq 0.05$) was observed on plants treated with Linkimil 72 WP (12.1 %) compared to the negative control (59.6 %) (Figure 4.7). Significant differences ($P \leq 0.05$) on blast disease severity were observed in the interaction between blast management methods and four rice genotypes (Table 4.4). Rice blast disease severity was significantly lower on Supa (12.9 %) and Kihogo (12.9 %) rice genotypes when treated with Linkimil 72WP (Table 4.2).

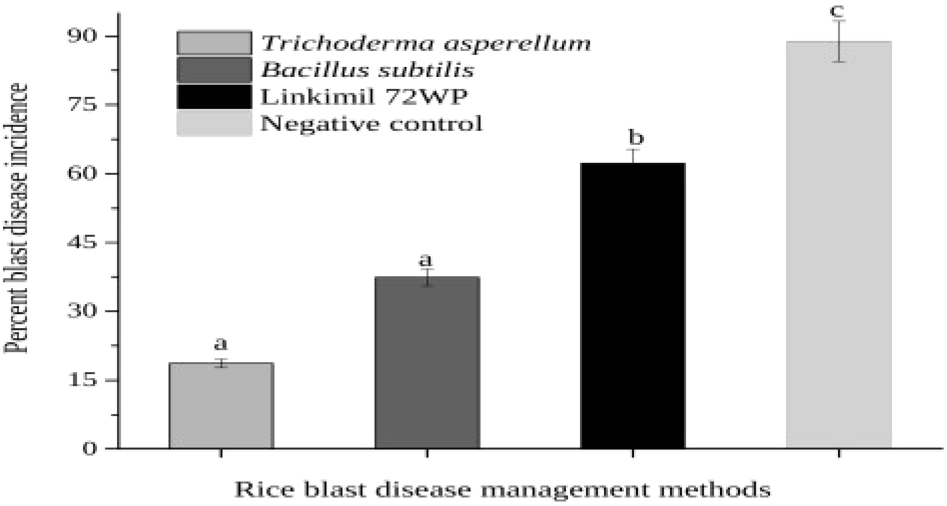

Figure 4.5: The effect of different blast disease management methods on the incidence of rice blast disease on different rice genotypes. Bar values are means ± standard error of means. Means with the same letters are not significantly different according to Tukey's HSD test at P < 0.05.

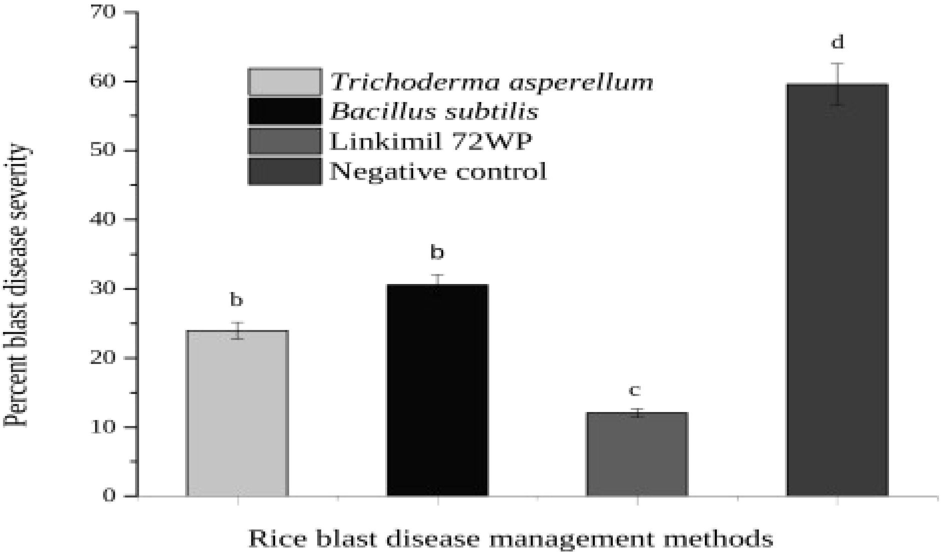

Figure 4. 6: The effect of different blast disease management methods on the severity of rice blast disease on different rice genotypes. Bar values are means ±

standard error of means. Means with the same letters are not significantly different according to Tukey’s HSD test at P < 0.05.

4.3.2.2 Number and size of lesions per leaf

The average number of lesions per leaf differed significantly (P ≤ 0.05) between different rice blast disease management treatments. The number of lesions per leaf was low on rice plants treated with *T. asperellum (*8) followed by Linkimil 72 WP (11) and *B. subtilis* (25) compared to the negative control (58) (Table 4.2).

The lesion size on plants treated with *T. asperellum B. subtilis* and Linkimil 72 WP did not differ significantly (P ≤ 0.05), while the largest lesion size was on the rice plants treated with negative control (46.3 mm) (Table 4.2). The lesion size on the interaction between blast management methods and rice genotypes was not significantly different (P ≤ 0.05). However, the lesion size (11.0 mm) on the interaction between the negative control and Lunyuki rice genotype was significantly smaller (P ≤ 0.05) than those on rice genotypes Supa (54.0 mm), Kihogo (58.4 mm) and Usiguse (61.6 mm) (Table 4.2).

Table 4.2: The interaction effect of microbial agents and rice genotypes on blast disease incidence, severity and lesion size

	Blast Disease Incidence (%)				**Blast Disease Severity (%)**				**Lesion Size (mm)**			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Supa	15.2 a	18.7 a	36.2 abc	85.5 d	25.4 abcd	38.4 cd	12.9 a	68.8 ef	4.4 a	7.1 a	5.2 a	54.0 b
Kihogo	18.7 a	52.9 abc	84.1 abcd	99.5 bcd	36.7 bcd	38.6 cde	12.9 a	70.5 f	5.4 a	11.9 a	9.7 a	58.4 b
Usiguse	31.2 ab	39.4 abc	77.3 abcd	99.9 cd	29.5 abcd	43.4 def	12.9 a	68.6 ef	4.9 a	6.6 a	6.5 a	61.6 b
Lunyuki	15.9 a	42.2 abc	51.6 abc	85.7 abcd	14.4 ab	17.0 abc	11.5 a	46.0 d	2.5 a	2.1 a	1.3 a	11.0 a

For each column, means followed by a common letter are not significantly different at $P \leq 0.05$ according to Tukey’s test

T1 = *Trichoderma asperellum*, T2 = *Bacillus subtilis*, T3 = Linkimil 72 WP, T4 = Negative control

4.3.2.3 Number of effective tillers per rice plant

The number of effective tillers per plant did not differ considerably between blast management methods ($P \leq 0.05$). The lowest number of tillers per plant (2 tillers) was observed on rice plants treated with the negative control (Table 4.3).

4.3.2.4 Percentage filled and unfilled grains

A significant difference between blast management methods ($P \leq 0.05$) was observed on the percentage of filled grains. However, the percentage of filled grains of rice plants treated with *T. asperellum* (71.6 %), *B. subtilis* (73.8 %) and Linkimil 72 WP (73.3 %) were higher than the negative control (57.4 %) (Table 4.3). Significant differences ($P \leq 0.05$) between blast management methods were observed in the percentage of unfilled grains (Table 4.3).

4.3.2.5 Panicle and 1,000 grain weight

The average weight of panicles did not differ significantly ($P \leq 0.05$) for rice plants treated with *T. asperellum B. subtilis* and Linkimil 72 WP. The lowest panicle weight was observed on rice plants treated with the negative control (5.8 g) (Table 4.3). The same trend was observed on 1,000 grains weight where rice plants treated with *T. asperellum* (8.7 g), *B. subtilis* (10.2 g) and Linkimil 72 WP (10.1 g) did not differ significantly ($P \leq 0.05$) (Table 4.3).

4.3.2.6 Dry shoot weight

Results indicate significant differences in dry shoot weight among blast management methods ($P \leq 0.05$). However, there were no significant differences between *T. asperellum*

(23.6 g), *B. subtilis* (26.3 g) and Linkimil 72 WP (23.3 g) and the negative control (Table 4.3).

Table 4.3: The effect of different rice blast disease management methods on per cent filled grains, unfilled grains, panicle weight, grain weight and dry shoot weight

Source of variation	**Number of lesions/leaf**	**Lesion size (mm)**	**Number of tillers/plant**	**Filled grains (%)**	**Unfilled grains (%)**	**Panicle weight (g)**	**Grain weight (g)**	**Dry shoot weight (g)**
Trichoderma asperellum	8.0 a	4.3 a	3.0 a	71.6 b	29.0 a	9.6 b	8.7 b	23.6 b
Bacillus subtilis	25.0 b	6.9 a	3.0 a	73.8 b	27.5 a	11.0 b	10.2 b	26.3 b
Linkimil 72WP	11.0 a	5.7 a	3.0 a	73.3 b	27.6 a	10.5 b	10.1 b	23.3 b
Negative control	58.0 c	46.3 b	2.0 a	57.4 a	43.2 b	5.8 a	5.4 a	17.5 a
Mean	19	15.8	2.51	69	31.8	9.2	8.6	22.7
S.E.	2.2	12.8	0.13	0.1	12.6	2.5	2.5	4.1
CV%	26.9	81.3	32.4	14.3	39.7	27.5	28.5	18.1

For each column, means followed by a common letter are not significantly different at $P \leq 0.05$ according to Tukey’s test

4.4 Discussion

Biological control using antagonistic fungi has been widely used to manage several plant disease-causing pathogens. Fungi belonging to the genus *Trichoderma* and bacteria such as *Bacillus subtilis* have been reported as the most effective biocontrol agents against a wide range of plant pathogens (Bhattacharjee and Dey, 2014). In this study, different concentrations of *T. asperellum* showed similar inhibition of radial growth of *P. oryzae* within five days of incubation. Such findings are in agreement with the study by Ali and Nadarajah (2014) which indicated that the dual culture assays of *Trichoderma* had the highest degree of inhibition of *P. oryzae In vitro* within four days. *Trichoderma* spp. has been reported to use more than one mechanism in the biocontrol activity. Such mechanisms include competition for nutrients, predation against pathogens (mycoparastism), stimulation of plant growth and immune response to induce resistance to diseases and the release of volatile antibiotics and hydrolytic enzymes such as chitinase and β - 1, 3 - glucanase (Awad, 2015; Krishna, 2016).

Hydrolytic enzymes have been reported to degrade the pathogen cell wall that aid to mycoparasitism (Krishna, 2016). The inhibition of mycelia growth could be due to competition for nutrients, antibiosis or mycoparasitism. In this study, Linkimil 72 WP had the lowest percentage of radial growth inhibition of *P. oryzae* (Figure 4.5). According to Ru and Di (2012), the percentage radial growth inhibition of *P. oryzae* by *T. asperellum* and *B. subtilis* in the current study indicated high antagonist activity. These results are in agreement with those reported by de Oliveira *et al.* (2016). This indicates that both *T. asperellum* and *B. subtilis* were the best in inhibition of radial growth of *P. oryzae* in dual inoculation.

This study indicated that the incidence of rice blast disease was reduced on plants treated with *T. asperellum* followed by *B. subtilis* and Linkimil 72 WP compared to the negative control. On disease severity, the percentage decrease in disease severity on *T. asperellum* and *B. subtilis* treated rice plants were lower than Linkimil 72 WP treated plants. These results agree with previous studies (Suprapta *et al.*, 2014; Krishna, 2016) which showed that reduction in blast disease incidence was significantly lower in rice plants treated with a fungicide (Benlate) than those treated with a bioagent (*Trichoderma viride*). In this study, significant differences in disease incidence were not observed in the interactions between *T. asperellum B. subtilis,* Linkimil 72WP and four rice genotypes, indicating that *T. asperellum* and *B. subtilis* were able to reduce rice blast disease incidence regardless of rice genotypes grown.

The number of lesions per leaf did not differ significantly on rice plants treated with *T. asperellum* and Linkimil 72 WP ($P \leq 0.05$). However, the number of lesions per leaf on rice plants treated with *B. subtilis* was higher than those on *T. asperellum* and Linkimil 72 WP treated plants (Table 4.2). The increased number of lesions per leaf reduces the photosynthetic rate by reducing the leaf area (Bastiaans and Roumen, 1993). The enlargement of the lesion on rice leaves due to blast disease has been reported to reduce photosynthesis through a reduction in the green leaf area and green leaf tissues surrounding the lesions (Debona *et al.*, 2014). The results further showed that lesion size decreased on rice plants treated with *T. asperellum* followed by Linkimil 72 WP and *B. subtilis* compared to rice plants which were not sprayed. The reduction of rice blast disease lesion size by application of *T. harzianum* and *T. viride* has also been reported by other workers (Krishna, 2016).

The effect of different rice blast management methods on the number of tillers per plant did not differ significantly ($P \leq 0.05$). The smallest number of tillers per plant was observed on rice plants which were not sprayed. The small differences observed on the number of tillers per plant could be due to a low range of genetic characteristics of upland rice genotypes (Sester *et al.*, 2008). However, furher studies are needed to confirm such preposition.

Results of this study indicated significant differences ($P \leq 0.05$) between blast management methods on the percentage of filled grains. However, there were no statistically significant differences on the percentage of filled grains observed between *T. asperellum*, *B. subtilis* and Linkimil 72 WP, indicating that the two microbial agents were similar with Linkimil 72 WP in reducing the effects of rice blast disease on grain filling (Table 4.3). The same trend was observed in the percentage of unfilled grains. However, the highest percentage of unfilled grains were observed on rice plants which were not sprayed (Table 4.3). Rice blast disease affected the photosynthetic rate and have been reported to contribute to the high percentage of unfilled grain due to reduced carbohydrate supply during grain filling (Bastiaans, 1993a; Chuwa *et al.*, 2015).

This study showed that the average rice panicle weight did not differ significantly ($P \leq 0.05$) between rice blast management methods for rice plants treated with *T. asperellum*, *B. subtilis* and Linkimil 72 WP. However, significantly low panicle weight was observed on rice plants which were not treated (Table 4.3). The same trend was observed on grains weight (Table 4.3). This indicates that both panicle and grain weight was greatly affected by rice blast disease on leaves. This affected the rate of photosynthesis and contributed to reduced grain filling. Chuwa *et al.* (2015) also reported similar findings.

Significant differences in dry shoot weight were not observed between rice plants treated with *T. asperellum B. subtilis* and Linkimil 72 WP. However, high, dry shoot weight was observed on rice plants treated with *B. subtilis*. *Bacillus subtilis* have been reported to have a bio-fertilizing effect that enhances the capacity of roots to mobilize and take up nutrients and substances that improve plant growth and accumulation of biomass (Yao *et al.*, 2006). Dry shoot weight was significantly lower ($P \leq 0.05$) on rice plants which were not sprayed (Table 4.3). Such findings may be attributed by high rice blast disease severity observed in this study and accelerated senesces on infected leaf tissues that affected the accumulation of dry matter (Bastiaans, 1993a).

4.5 Conclusion

In Tanzania, *Trichoderma asperellum* and *Bacillus subtilis* have been used for controlling soil-borne disease of ornamental and vegetable plants. The potential of two commercial bioagents for management of other plant diseases such as rice blast disease was not yet evaluated in Tanzania. This study aimed at evaluating the efficacy of *Trichoderma asperellum* and *Bacillus subtilis* for inhibiting the growth of *P. oryzae* and reducing the incidence and severity of rice blast disease.

In this study, *T. asperellum* and *B. subtilis* showed high antagonistic activity against *P. oryzae* in dual culture inoculation. The effective concentrations of the two microbial agents were ranging from 0.5 - 2.0 ml/L. In the screen house experiment, *T. asperellum* and *B. subtilis* were effective in reducing rice blast disease incidence, severity, number of lesions per leaf, and size of the lesion. The two microbial agents were also effective in increasing the panicle weight, percentage of filled grains and grains weight. Therefore, these commercial *T. asperellum* and *B. subtilis* may be recommended for the integrated management of rice blast disease in Tanzania.

4.6 Acknowledgment

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References

Akagi, A., Jiang, C. J. and Takatsuji, H. (2015). *Magnaporthe oryzae* inoculation of rice seedlings by spraying with a spore suspension. *Bioprotocol* 5 (11): 1 - 5.

Ali, H. and Nadarajah, K. (2014). Evaluating the efficacy of *Trichoderma* spp. and *Bacillus substilis* as biocontrol agents against *Magnaporthe grise*a in rice. *Australian Journal of Crop Science* 8 (9): 1324 - 1335.

Awad, H., M., Hamed, E. R., Ghazi, E. A., El-Gamal, N. G. and Shehata, H. S. (2015). *Trichoderma asperellum* isolated from salinity soil using rice straw waste as biocontrol agent for cowpea plant pathogens. *Journal of Applied Pharmaceutical Science* 5 (2): 91 – 98.

Bastiaans, L. (1993a). Effects of leaf blast on photosynthesis of rice. *Netheland Journal of Plant Pathology* 99: 197-203.

Bastiaans, L. and Roumen, E. C. (1993). Effect on leaf photosynthetic rate by leaf blast for rice cultivars with different types and levels of resistance. *Euphytica* 66: 81-87.

Bhattacharjee, R. and Dey, U. (2014). An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. *African Journal of Microbiology Research* 8 (17): 1749 -1762.

Chuwa, C., Mabagala, R. and Reuben, M. S. O.W. (2015). Assessment of grain yield losses caused by rice blast disease in major rice growing areas in Tanzania. *International Journal of Science and Research* 4 (10): 2211-2218.

de Oliveira, N. I., Rodrigues, A. A. C., Moraes, F. H., de Sousa, F., Arruda, a., Corsi, M.C.F. and de Moraes Catarino, A. (2016). Isolation, identification and *In vitro* evaluation of *Bacillus* spp. in control of *Magnaporthe oryzae* comparing evaluation methods. *African Journal of Agricultural Research* 11 (19): 1743-1749.

Debona, D., Rodrigues, F. A., Rios, J. A., Martins, S. C.V., Pereira, L. F. and DaMatta, F. M. (2014). Limitations to photosynthesis in leaves of wheat plants Infected by *Pyricularia oryzae*. *Phytopathology* 104: 34 - 39.

Faivre-Rampant, O., Genies, L., Piffaneli, P. and Thareau, D. (2013). Transmission of rice blast from seeds to adult plants in a non-systemic way. *Plant Pathology* 62: 879 – 887.

Faruq, A., Amin, M., Islam, M., Islam, M. and Alam, M. (2015). Evaluation of some selected seed treatments against leaf blast, brown spot and narrow brown leaf spot diseases of hybrid rice. *Advance in Agriculture and Biology* 4 (1): 8 -15.

Gana, A. S., Dangana, S. S., Tsado, E. K. and Maji, E. A. (2014). Rice blast pathogenicity and its effect on some rice cultivars in Nigeria. *Journal of International Scientific Publications: Agriculture and Food* 2: 30 - 35.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research. John Wiley and Sons Inc. London 680 pp.

Hubert, J., Mabagala, R. B. and Mamiro, D. P. (2015). Efficacy of selected plant extracts against *Pyricularia grisea*, causal agent of rice blast disease. *American Journal of Plant Sciences* 6: 602 - 611.

IRRI (1996). Standard evaluation system for rice, 4th ed, Manila, Philippines, p. 7.

IRRI. (2016). *Magnaporthe oryzae: Rice Blast*. International Rice Research Institute Accessed on 10/06/ 2016.

Jayaraj, J., Yi, H., Liang, G., Muthukrishnan, S. and Velazhahan, R. (2004). Foliar application of *Bacillus subtilis* AUBS1 reduces sheath blight and triggers defense mechanisms in rice. *Journal of Plant Diseases and Protection* 111 (2): 115 - 125.

Jia, Y. (2009). A user-friendly method to isolate and single spore the fungi *Magnaporthe oryzae* and *Magnaporthe grisea* obtained from diseased field samples. Online. *Plant Health Progress* 2019: 1 - 5.

Kato, H. (2001). Rice blast disease. *Pesticide Outlook* 12 (1): 23 - 25.

Kipngeno, P., Losenge, T., Maina, N., Kahangi, E. and Juma, P. (2015). Efficacy of *Bacillus subtilis* and *Trichoderma asperellum* against *Pythium aphanidermatum* in tomatoes. *Biological Control* 90: 92 - 95.

Krishna, K. P. (2016). *In vitro* and *in vivo* studies on effect of *Trichoderma* species, to control the rice sheath blight disease *Rhizoctonia solani*. *Journal of Environmental Science, Computer Science and Engineering and Technology* 5 (1): 52 – 62.

Magar, P. B., Acharya, B. and Pandey, B. (2015). Use of chemical fungicides for the management of rice blast (*Pyricularia grisea*) disease at Jyotinagar, Chitwan, Nepal. *International Journal of Applied Sciences and Biotechnology* 3 (3): 474 - 478.

Mwangi, M. W., Monda, E. O., Okoth, S. A. and Jefwa, J. M. (2011). Inoculation of tomato seedlings with *Trichoderma harzianum* and arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings. *Brazilian Journal of Microbiology* 42 (2): 508 - 513.

Pooja, K. and Katoch, A. (2014). Past, present and future of rice blast management. *Plant Science Today* 1 (3): 165 - 173.

Raveloson, H., Ratsimiala Ramonta, I., Tharreau, D. and Sester, M. (2018). Long term survival of blast pathogen in infected rice residues as major source of primary inoculum in high altitude upland ecology. *Plant Pathology* 67 (3): 610 - 618.

Ru, Z. and Di, W. (2012). *Trichoderma* spp. from rhizosphere soil and their antagonism against *Fusarium sambucinum*. *African Journal of Biotechnology* 11 (18): 4180 - 4186.

Sester, M., Raboin, L. M., Ramanantsoanirina, A. and Tharreau, D. (Ed.) (2008). *Toward an integrated strategy to limit blast disease in upland rice Diversifying crop protection, La Grande-Motte, France*, 2008

Sharfuddin, C. and Mohanka, R. (2012). *In vitro* antagonism of indigenous *Trichoderma* isolates against phytopathogen causing wilt of lentil. *International Journal of Life Science and Pharma Research* 2: 195 -202.

Singh, P., Singh, A., Singh, H. and Dhakad, B. (2012). Biological control of rice blast disease with *Trichoderma harzianum* in direct seeded rice under medium low land rainfed conditions. *Environmental Ecology* 30 (3B): 834 -837.

Soytong, K. (1988). Species of Chaetomium in the Philippines and screening for their biocontrol properties against seedborne fungi for rice.Thesis for Award of PhD at UPLB, Los Banos, Philippines, 189 pp.

Sun, S., Tao, R., Sun, G., Zhang, Z. and Zheng, Z. (1997). Effects of weed-hosts of *Pyricularia* on incidence of rice blast diseases. *Acta Phytopathologica Sinica* 27 (4): 327-332.

Suprapta, D.N., Quintao, V. and Khalimi, K. (2014). Effectiveness of rhizobacteria to reduce rice blast disease intensity. *Journal of Biology, Agriculture and Healthcare* 4 (3): 35 - 41.

Webster, R. K. 2000. *Rice blast disease identification guide*. University of Calfornia, Davis. https://www2.ipm.ucanr.edu/agriculture/rice/Rice-Blast/. Accessed on 05/08/ 2017.

Yao, A., Bochow, H., Karimov, S., Boturov, U., Sanginboy, S. and Sharipov, A. (2006). Effect of FZB 24® *Bacillus subtilis* as a biofertilizer on cotton yields in field tests. *Archives of Phytopathology and Plant Protection* 39 (4): 323 -328.

Zarandi, M.E., Bonjar, G.S., Dehkaei, F.P., Moosavi, S.A., Farokhi, P.R. and Aghighi, S. (2009). Biological control of rice blast (*Magnaporthe oryzae*) by use of *Streptomyces sindeneusi*s isolate 263 in greenhouse. *American Journal of Applied Sciences* 6 (1): 194 -199.

CHAPTER FIVE

5.0 REDUCTION OF INITIAL OCCURRENCE OF RICE BLAST (*Pyricularia oryzae*) INOCULA ON SEEDS BY MICROBIAL AND HOT WATER SEED TREATMENTS

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Abstract

Rice blast disease resulting from infected rice seed can be avoided by using treated seeds. Seed treatment using chemical fungicide has many limitations such as the development of resistance to pathogens and damage to the natural environment and health of farmers and consumers. Such limitations have raised the need for alternative non-chemical seed treatment methods such as the use of antagonist microbial agent and hot water. Laboratory and screen house experiments were carried out to evaluate the efficacy of *Trichoderma asperellum*, *Bacillus subtilis* and hot water (50°C/15 min) seed treatments against *Pyricularia oryzae* inocula on rice seeds. The results showed that seeds treated with microbial pesticides (*T. asperellum* and *B. subtilis*) and hot water reduced the percentage of infected rice seeds by 4.3% and 52.7%, respectively, relative to non-treated seeds. The germination per cent and rice seedling vigour index increased by 17.1% and 12.3%. Rice seeds treated with *B. subtilis* reduced the incidence and severity of rice blast disease by 10% and 72.4%, respectively. Seed treatment using *B. subtilis* followed by *T. asperellum* was the most successful in reducing the number of infected seeds and rice blast disease incidence and severity on rice seedlings. Therefore, the use of these microbial agents has the potential for effective management of rice blast disease.

Keywords: *Bacillus subtilis,* hot water, *Pyricularia oryzae,* Seed treatment, *Trichoderma asperellum*

5.1 Introduction

Rice is the second most-produced crop in the world after maize (FAOSTAT, 2016). It is a staple food security crop of economic importance in Asian and many African countries (FAO, 2017). Some of the diseases affecting rice production include rice blast disease (RBD) caused by *Pyricularia oryzae* Cav. The disease is the main constraint to rice production worldwide (Kihoro *et al.*, 2013; Aravindan *et al.*, 2016; Mgonja *et al.*, 2016). In the absence of control measures and where susceptible cultivars are grown, *P. oryzae* can cause yield losses ranging from 60% to 100% (Kihoro *et al.*, 2013; Aravindan *et al.*, 2016).

When *P. oryzae* infects rice plants in the field at the reproductive stage (flowering and maturity) infested rice seeds are produced (Faivre-Rampant *et al.*, 2013). When used for planting, infected seeds act as the primary source of inocula resulting in poor germination and abnormal seedlings (Imolehin, 1983; Webster, 2000; Long *et al.*, 2001; Faivre-Rampant *et al.*, 2013). Spores of *P. oryzae* produced on contaminated rice seeds are transmitted to newly formed leaves and roots during seed germination (Faivre-Rampant *et al.*, 2013). Rice blast disease resulting from infested and infected rice seeds can be avoided by using treated seeds. Currently, seed treatment using chemical fungicide has resulted in many limitations such as the development of resistance to pathogens and damage to the natural environment as well as the health of farmers and consumers (Burgess and Keane, 1997; Jensen *et al.*, 2000). Such effects have raised the need for alternative non-chemical seed treatment methods such as antagonist microbial agent and hot water. Hot water seed treatment has been used successfully on different vegetable crops against different pathogens (Nega *et al.*, 2003; du Toit and Hernandez-Perez, 2005; Mtui *et al.*, 2010; Koch and Roberts, 2014){Nega, 2003 #143;Koch, 2014 #148;du Toit, 2005 #145;Mtui, 2010 #133}. Microbial seed treatment has been reported to control seed-borne pathogens and provide protection against soil-borne pathogens (Chung *et al.*, 2008; Raaijmakers *et al.*, 2009; Goudjal *et al.*, 2014). However, few reports are showing the application of hot water and microbial seed treatment for the management of rice blast disease (Faruq *et al.*, 2015). Several studies on alternative management methods of rice blast disease have focused mainly on foliar application of microbial agents (Jayaraj *et al.*, 2004; Kumar *et al.*, 2012; Singh *et al.*, 2012). The present study, therefore, was undertaken to test the efficacy of *T. asperellum, B. subtilis* and hot water seed treatment for the reduction of rice blast inocula on rice seeds.

5.2 Materials and methods

5.2.1 Plant materials

Rice genotype (Supa), susceptible to rice blast disease, was used (Chuwa *et al.*, 2015). The genotype was obtained from the International Rice Research Institute (IRRI), Dakawa, Morogoro, Tanzania.

5.2.2 Location and treatments

The experiment was conducted in the laboratory and screen house at the African Seed Health Centre, Sokoine University of Agriculture, Morogoro, Tanzania. Commercial microbial pesticides (*T. asperellum* and *B. subtilis*) were obtained from Real IPM Pvt., Nairobi, Kenya. Apron Star® (20% Thiamethoxam+2% Metalaxyl +2% Difenoconazole) was obtained from the agrochemical shop in Morogoro, Tanzania. Apron star is a commonly used seed coating fungicide in Tanzania.

5.2.3 Inoculum preparation and inoculation

Rice blast was isolated in May 2017 from infected rice leaves collected in rice fields in Morogoro and Tanga regions. Isolation and sporulation of *P. oryzae* were done as described by Mathur and Kongsdal (2003). Rice seeds were surface sterilized with 0.1% sodium hypochlorite solution for 2 minutes thereafter, rinsed with distilled water and dried on blotter paper as described by Singh *et al.* (2012). Inoculation was done by soaking rice seeds in *P. oryzae* suspension adjusted to 1×10^5 spores/ml using a Haemocytometer. Inoculated seeds were then allowed to dry on three layers of sterile blotting paper placed under the laminar flow chamber for one hour.

5.2.4 Application of seed treatments

Rice seeds were treated with a liquid suspension of *T. asperellum* (1 ml/L), *B. subtilis (*1 ml/L), hot water (50°C/15 min) and Apron Star®. Rice seeds (200 seeds per treatment) pre-inoculated with *P. oryzae* as described above, were soaked separately in suspensions of each *Trichoderma asperellum* (1 ml/L), *Bacillus subtilis* (1 ml/L) and Apron Star® (2.5 g/Kg) for one hour, as per the suppliers' recommendation. Hot water treatment was carried out by dipping seeds on a water bath at 50°C for 15 min as described by Faruq *et al.* (2015). Rice seeds soaked in sterile distilled water were used as a control. Treated seeds were dried on sterile blotter paper placed under the laminar flow chamber and allowed to dry for one hour.

5.2.5 Detection of *Pyricularia oryzae* on seeds

The blotter method and direct plating on potato dextrose agar (PDA) techniques were used to detect *P. oryzae* on pre-inoculated and treated rice seeds. Each treatment was made up of two hundred seeds (Mathur and Kongsdal, 2003). The blotter method consisted of 25 seeds per dish placed on three layers of moist sterile filter paper in 90 mm diameter Petri dishes as described by Mathur and Kongsdal (2003). Direct plating on PDA was done using 25 seeds per dish in 90 mm diameter Petri dishes. The inoculated plates were arranged into a completely randomized design (CRD) with four replications and incubated at 25°C to 26°C. After 7 days of incubation, rice seeds were examined under the stereo and compound microscopes for the presence of *P. oryzae* as described by Mathur and Kongsdal (2003).

5.2.6 Rice seed germination test

The pre-inoculated and treated seeds as described above were tested for germination using the between paper method as described by Mathur and Kongsdal (2003). Fifty seeds per treatment were placed on water-soaked filter papers. Thereafter, seeds were loosely covered with another moistened filter paper rolled together and tied with a rubber band at each end. The experiment was arranged into a completely randomized design (CRD) with four replications and incubated at 25°C to 26°C. After 10 days, the number of germinated and dead seeds, number of normal and abnormal (seedlings with deformed roots or shoots) were counted. Seedling vigour index (Vi) was determined after measuring the seedling shoot length and root length and calculated using the equation as described by Joe (2012) as shown below:

$Vi = (RL + SL) \times GP$

Where RL = root length (cm), SL = shoot length (cm) and GP = germination percentage

5.2.7 Seedling emergence, rice blast disease incidence and severity

Pre- inoculated and treated rice seeds as described above were planted in trays (24 cm x 14 cm x 48 cm), 20 seeds/tray arranged in CRD with four replications. The trays were kept under screen house conditions (26˚C to 30˚C and 75% to 90% RH). Seedlings emergence was assessed 15 days after sowing based on the presence of aboveground hypocotyls. Rice blast disease incidence and severity on rice seedlings were assessed 35 days after emergence. The percentage of disease incidence was calculated as follows:

$$\text{Percentage disease incidence} = \frac{\text{Number of plants deseased}}{\text{Total number of plants inspected}} \text{ x } 100\,\%$$

Rice blast disease severity was scored using the 0 - 9 scale (IRRI (1996). The scores were converted into per cent disease severity using the formula below:

$$\text{Percentage disease severity} = \frac{\text{Sum of scores x } 100\,\%}{\text{Total number of observations x highest number on the rating scale}}$$

5.2.8 Statistical data analysis

Analysis of variance was performed on percentage seed germination, abnormal seedlings, dead seeds, seedling vigour index, percentage disease incidence and severity. Tukey's honestly significant difference (HSD) test was used to compare means at $P \leq 0.05$. To obtain homogeneity of variance, data on percentage seed germination, abnormal seedlings, dead seeds and disease incidence were ArcSine transformed before analysis, while data on percentage seedling emergence were square root transformed before analysis (Gomez and Gomez, 1984). All analyses were conducted using Genstat software 15th Edition.

5.3 Results

5.3.1 Effects of seed treatments on infected rice seeds

Results showed that rice seeds treated with *B. subtilis, T. asperellum,* Apron star and hot water gave a similar percentage of infected seeds detected in both blotter and PDA plating techniques (Figures 5.1, 5.2 and 5.3). The percentage of infected rice seeds on microbial agent and hot water treated seeds were significantly lower (P ≤ 0.05) than the negative control (untreated seeds).

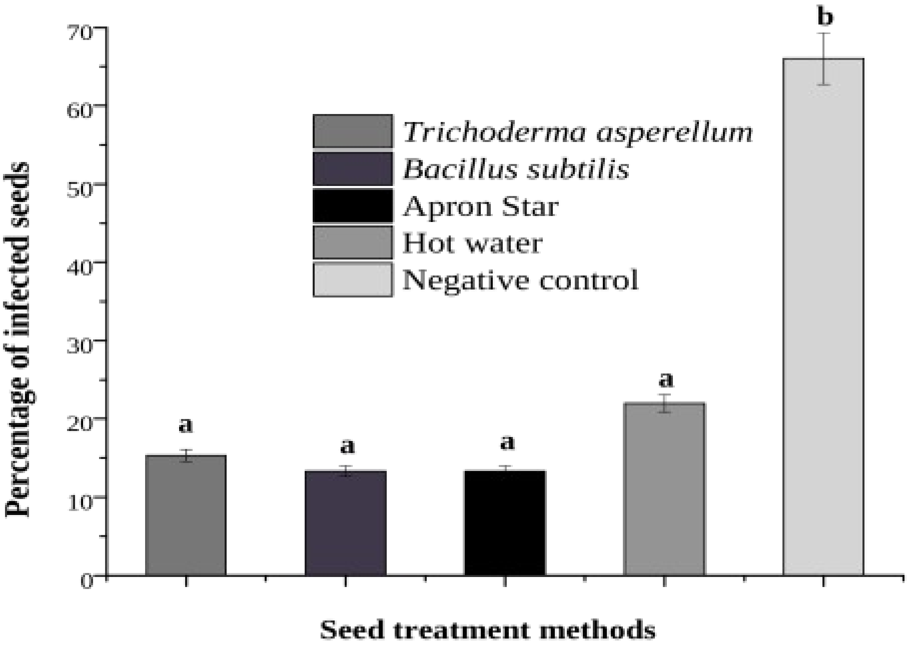

Figure 5.1: Effect of seed treatments on the percentage of *Pyricularia oryzae* infected rice seeds detected using the Blotter test method. Bar values are means ± standard error of means. Means with the same letters are not significantly different according to Tukey's HSD test at P < 0.05.

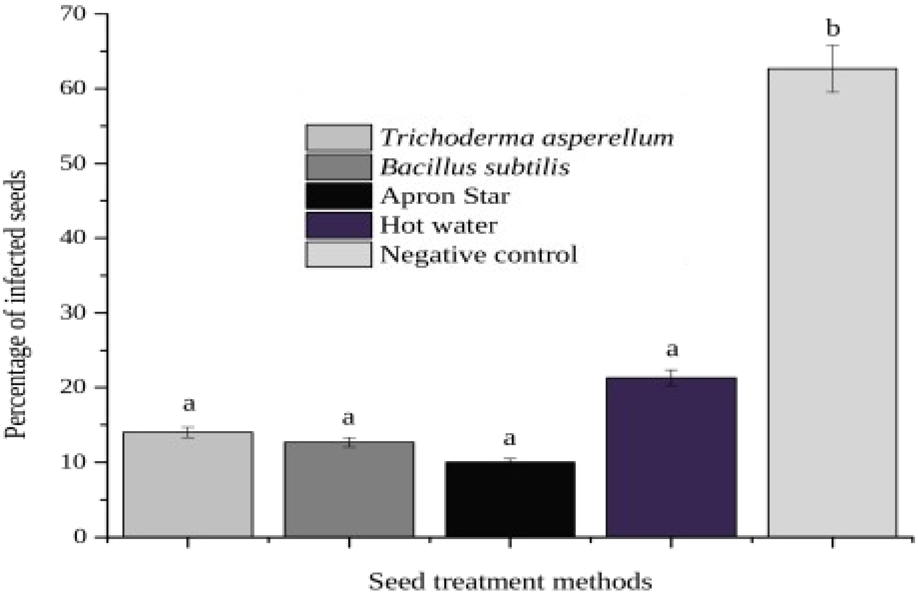

Figure 5.2: Effect of seed treatments on the percentage of *Pyricularia oryzae* infected rice seeds detected using the potato dextrose agar test. Bar values are means ± standard error of means. Means with the same letters are not significantly different according to Tukey's HSD test at P < 0.05.

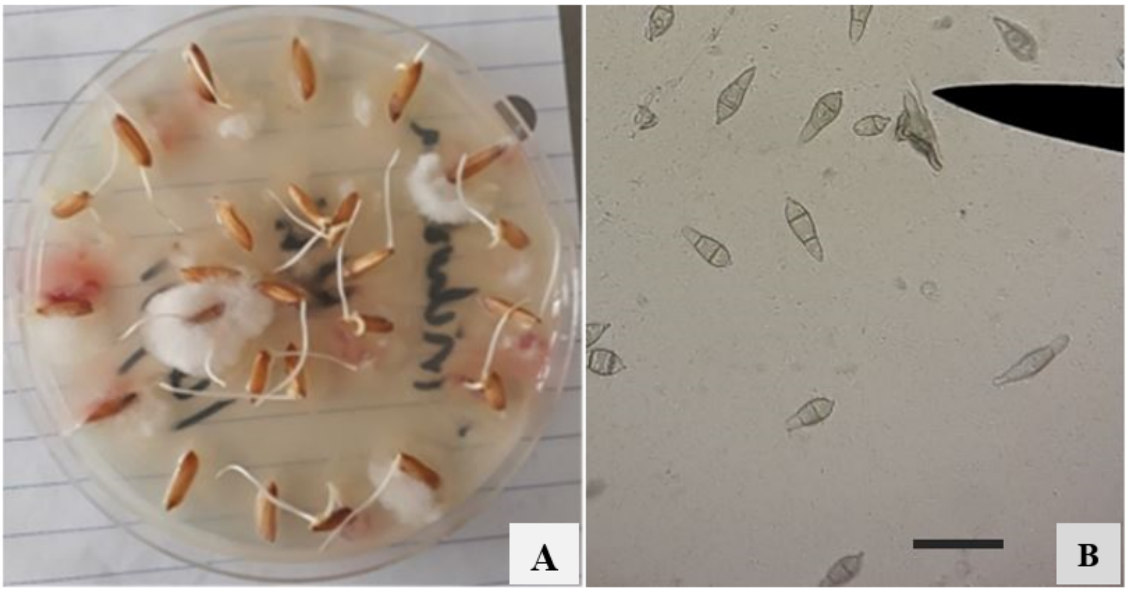

Figure 5.3: Colonies (A) and spores (B) of *Pyricularia oryzae* isolated from inoculated rice seeds on potato dextrose agar media. The mycelia and spores were visible at 5 – 10 days after incubation. Scale bar = 10µ.

5.3.2 The effect of seed treatments on seed germination and seedling performance

Results indicated that there were significant differences (P ≤ 0.05) between treatments in rice seed germination and performance of seedlings (Table 5.1). The highest seed germination was recorded when the seeds were treated with *B. subtilis* (98%) followed by Apron star® (94.9%), hot water (87%) treatments and the control (80.9%). There were significant differences (P ≤ 0.05) between seed treatments in the number of normal and abnormal seedlings, per cent of dead seeds, root length and seedling vigour index. Treatment of rice seeds with *B. subtilis, T. asperellum*, Apron star and hot water gave similar results for root length and seedling vigour. Higher levels of dead seeds were observed in hot water treated ric e seeds and the control than in rice seeds treated with *B. subtilis, T. asperellum* and Apron star (Table 5.1).

5.3.3 Seedling emergence and rice blast disease incidence and severity

Significant differences (P ≤ 0.05) were observed among rice seeds treated with microbial agents, hot water treatment, Apron Star and the negative control on disease incidence and severity (Table 5.2). Rice seeds treated with *B. subtilis, T. asperellum,* Apron star and hot water significantly reduced disease incidence and severity compared to the control (Table 5.2).

Table 5.1 Analysis of seed germination and seedling morphology after 10 days following seed treatment with *Trichoderma asperellum*, *Bacillus subtilis* and hot water

Treatments	**Germination (%)**	**Seedling performance**		**Dead seeds (%)**	**Root length (cm)**	**Shoot length (cm)**	**Seedling vigour index**
		Normal seedlings (%)	**Abnormal seedlings (%)**				
Trichoderma asperellum	94.9 bc	76.2 b	23.8 a	3.5 ab	15.2 b	12.3 a	126.5 b
Bacillus subtilis	98.0 c	82.8 b	17.2 a	1.0 a	14.6 b	12.5 a	126.1 b
Hot water	87.0 ab	58.8 ab	41.2 ab	12.8 bc	14.2 ab	11.8 a	122.1 ab
Apron Star®	98.0 c	79.6 b	18.6 a	0.5 a	15.0 b	12.7 a	126.7 b
Negatives control	80.9 a	33.5 a	64.1 b	18.7 c	12.4 a	9.3 a	113.8 a
P value	<.001	<.001	<.001	<.001	0.005	0.091	0.016
Mean	91.8	66.2	32.9	7.3	14.3	12.3	127.4
C.V	2.3	15.4	23.5	47.9	6.5	15.1	4.3
SE +/-	0.22	0.15	0.14	1.09	0.93	1.77	5.32

C.V = Coefficient of variation, S.E = Standard error of mean

Means on the same column followed by different letters are significantly different according to Tukey’s test at P ≤ 0.05.

Table 5.2: Effects of seed treatment on seedling emergence, rice blast disease incidence and severity under screen house conditions

Treatments	Emergence (%)	RBD incidence (%)	RBD severity (%)
Trichoderma asperellum	99.0 a	15.1 a	5.6 a
Bacillus subtilis	99.0 a	12.9 a	4.5 a
Hot water	95.9 a	17.7 a	5.6 a
Apron Star	99.0 a	14.4 a	5.0 a
Negative control	91.9 a	98.1 b	15.7 b
P value	0.196	<0.001	<0.001
Mean	97	31.6	7.3
C.V	2.5	17.0	23.3
SE +/-	0.24	0.10	1.69

RBD = Rice blast disease, C.V = Coefficient of variation, S.E = Standard error of mean
Means in the same column followed by the same letters are not significantly different according to Tukey’s test at P ≤ 0.05.

5.4 Discussion

Seed treatment has been reported to reduce the population of the target pathogen on the seeds. The percentage of rice seeds infected with *P. oryzae* detected using the blotter and PDA methods were similar when *B. subtilis, T. asperellum* hot water and Apron star seed treatments were used. However, the application of *B. subtilis* and Apron star on infected rice seed was more effective in reducing the percentage of infected seeds detected than *T. asperellum* and hot water treatments. A similar effect of *Bacillus* spp. and chemical fungicide on controlling rice blast disease has previously been documented (Shan *et al.*, 2013; Meng *et al.*, 2015). In this study, seeds treated with microbial agents and hot water reduced the percentage of *P. oryzae* infected rice seeds by 4.3% to 52.7% compared to the negative control. This implies that the two microbial agents and hot water treatments were effective in reducing *P. oryzae* inocula on rice seeds.

The germination of rice seeds treated with *B. subtilis* significantly increased by 17.1% followed by seeds treated with *T. asperellum (*14.0%) and hot water (7.1%).

Some of the rice seeds died due to blast disease establishment on emerging coleoptiles and primary roots. A significantly low ($P \leq 0.01$) percentage of dead seeds was observed on rice seeds treated with *B. subtilis* and Apron star. In a previous study, Faivre-Rampant *et al.* (2013) indicated that dead seeds and seedlings in the soil were the sources of *P. oryzae* infecting healthy seedlings. This indicates that rice seed treatment can reduce the percentage of dead seeds, which may act as a primary source of inocula for blast disease.

A significant increase in seedling vigour index (> 12.3%, $P \leq 0.016$) was found on rice seeds treated with *B. subtilis* and *T. asperellum*. The increased rice seedling vigour index may be attributed by increase in germination percentage in rice seeds treated with *B. subtilis* and *T. asperellum*. The fungicide Apron star was also found to increase rice seedling vigour index (12.9 %, $P \leq 0.016$). The effects of growth-promoting and antifungal activity of microbial agents have been reported to increase seedling vigour index (Andresen *et al.*, 2015). The increased seedling vigour index on *B. subtilis* and *T. asperellum* treated seeds can be partly caused by antifungal activity and growth-promoting effect of these microbial agents. *Trichoderma* spp. and *B. subtilis* have been reported to induce the growth of young rice seedlings (Ali and Nadarajah, 2014).

A significantly lower ($P < 0.001$) incidence and severity of rice blast disease were observed on microbial and hot water seed treatments than on untreated rice seeds. Together, *B. subtilis*, *T. asperellum* hot water and Apron star were significantly ($P < 0.001$) more effective in reducing the incidence and severity of rice blast disease on rice seedlings than the control. This implies that treating rice seeds with microbial and hot water reduced rice blast disease on young rice seedlings. Roumen *et al.* (1992) and Faivre-Rampant *et al.* (2013) indicated that young rice seedlings were more susceptible to blast disease than older rice plants. All rice seed treatments used in this study reduced blast disease on rice seedlings.

5.5 Conclusion

This study showed the potential of using microbial agents and hot water for reducing inoculum causing rice blast disease on rice seeds. The study indicated that infested rice seeds treated with microbial pesticides increased the germination per cent and seedling vigour index, while it reduced the incidence and severity of rice blast disease on rice seedlings. Further studies to determine the incidence and severity of rice blast disease on combinations of seed treatment and foliar application of microbial pesticides are needed. Although hot water (50°C for 15 minutes) seed treatment is simple and practical for use by farmers, there is a need to standardize the temperature and time for hot water seed treatment on different rice genotypes, to reduce any side effect that may occur on the seeds.

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References

Ali, H. and Nadarajah, K. (2014). Evaluating the efficacy of *Trichoderma* spp and *Bacillus substilis* as biocontrol agents against *Magnaporthe grise*a in rice. *Australian Journal of Crop Science* 8 (9): 1324 - 1335.

Andresen, M., Wulff, E. G., Mbega, E. R., Stokholm, M. S., Glazowska, S. E., Zida, P. E., Mabagala, R.B. and Lund, O.S. (2015). Seed treatment with an aqueous extract of *Agave sisalana* improves seed health and seedling growth of sorghum. *European Journal of Plant Pathology* 141 (1): 119 -132.

Aravindan, S., Yadav, M. K. and Sharma, P. (2016). Biological control of rice blast disease with *Trichoderma* spp. under upland rice System. *ORYZA-An International*

Burgess, D. R., Keane, P. J. (1997). Biological control of Botrytis cinerea on chickpea seed with *Trichoderma* spp. and *Gliocladium roseum*: indigenous versus non-indigenous isolates. *Plant Pathology* 46: 910 –918.

Chung, S., Kong, H., Buyer, J. S., Lakshman, D.K., Lydon, J., Kim, S. D. and Roberts, D. P. (2008). Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soilborne pathogens of cucumber and pepper. *Applied Microbiology and Biotechnology* 80 (1): 115 -123.

Chuwa, C., Mabagala, R. and Reuben, M. S. O. W. (2015). Assessment of grain yield losses caused by rice blast disease in major rice growing areas in Tanzania. *International Journal of Science and Research* 4 (10): 2211 - 2218.

du Toit, L. J. and Hernandez-Perez, P. (2005). Efficacy of hot water and chlorine for eradication of *Cladosporium variabile, Stemphylium botryosum,* and *Verticillium dahliae* from spinach seed. *Plant Disease* 89 (12): 1305 - 1312.

Faivre-Rampant, O., Genies, L., Piffaneli, P. and Thareau, D. (2013). Transmission of rice blast from seeds to adult plants in a non-systemic way. *Plant Pathology* 62: 879 – 887.

FAO (2017). Rice market monitor. Food and Agriculture Organization of the United Nation, 39 p.

FAOSTAT. (2016). Food and Agriculture Organization of the United Nations. Accessed on 01/03/ 2018.

Faruq, A., Amin, M., Islam, M., Islam, M. and Alam, M. (2015). Evaluation of some selected seed treatments against leaf blast, brown spot and narrow brown leaf spot diseases of hybrid rice. *Advance in Agriculture and Biology* 4 (1): 8 - 15.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research. John Wiley and Sons Inc. London. 680 pp.

Goudjal, Y., Toumatia, O., Yekkour, A., Sabaou, N., Mathieu, F. and Zitouni, A. (2014). Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic *Actinomycetes* isolated from native plants of Algerian Sahara. *Microbiological Research* 169 (1): 59 - 65.

Imolehin, E. (1983). Rice seedborne fungi and their effect on seed germination. *Plant Disease* 67 (12): 1334 - 1336.

IRRI (1996). Standard Evaluation System for Rice. 4th ed., Manila, Philippines, p. 7.

IRRI. (2016). *Magnaporthe oryzae: Rice Blast*. International Rice Research Institute Accessed on 10/06/ 2016.

Jayaraj, J., Yi, H., Liang, G., Muthukrishnan, S. and Velazhahan, R. (2004). Foliar application of *Bacillus subtilis* AUBS1 reduces sheath blight and triggers defense mechanisms in rice. *Journal of Plant Diseases and Protection* 111 (2): 115 - 125.

Jensen, B., Knudsen, I. M. and Jensen, D. F. (2000). Biological seed treatment of cereals with fresh and long-term stored formulations of *Clonostachys rosea*: Biocontrol efficacy against *Fusarium culmorum*. *European Journal of Plant Pathology* 106: 233 – 242.

Joe, M. M., Rashedul, I., Karthikeyan, B., Bradeepa, K., Sivakumaar, P. K. and Sa, T. (2012). Resistance responses of rice to rice blast fungus after seed treatment with the endophytic *Achromobacter xylosoxidans* AUM54 strains. *Crop Protection* 42: 141 - 148.

Kihoro, J., Bosco, N. J., Murage, H., Ateka, E. and Makihara, D. (2013). Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. *SpringerPlus* 2 (1): 308 - 318.

Koch, E. and Roberts, S. J. (2014). Non-chemical seed treatment in the control of seed-borne pathogens, Global perspectives on the health of seeds and plant propagation material. Springer, Dordrecht, pp. 105 - 123.

Kumar, K. V. K., Yellareddygari, S. K., Reddy, M., Kloepper, J., Lawrence, K., Zhou, X., Sudini, H., Groth, D., Raju, S.K. and Miller, M. (2012). Efficacy of *Bacillus subtilis* MBI 600 against sheath blight caused by *Rhizoctonia solani* and on growth and yield of rice. *Rice Science* 19 (1): 55 - 63.

Long, D., Correll, J., Lee, F. and TeBeest, D. (2001). Rice blast epidemics initiated by infested rice grain on the soil surface. *Plant Disease* 85 (6): 612 - 616.

Mathur, S. B. and Kongsdal, O. (2003). Common laboratory seed health testing methods for detecting fungi. Bassersdorf, Switzerland. 89 pp.

Meng, X., Yu, J., Yu, M., Yin, X. and Liu, Y. (2015). Dry flowable formulations of antagonistic *Bacillus subtilis* strain T429 by spray drying to control rice blast disease. *Biological control* 85: 46 - 51.

Mgonja, E. M., Balimponya, E. G., Kang, H., Bellizzi, M., Park, C. H., Li, Y., Mabagala, R., Sneller, C., Correll, J. and Opiyo, S. (2016). Genome-wide association mapping of rice resistance genes against *Magnaporthe oryzae* isolates from four African countries. *Phytopathology* 106 (11): 1359 - 1365.

Mtui, H. D., Bennett, M. A., Maerere, A. P., Miller, S.A., Kleinhenz, M. D. and Sibuga, K. P. (2010). Effect of seed treatments and mulch on seedborne bacterial pathogens and yield of tomato (*Solanum lycopersicum* Mill.) in Tanzania. *Journal of Animal and Plant Sciences* 8 (3): 1006 - 1015.

Nega, E., Ulrich, R., Werner, S. and Jahn, M. (2003). Hot water treatment of vegetable seed an alternative seed treatment method to control seed-borne pathogens in organic farming. *Journal of Plant Diseases and Protection* 110 (3): 220 - 234.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C. and Moënne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* 321 (1-2): 341 - 361.

Roumen, E., Bonman, J. and Parlevliet, J. (1992). Leaf age related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measured by the number of sporulating lesions. *Phytopathology* 82 (12): 1414 -1417.

Sester, M., Raboin, L. M., Ramanantsoanirina, A. and Tharreau, D. (Ed.) (2008). Toward an integrated strategy to limit blast disease in upland rice. *In: Diversifying crop protection, La Grande-Motte, France*, 2008.

Shan, H., Zhao, M., Chen, D., Cheng, J., Li, J., Feng, Z., Ma, Z. and An, D. (2013). Biocontrol of rice blast by the phenaminomethylacetic acid producer of *Bacillus methylotrophicus* strain BC79. *Crop Protection* 44: 29 - 37.

Singh, P., Singh, A., Singh, H. and Dhakad, B. (2012). Biological control of rice blast disease with *Trichoderma harzianum* in direct seeded rice under medium low land rainfed conditions. *Environmental Ecology* 30 (3b): 834-837.

Webster, R. K. 2000. *Rice blast disease identification guide*. University of Calfornia, Davis. https://www2.ipm.ucanr.edu/agriculture/rice/Rice-Blast/. Accessed on 05/08/2017.

CHAPTER SIX

6.0 EFFECT OF RICE BLAST DISEASE ON GRAIN YIELD OF SELECTED UPLAND RICE GENOTYPES IN TANZANIA

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Abstract

In Tanzania, rice blast disease caused by *Pyricularia oryzae* Cav. occurs in both lowland and upland ecologies. However, its effects on grain yield of upland rice are not well documented. Field trials to establish the effect of rice blast disease on six upland rice genotypes were established in Mvomero, Morogoro and Muheza in 2017/2018 and 2018/2019 rice growing seasons. The experiment was laid out in a randomized complete block design in a paired block (*P. oryzae* naturally inoculated and fungicide sprayed) with three replications. In the two seasons, the highest (41.5%) grain yield loss was recorded in Mvomero and the lowest (31.9%) was in Muheza. In 2017/2018 rice growing season, the highest (52.1%) grain yield loss was recorded on Kalongole followed by Kigunia (45.3%), Supa (40.5%), Kihogo (37.1%), NERICA 7 (30.7%) and WAB 450 (26.6%) genotypes. The same trend was followed in 2018/2019 rice growing season except that grain yield loss for NERICA 7 (34.0%) and WAB 450 (33.5%) genotypes were higher than in 2017/2018 season.

Yield loss differences per genotype and season would be linked to seasonal changes of disease severity due to changes in weather conditions. Further study on the effect of rice blast disease on upland rice genotypes through artificial inoculation is recommended.

Keywords: Blast disease, genotypes, grain yield, upland rice

6.1 Introduction

Rice (*Oryza sativa* L.) is a principal staple food and cash crop consumed by about half of the world’s population (Mohanty, 2013). Apart from Asian countries where food diversity is changing the trend in rice consumption, in Sub-Saharan Africa (SSA) rice consumption is expected to increase due to rising income and changing food preference for rice among urban and Rural inhabitants (Mohanty, 2013). In Tanzania, in 2017 rice was planted on 1,169,943 ha which produced about 2,871,963 tones (FAOSTAT, 2017). The majority of which was planted under low land rain-fed ecology, where serious losses of productivity due to rice blast disease have been reported (Balimponya, 2015; Chuwa *et al.*, 2015; Mgonja *et al.*, 2016).

Rice blast caused by an Ascomycete fungus *Pyricularia oryzae* Cav. (anamorph) *Magnaporthe grisea* Sacc (Teleomorph) is the most widespread disease in all rice-growing environment (Bonman, 1992). In severe outbreaks, the yield loss ranging from 20 to 80% have been reported (Séré *et al.*, 2013). The importance of rice in human food security and significant yield losses caused by blast disease has made blast disease widely studied in the world (Valent, 1990).

The damage caused by leaf blast includes the reduction in the active photosynthetic area during the growing stage, which results into decreased tillering, number of panicles per plant and number of grains per panicle (Bastiaans, 1991). Under severe conditions, rice blast disease can kill the whole plant. Panicle or neck blast is the most destructive form of blast disease due to the reduced grain filling and hence grain yield loss (Ou, 1980).

The selection of varieties is one of the most efficient practices to limit disease development and the impact on the crop (Titone *et al.*, 2015). This is even more important in Tanzania, where the majority of the upland rice-growing areas are cultivated with local varieties that are highly susceptible to rice blast disease but are more preferred because of their marketing and qualities such as milling, aroma and palatability (Hashim *et al.*, 2018b). Although upland rice makes about 20% of Tanzanian rice (Kanyeka *et al.*, 1994) and weather conditions favour the proliferation of rice blast disease; there is no detailed information about the effect of rice blast disease on grain yields of upland rice genotypes grown in Tanzania. This study was performed to investigate the effect of rice blast disease on grain yield loss of selected upland rice genotypes grown in Tanzania. The information obtained in this study will help the rice stakeholders to know the extent of the disease and guide on the decisions in integrated disease management programs.

6.2 Materials and Methods

6.2.1 Location

Field experiments were conducted in rice blast disease hot spot areas in Morogoro, Mvomero and Muheza districts. These sites are located between 6°14′8.22″S and

38°41′37.49″E, 6°49′15″S and 37°39′40″E and 5°00′00″S and 38°55′00″E, respectively.

6.2.2 Weather conditions during the period of the experiment

The area receives two rainfall seasons; the short season in Mvomero and Morogoro starting from November ends in January and Muheza it starts from October and ends in December. The long rain season extends from February to May which is the rice main growing season. Weather conditions during the current field experiment are presented in Table 6.1. The average maximum and minimum temperature during the two rice growing seasons (March - July 2018 and 2019) ranged between 29 to 30°C in Morogoro and Mvomero and 29 to 31°C in Muheza. Rainfall varied with location and season with 532.9 mm and 486.5 mm in Morogoro and Mvomero in 2017/2018 and 2018/2019 seasons, respectively. In the Muheza, the amount of rainfall was 738.0 mm in 2017/2018 and 578.9 mm in 2018/2019 rice growing season. The relative humidity ranged from 83 – 88% in Morogoro and Mvomero and 80 – 83% in Muheza (Table 6.1).

Table 6.1: Weather conditions in the rice cropping seasons from May to July 2018 and 2019 in the three experimental sites

Site	Season	T_{av} °C		R_{cum} (mm)	RH_{av}%
		Max	Min		
Morogoro	2017/2018	29.0	19.0	532.9	88.0
	2018/2019	30.0	20.0	486.5	83.0
Mvomero	2017/2018	29.0	19.0	532.9	88.0
	2018/2019	30.0	20.0	486.5	83.0
Muheza	2017/2018	29.0	22.0	738.0	83.0
	2018/2019	31.0	23.0	578.9	80.0

T_{av} = average temperature, RH_{av} = average relative humidity and R_{cum} = cumulated rainfall.

Source: Tanzania Meteorological Agency (2019)

6.2.3 Plant materials

Six preferred upland rice genotypes (Supa, Kihogo, Kalongole, Kigunia, WAB450 and NERICA 7) were used. Supa is a local rice genotype released in Tanzania due to its superior characteristics such as aroma, good taste and grain quality. WAB 450 and NERICA 7 are among the improved upland rice genotypes successfully released in Tanzania, mostly adopted due to high yielding and early maturity.

6.2.4 The experimental design

The experiments were conducted in the 2017/2018 and 2018/2019 rice growing seasons. The field was prepared by ploughing the land using a tractor and thereafter harrowing was done manually by a hand hoe. Four to five seeds were planted at a spacing of 20 cm x 20 cm under rain-fed conditions in a 1.5 m x 3 m plot. The experiment was set up in a randomized complete block design (RCBD) with three replications. The trial was conducted in a paired block with *P. oryzae* naturally inoculated and fungicide (Mancozeb 64% and Metalaxyl 8%) sprayed blocks (Nwanosike *et al*., 2015). Locations, treatments, replications and experimental design were similar for the two rice growing seasons. The fungicide was sprayed three times in the fungicide sprayed block beginning at 30 days after emergence and subsequently at 30 days interval to prevent the occurrence of rice blast disease. All recommended agronomical practices were performed to raise the crop as described by Kanyeka *et al.* (2007).

6.2.5 Data collection

6.2.5.1 Blast disease assessment

In this experiment, blast disease occurred through natural inoculation. Ten plants from the central rows were selected from each plot and tagged for blast disease assessments. Leaf blast severity was scored at weekly intervals starting at the tillering stage to flowering stage. Panicle blast severity was scored at a weekly interval from flowering to maturity using a 1- 9 scale described by IRRI (1996). The total number of tillers per hill and number of tillers with a typical rice blast symptom were counted. The percentage of disease incidence was calculated using formula (i) and disease severity scores were converted into percentage disease severity using formula (ii) described by Magar *et al.* (2015).

$$\text{\% Blast disease incidence} = \frac{\text{Number of diseased plants X 100\%}}{\text{Total number of plants inspected}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

$$\text{\% Disease severity} = \frac{\text{Sum of scores X 100\%}}{\text{Number of plants observed x highest number on the rating scale}} \ldots\ldots \text{(ii)}$$

The percentage blast disease severity of an experimental unit is the mean of leaf blast severity of 10 plants evaluated. Other data collected included panicle weight, grain weight /plant, number of filled grains/panicle, number of unfilled grains/panicle and straw yield/plant. Rice grain yield was determined at 12% grain moisture content and percentage grain yield losses due to rice blast disease for each rice genotype was determined using formula (iii) as described by Bregaglio *et al.* (2017).

$$\text{Yield loss (\%)} = \frac{\text{Yield of sprayed plants - Yield of unsprayed plants}}{\text{Yield of sprayed plants}} \text{ x 100\%} \ldots\ldots \text{(iii)}$$

6.2.6 Data analysis

Data from both trials were subjected to analysis of variance (ANOVA) to determine the significant differences of treatments. Where treatments differences were found significant, means were compared using the Tukey Honesty Significant Difference (HSD). All statistical analysis was performed using R software version 3.4.4 (R Development Core Team, 2015).

6.3 Results

6.3.2 Rice blast disease incidence and severity

Results showed that in the 2017/2018 rice growing season, there was no significant difference ($P \leq 0.05$) observed on leaf blast disease incidence and severity, panicle blast severity, panicle weight and grain weight between different rice genotypes (Table 6.2). However, the highest percentage leaf blast disease incidence (64.4%) and severity (39.2%) were recorded on Supa genotype (susceptible check) followed by Kihogo (62.5%, 35.3%) and Kigunia (61.4%, 35.4%). The lowest leaf blast disease incidence (54.3%) and severity (30.9%) were recorded on NERICA 7. Panicle blast disease severity was higher (58.6%) on Supa genotype and lower (36.2%) on NERICA 7 than the rest of the genotypes (Table 6.2). The highest grain weight (14.9 g/plant) was recorded on Kalongole and Supa genotypes and the lowest grain weight (11.9 g/plant) was recorded on Kihogo.

Significant differences ($P \leq 0.05$) were observed in the panicle blast disease incidence, percentage filled and unfilled grains and straw weight (Table 6.2). The highest percentage of unfilled grains (51.9%) was observed on Kihogo and the lowest (30.5%) was on WAB 450 (Table 6.2). Kihogo and Kigunia had high (72.7 g/plant) straw weight than the rest of the genotypes. The lowest straw weight (39.8 g/plant) was obtained from WAB 450.

In 2017/2018 rice growing season, significant differences ($P \leq 0.05$) were observed for leaf blast disease incidence and severity and panicle blast disease severity, percentage filled and unfilled grains and straw weight (Table 6.2). Leaf blast disease incidence between NERICA 7 (24.5%), Kalongole (39.8%) and Supa (55.5%) showed significant differences ($P \leq 0.05$). Panicle blast disease severity (64.2%) was significantly higher on Supa than on other genotypes tested (Table 6.2). The percentage of unfilled grains (47.7%) was high on Kigunia than on Kihogo (46.2%), WAB 450 (44.1%) and NERICA 7 (38.1%). A significantly ($P \leq 0.05$) higher straw weight (56.0 g/plant) was recorded on Kigunia than the other rice genotypes (Table 6.2).

Table 6.2: Effect of rice leaf and panicle blast disease incidence and severity and yield parameters of different rice genotypes during the 2017/2018 and 2018/2019 rice growing seasons

Genotypes	LBI	LBS	PBI	PBS	PW (g)	GW (g)	FG (%)	UG (%)	SW (g)
Season 2017/2018									
NERICA 7	54.3 a	30.9 a	47.4 a	36.2 a	19.4 a	13.9 a	61.3 b	38.7 a	50.7 b
WAB 450	56.9 a	33.8 a	55.3 ab	41.4 a	18.8 a	13.2 a	55.9 ab	44.1 ab	39.8 ab
Kalongole	56.2 a	34.4 a	47.4 a	38.2 a	20.8 a	14.9 a	59.3 ab	40.7 ab	72.7 b
Kigunia	61.4 a	35.4 ab	61.7 ab	39.8 a	17.2 a	12.1 a	52.3 a	47.7 b	72.7 b
Kihogo	62.5 a	35.3 ab	60.3 ab	51.7 a	18.2 a	11.9 a	53.8 ab	46.2 ab	66.0 a
Supa	66.4 a	39.2 a	83.3 b	58.6 a	21.2 a	14.9 a	61.9 b	38.1 a	64.7 b
Mean	59.6	34.8	59.2	44.3	19.3	13.5	57.4	42.6	61.1
CV%	18.1	9.3	35.4	10.4	18.8	23.9	10.2	13.7	28.9
Season 2018/2019									
NERICA 7	24.5 a	26.3 a	36.7 a	20.0 a	18.3 a	15.0 a	63.3 ab	36.7 bc	28.7 a
WAB 450	28.7 ab	31.4 ab	45.6 a	29.1 a	19.8 a	14.3 a	69.5 a	30.5 c	26.8 a
Kalongole	39.8 b	32.1 ab	33.2 a	26.0 a	20.8 a	15.1 a	66.5 ab	33.5 bc	38.0 ab
Kigunia	38.4 ab	35.3 ab	29.0 a	22.1 a	17.2 a	12.9 a	54.8 bc	45.2 ab	56.0 c
Kihogo	42.1 bc	36.5 b	39.2 a	22.3 a	18.2 a	14.9 a	48.1 c	51.9 a	49.8 bc
Supa	55.5 c	37.3 b	61.2 a	64.2 b	21.2 a	15.2 a	65.7 ab	34.3 bc	38.9 ab
Mean	38.1	33.2	40.8	30.6	19.3	14.6	61.3	38.7	39.7
CV%	26.9	24.2	5.1	19.0	18.9	20.1	16.3	25.9	28.9

LBI = leaf blast incidence, LBS = leaf blast severity, PBI = panicle blast incidence, PBS= panicle blast severity, PW = panicle weight, GW = grain weight, FG = filled grains, UG = unfilled grains, SW = straw weight. Means in the same column followed by the same letters are not significantly different at $P \leq 0.05$.

6.3.3 Effect of rice blast disease on grain yield loss

Significant differences (P ≤ 0.05) were observed on the percentage of grain yield loss between different rice genotypes studied (Table 6.3). The effect of rice blast disease in different seasons and locations on the percentage grain yield loss did not differ significantly (P ≤ 0.05). The interaction of season by location and genotype by season were significantly different at P ≤ 0.05 (Table 6.3). There was no significant difference (P ≤ 0.05) on percentage grain yield loss in the interaction between genotype by location and genotype by season by location (Table 6.3).

Table 6.3: Analysis of variance on the effect of rice blast disease on grain yield loss on different rice genotypes, locations and seasons

Source of variation	Df	MS	F	P-value
Seasons	1	95.57	0.59	0.442
Locations	2	422.21	2.64	0.078
Genotypes	5	498.24	3.11	0.014**
Season*Location	2	948.55	5.927	0.004***
Genotypes *Season	5	385.72	2.411	0.044**
Genotypes *Location	10	132.15	0.825	0.605
Genotypes * Season *Location	10	114.35	0.714	0.707
Residuals	70	160.01		

Df = degree of freedom, MS = mean sum of squares, F = F-statistics, ***significant different at P ≤ 0.01, *** P < 0.05.

The highest percentage grain yield loss (40.8%) was recorded in Mvomero in the 2017/2018 rice growing season followed by Morogoro (38.7%) and Muheza (36.6%) (Figure 6.1). In the 2018/2019 rice growing season, the percentage grain yield loss in Mvomero (42.5%) and Morogoro (40.9%) did not differ significantly (P ≤ 0.05) and the lowest percentage grain yield loss (27.1%) was recorded in Muheza. In both seasons, the highest and the lowest percentage grain yield losses were recorded in Mvomero and Muheza sites, respectively (Figure 6.1).

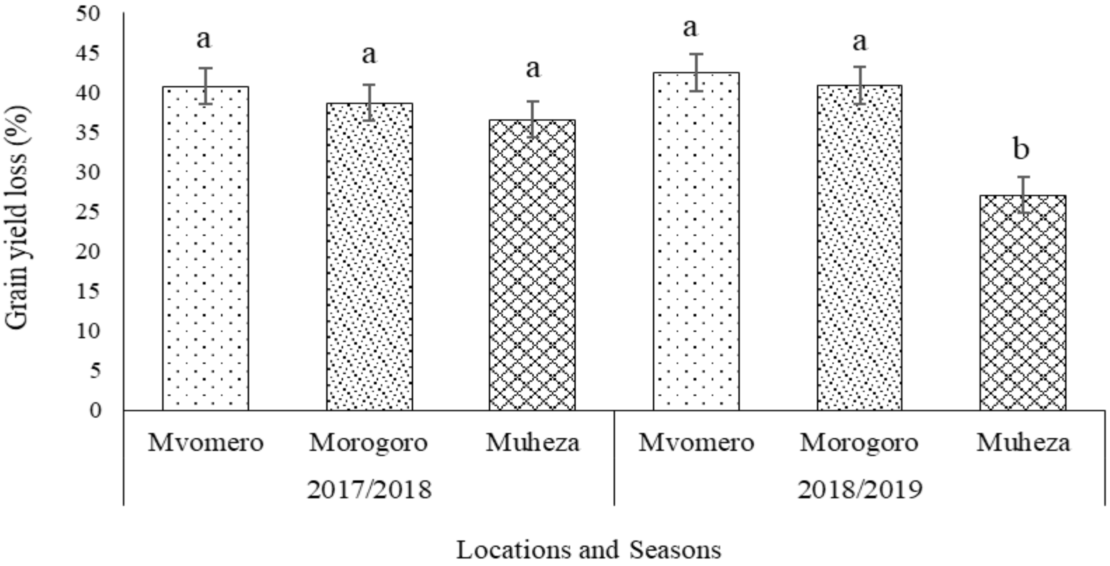

Figure 6.1: Effect of rice blast disease on percentage grain yield loss in 2017/2018 and 2018/2019 rice growing seasons at different locations. Bar values are means ± standard error of means. Means with the same letters are not significantly different according to Tukey's HSD test at P < 0.05.

The effect of rice blast disease on the percentage grain yield loss of different rice genotypes in the 2017/2018 rice growing season differed significantly ($P \leq 0.05$) (Figure 2). The highest percentage grain yield loss was 52.1% on Kalongole genotype followed by Kigunia (45.3%), Supa (40.5%), Kihogo (37.1%), NERICA 7 (30.7%) and WAB 450 (26.6%). In the 2018/2019 rice growing season, the percentage yield loss between different rice genotypes was not significantly different ($P \leq 0.05$). However, genotypes such as Kalongole, Kigunia and Kihogo had the highest percentage grain yield loss (41.4%), (39.7%) and (37.4%), respectively. Whereas; NERICA 7 and WAB 450 genotypes had the lowest percentage grain yield loss (34.0%) and (33.5%), respectively (Figure 6.2).

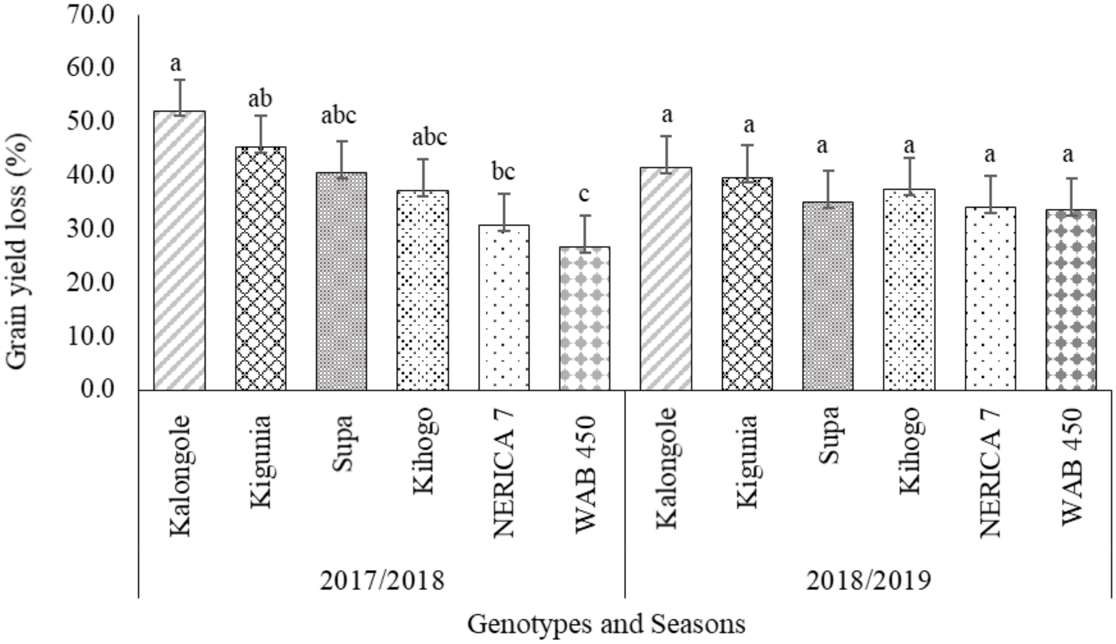

Figure 6.2: Effect of rice blast disease on percentage grain yield loss on different upland rice genotypes in the 2017/2018 and 2018/2019 rice growing seasons. Bar values are means ± standard error of means. Means with the same letters are not significantly different according to Tukey's HSD test at P < 0.05.

6.4 Discussion

In all tested six rice genotypes, a natural inoculum of *P. oryzae* caused rice blast disease incidence ranging from 24.5 to 66.4% and from 29.0 to 83.3% on leaves and panicles, respectively. In the 2017/2018 rice growing season, the severity of leaf and panicle blast disease between the improved (NERICA 7 and WAB 450) and local (Supa, Kihogo, Kigunia and Kalongole) rice genotypes was not significantly different ($P \leq 0.05$), indicating that such improved rice genotypes were not resistant to rice blast disease (Table 6.2). The differences in leaf and panicle blast disease severity were observed during the 2018/2019 rice growing season, indicating that there were differences in rice blast disease pressure within the two rice growing seasons. The variations of magnitudes of rice blast disease due to differences in disease pressure across different locations have been reported by Abebrese *et al.* (2019). The low panicle blast severity on NERICA 7 and WAB 450 compared to Supa (susceptible check), may be due to early maturity (about 90 days) which enabled them to escape the disease even under favourable conditions for severe panicle blast disease development. Such conditions include high relative humidity and high temperature at maturity. On the other hand, the high panicle blast severity on genotypes Supa and Kihogo have been attributed to late maturing (about 130 days) and high inocula build-up from the lesions on upper rice leaves (Kobayashi *et al.*, 2016).

The severity of rice blast disease has been reported to vary within rice genotypes and rice growing seasons (Tuhina-Khatun *et al.*, 2015). In this study, high (44.3%) average panicle blast severity in 2017/2018 than (30.2%) in the 2018/2019 rice growing season, may be influenced by the changes in weather conditions such as rainfall in Morogoro and Mvomero (532.9 mm to 486.5 mm) and Muheza (738.0 mm to 578.9 mm), high relative humidity in Morogoro and Mvomero (88% to 83%) and Muheza (83% to 80%) (Table 6.1). According to Luo *et al.* (1998), the changes in ambient temperature and susceptibility of local rice genotypes to rice blast disease are among the factors affecting the disease development and results in increased yield loss. The variations in leaf and panicle blast severity indicate that genetic variability exists between different rice genotypes tested. Such a phenomenon has also been reported by Zelalem *et al.* (2017).

The magnitude of yield reduction by rice blast disease has been reported to depend on the disease incidence, severity and genetic make-up of a given rice genotype (Khan *et al.*, 2014). Results from the current study showed that there was no significant difference in grain weight between the 2017/2018 and the 2018/2019 rice growing seasons. However, grain weight in the 2018/2019 rice growing season was higher than in 2017/2019 rice growing season (Table 6.2). Panicle blast disease severity has been reported as the most destructive symptoms and can be used to establish the prediction of grain yield loss (Bregaglio *et al.*, 2017). In this study, high panicle blast severity in the 2017/2018 rice growing season may have increased the percentage of grain sterility, the percentage of unfilled grain and reduced grain yield. Khan *et al.* (2014) reported similar findings that panicle blast disease severity reduced grain yield.

According to Bregaglio *et al.* (2017), rice growing season and variety are the first and second factors, respectively, contributing to the losses of grain yield and milling qualities. This agreed with the result from this study which showed that grain yield loss differed significantly ($P \leq 0.05$) between different rice genotypes and the interaction between genotype and season (Table 6.4). Generally, the disease caused 26.6 to 52.1% grain yield reduction in the two rice growing seasons. This loss is higher than the range of 10 to 41% reported in Ethiopia (Getachew *et al.*, 2014) and about 38% in low land rice in Tanzania (Chuwa *et al.*, 2015). Grain yield loss of about 60% under irrigated rice has been reported in Kenya (Kihoro *et al.*, 2013). Grain yield loss and milling qualities are highly associated with blast disease severity, with panicle blast severity being the most influencing indicator (Bregaglio *et al.*, 2017).

6.5 Conclusion and Recommendation

This study highlighted the effects of rice blast disease on selected upland rice genotypes grown under rain-fed conditions. The magnitude of the effects of rice blast disease depended on the disease pressure, which differed across locations, rice growing seasons and genotypes. Grain yield loss due to rice blast disease differed across different rice genotypes and the interaction between rice genotypes and rice growing seasons. In general, the disease caused 26.6 to 52.1% grain yield reduction in the two rice growing seasons. This study also showed that improved rice genotypes such as NERICA 7 and WAB 450 were susceptible to rice blast disease in the study area. Under field conditions, rice genotype may have been exposed to different inoculum density of *P. oryzae*. Further studies on the effect of rice blast disease on upland rice genotypes through quantified artificial inoculation is recommended.

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References

Abebrese, S.O., Yeboah, A., Dogbe, W., Dartey, P.K.A., Akromah, R., Gracen, V.E., Offei, S.K. and Danquah, E.Y. (2019). Evaluation of yield, reaction to diseases, and grain physical attributes of some introduced rice hybrids in Ghana. *International Journal of Agronomy* 1: 1 - 8.

Balimponya, E. (2015). Application of genomic selection and association mapping to breeding for resistance to rice blast and bacterial blight of rice (*Oryza* *sativa* L.), Dissertation for Award of Master of Science at Ohio State University, 122 p.

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 (6): 611 - 615.

Bonman, J. (1992). Durable resistance to rice blast disease - environmental influences, breeding for disease resistance. In: *Breeding for Disease Resistance*. (Edited by R. Johnson and G.J. Jellis) Springer, Dordrecht. pp. 115 - 123.

Bregaglio, S., Titone, P., Hossard, L., Mongiano, G., Savoini, G., Piatti, F. M., Paleari, L., Masseroli, A. and Tamborini, L. (2017). Effects of agro-pedo-meteorological conditions on dynamics of temperate rice blast epidemics and associated yield and milling losses. *Field Crops Research* 212: 11 - 22.

Chuwa, C., Mabagala, R. and Reuben, M. S. O.W. (2015). Assessment of grain yield losses caused by rice blast disease in major rice growing areas in Tanzania. *International Journal of Science and Research* 4 (10): 2211 - 2218.

FAOSTAT. (2017). *Food and Agriculture Organization of the United Nations*. [http://www.fao.org/faostat/en/#data/QC/visualize]. Accessed on 28 / 01/2019.

Getachew, G., Tesfaye, A. and Kassahun, T. (2014). Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelial growth inhibition of *Pyricularia grisea* isolates using biological antagonists and fungicides *In vitro* condition. *Journal of Applied Biosciences* 73: 5883 - 5901.

Hashim, I., Mamiro, D. P., Mabagala, R. B. and Tefera, T. (2018b). Smallholder farmers' knowledge, perception and management of rice blast disease in upland rice production in Tanzania. *Journal of Agricultural Science* 10 (7): 137 - 145.

IRRI (1996). Standard evaluation system for rice, 4th ed, Manila, Philippines, 7 p.

Kanyeka, E., Kamala, R. and Kasuga, R. (2007). Improved Agricultural Technologies in Tanzania. (1st Edition), in: COOPERATIVES, M. O. A. F. S. A. (Ed.), Dar es Salaam, Tanzania, 125 p.

Kanyeka, Z., Msomba, S., Kihupi, A. and Penza, M. (1994). Rice ecosystems in Tanzania: characterization and classification. *Tanzania Agricultural Research and Training Newsletter* 9 (1-2): 13 - 15.

Khan, M. A. I., Bhuiyan, M. R., Hossain, M. S., Sen, P. P., Ara, A., Siddique, M. A. and Ali, M. A. (2014). Neck blast disease influences grain yield and quality traits of aromatic rice. *Comptes Rendus Biologies* 337 (11): 635 - 641.

Kihoro, J., Bosco, N. J., Murage, H., Ateka, E. and Makihara, D. (2013). Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. *SpringerPlus* 2 (1): 308 - 318.

Kobayashi, T., Sasahara, M., Kanda, E., Ishiguro, K., Hase, S. and Torigoe, Y. (2016). Assessment of rice panicle blast disease using airborne hyperspectral imagery. *The Open Agriculture Journal* 10 (1): 28 - 34.

Luo, Y., Teng, P., Fabellar, N. and TeBeest, D. (1998). Risk analysis of yield losses caused by rice leaf blast associated with temperature changes above and below for five Asian countries. *Agriculture, Ecosystems and Environment* 68 (3): 197 - 205.

Magar, P. B., Acharya, B. and Pandey, B. (2015). Use of chemical fungicides for the management of rice blast (*Pyricularia grisea*) disease at Jyotinagar, Chitwan, Nepal. *International Journal of Applied Sciences and Biotechnology* 3 (3): 474 -

Mgonja, E. M., Balimponya, E. G., Kang, H., Bellizzi, M., Park, C. H., Li, Y., Mabagala, R., Sneller, C., Correll, J. and Opiyo, S. (2016). Genome-wide association mapping of rice resistance genes against *Magnaporthe oryzae* isolates from four African countries. *Phytopathology* 106 (11): 1359 - 1365.

Mohanty, S. (2013). Trends in global rice consumption. *Rice Today* 12 (1): 44 - 45.

Nwanosike, M. R. O., Mabagala, R. B. and Kusolwa, P. M. (2015). Effect of northern leaf blight (*Exserohilum turcicum*) severity on yield of maize (*Zea mays* L.) in Morogoro, Tanzania. *International Journal of Science and Research* 4 (9): 466 - 475.

Ou, S. (1980). Pathogen variability and host resistance in rice blast disease. *Annual Review of Phytopathology* 18 (1): 167 - 187.

R Development Core Team. (2015). *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing*. http://www. Rproject.org. Accessed on 10/9/2019.

Séré, Y., Fargette, D., Abo, M. E., Wydra, K., Bimerew, M., Onasanya, A. and Akator, S. K. (2013). Managing the Major Diseases of Rice in Africa. CAB International. Realizing Africa's Rice Promise pp 213 - 228.

Titone, P., Mongiano, G. and Tamborini, L. (2015). Resistance to neck blast caused by *Pyricularia oryzae* in Italian rice cultivars. *European Journal of Plant Pathology* 142 (1): 49 - 59.

Tuhina-Khatun, M., Hanafi, M.M., Rafii, M.Y. and Wong, M.Y. (2015). Reactions and diversity analysis of upland rice genotypes against blast disease of rice (*Oryza sativa* L.). *Australasian Plant Pathology* 44: 405 – 412.

Valent, B. (1990). Rice blast as a model system for plant pathology. *Phytopathology* 80 (1): 33 - 36.

Zelalem, Z., Paul, G., Jimmy, L. and Richard, E. (2017). Reaction of introduced Korean rice genotypes for resistance to rice blast in Uganda. *Journal of Plant Breeding and Crop Science* 9 (7): 98 -105.

CHAPTER SEVEN

7.0 GENERAL CONCLUSION AND RECOMMENDATIONS

7.1 General Conclusion

The purpose of this study was to enhancing rice blast disease management by gathering the baseline information about farmers' knowledge of the disease and management options used by farmers. Also, to establish the disease incidence, severity and its effect on yield of selected upland rice genotypes and the use of environmentally friendly methods such as bio-agents and hot water seed treatments.

This study revealed that farmers' lack of information, knowledge, inability to afford the cost of buying fungicides and unavailability of other effective rice blast disease management methods were among the factors limiting the effective rice blast disease management in Morogoro and Tanga regions. The continuous use of susceptible rice varieties/genotypes and improper agronomic practices were additional constraints to the management of rice blast disease in the study area.

In an attempt to establish the incidence and severity of rice blast disease in upland rice, it was noted that rice blast disease was widely distributed in the upland rice growing areas of Morogoro and Tanga regions with different magnitudes in different rice growing seasons. The highest rice blast disease incidence and severity were recorded in the 2017/2018 rice growing season. This study shows that Mvomero and Korogwe districts were the hot spot areas for this disease while Morogoro Rural and Muheza districts were the low disease severity areas. The results of this study provide bases for policy and research prioritization of rice blast disease management.

Evaluation of bio-agents for management of rice blast disease indicated that T. *asperellum* and *B. subtilis* had high antagonistic activity against the growth of *P. oryzae* in dual culture inoculation. The effective concentrations of the two bio-agents ranged from 0.5 to 2.0 ml/L. In the screen house experiments, *T. asperellum* and *B. subtilis* were effective in reducing rice blast disease incidence, severity, number of lesions per leaf and the size of blast disease lesions. The two bio-agents were also effective in increasing the panicle weight, percentage of filled grains and grains weight. Therefore, these commercial *T. asperellum* and *B. subtilis* were effective in reducing the effect of rice blast disease.

This study also evaluated the efficacy of bio-agent and hot water as seed treatments to reduce the initial inocula of rice blast disease on rice seeds. The study indicated that infested rice seeds treated with bio-agents increased the germination per cent and seedling vigour index, while it reduced the incidence and severity of rice blast disease on rice seedlings. The study showed the potential of using *T. asperellum* and *B. subtilis* and hot water for reducing inoculum of rice blast disease on rice seeds.

On the effects of rice blast disease incidence and severity on grain yield of upland rice genotypes, the magnitude of the effects of rice blast disease depended on the disease pressure, which differed across locations, rice growing seasons and rice genotypes. In general, the disease caused 26.6 to 52.1% grain yield reduction in the two rice growing seasons. Improved rice genotypes such as NERICA 7 and WAB 450 were also found susceptible to rice blast disease in the study area.

This study, therefore, gives highlights of the incidence and severity of rice blast disease, its management options using bio - agents (*T. asperellum* and *B. subtilis*) and the effects of the disease on grain yield of selected upland rice genotypes grown under rain-fed conditions in Morogoro and Tanga regions. Such information is important in designing rice blast disease management options.

7.2 Recommendations

Based on the current study, the following recommendations are put forward.

i. To improve upland rice grain yield, there is a need for strengthening the capacity of farmers through training on proper identification and management of rice blast disease.

ii. Due to high blast disease incidence in most of the surveyed areas, there is a need for regular rice blast disease surveillance to guide farmers the right time for application of appropriate disease management measures such as a combination of disease-resistant varieties, folia application and seed treatment with bio-agents and hot water to reduce the use of fungicides where needed.

iii. Commercial *T. asperellum* and *B. subtilis* had high antagonistic activity against the growth of *P. oryzae* in dual culture inoculation and reduced rice blast disease severity under screen-house conditions. Further, evaluation of the efficacy of the two bio-agents for management of rice blast disease under field conditions in Tanzania is recommended.

iv. Isolation and evaluation of locally available *T. asperellum* and *B. subtilis* for management of rice blast disease are required that in such isolates will be more adapted to upland rice growing areas of Tanzania and thus provide more effective control of the disease.

v. Further studies to determine the incidence and severity of rice blast disease on combinations of seed treatment and foliar application of *T. asperellum* and *B. subtilis* are needed to enhance their effectiveness for rice blast disease management.

vi. Hot water seed treatment was effective in reducing *P. oryzae* inocula on rice seeds. Optimization of temperature and time for hot water seed treatment on other/different rice genotypes is recommended, to reduce any side effect that may occur on the seeds.

vii. Under field conditions, rice genotypes may have been exposed to different inoculum density of *P. oryzae*. In-depth studies on the effect of rice blast disease on upland rice genotypes through quantified artificial inoculation is recommended.

APPENDICES

Appendix 1: Questionnaire used for interviewing famers during the surveys

Introductory and consent statement

Introductory and consent statement:

"*Dear Sir/Madam, I am a Student at Sokoine University. I am conducting a research survey to study farmers' knowledge and their management practices of rice blast disease in your village. Your response to these questions would remain* ***anonymous****. Taking part in this study is voluntary. If you choose not to take part, you have the right not to participate and there will be no consequences. Do you and your family consent to provide information? 1=yes, 0=No. Thank you for your kind co-operation*".

A: Geographical location

Region ……………… District ……………. Ward …………………Village ………………

B: Topography of the Area

Longitude …………………… Latitude ……….... Altitude…………....

C: Farmers information

(i) Name of the respondent……………………………………. Age ……...years

(ii) Sex…………... (1= Male 2= Female)

(iii) What is your Martial status? (1=Singe or Never married, 2=Married, 3=Widow/widowed, 4=divorced)

(iv) Do you have a phone? (1= Yes, 2= No). Phone number ……………………………

(v) Relationship with the head of the household……………. (1=head of the household, 2=Spouse, 3=Son/daughter 4=Grandson/daughter

(vi) Level of education ……………………. (1= none, 2= primary, 3= secondary, 4=tertiary 5=others)

(vii) The number of years in this area …………… years. Years spent in rice farming ………years

D: Rice production and source of inputs

(i) Size of all your farms in acres…………… Source 1= own, 2=inherited, 3=rented

(ii) Size of rice fields in acres…... …. Source 1= own, 2=inherited, 3=rented

(iii) Do you grow rice for (1= Food only, 2= Cash only 3= both)?

(iv) What rice varieties do you grow? (1= Local, 2= Improved)

(v) What are the sources of rice seeds you used? (1=own, 2= neighbours/friends 3=both 1and 2, 4= researchers 5= local market 6=others/specify)

(vi) During the last season in 2016, how many acres did you grow rice …………………….……...

(vii) What type of water source did you use (1=irrigation, 2=water with cans/bucket, 3=Rainfed)?

(viii) How far is your house from the nearest Agricultural input dealers/shops …… (1=walking distance, 2=travelling distance (4-5km) 3=no input shops)

(ix) How far is your house from the nearest Agricultural Extension office ……… (1=walking distance, 2=travelling distance (4-5km) 3=no extension office)

D: Knowledge on Rice blast disease and management

(i) Have you ever heard or observed rice blast disease (*shown in photos*) (1= yes …, 2= no…...)?

(ii) What is its local name? ………………………………………..…………………………

(iii) From where did you first hear or see this disease (1= friends/neighbours' field, 2=own field, 3= market place, 4= others/specify)

(iv) Has the disease ever been observed in your:

		If **YES**; when did the disease for the first time observed	
Farm	(1= yes, 2= no)	Month	Year
Village	(1= yes, 2= no)	Month	Year

(v) What type of blast do you find in your farm (*show the photo*)? 1) Panicle, 2) leaf, 3) neck, 4) stem

(vi) What are the major means of dissemination of the above disease? ………………….………

(vii) What proportion of your rice farm was infected by rice blast (*% in acre*) …...?

(viii) How severe were rice disease pests last year? 0=None, 1=Low, 2=Medium 3=High

(ix) Have you ever observed any other diseases in your farm? 1. Yes, 2. No

(x) If yes, which one 1= RYMV 2=Brow spot 3= Bacterial leaf blight (*specify*) …………………………….

(xi) How severe were above disease? 0=None, 1=Low, 2=Medium 3=High

(xii) Have you ever realized the occurrence of rice blast disease in other farms or neighbours farm? 1=Yes, 2= No

If **YES** indicates the month, year and distance of the farm from your farm.

Year	Month	Distance from your farm

(xiii) Does the disease affect the rice crop in your farm throughout the growing season? (Please tick as appropriate) 1=yes 2=No

If **NO**, indicate which month (s) of the year the disease is prevalent (*tick* in the box)

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec

(xiv) What factors do you think might have led to the presence of the disease in your farm?

1.
2.
3.

(xv) Does rice blast disease occur in your village/farm over past five years?

Year	Blast disease occurrence
2016	1= Yes 2=No
2015	1= Yes 2=No
2014	1= Yes 2=No
2013	1= Yes 2=No
2012	1= Yes 2=No

E: Control options by the farmers

(i) Is there any indigenous knowledge used/ known to control rice blast disease? 1=yes 2=No

(ii) What do you do to control rice blast disease in your farm?

(iii) 1=Burning diseased-straw and stubble, 2= Use of resistance variety 3= chemical use 4=abandon field 5=Split applications of nitrogenous fertilizer 6= other (specify)

(iv) Who informed you about these practices; (1=own, 2= neighbours/friends 3=both 1and 2, 4= researchers 5= extension officer, 6= others (specify))

(v) Is the method still used? 1=yes 2=No

(vi) If method stopped, give a reason for abandoning. 1=Worked very well, 2= Worked satisfactorily, 3 =Worked − but not well, 4= Did not work, 4 =I don't know

(vii) If using chemicals what kind of chemicals do you use to control blast disease? Name of product___________________________________ how much ……………………

(viii) From whom did you learn about the product? __________ 1) Extension staff, 2) ARI, 3) MoA, 4) fellow farmers, 5) TV, 6) Radio, 7) other (specify)

(ix) If not using any control method, give reasons why. 1= blast is a new disease in this place 2= lack of knowledge on blast control 3=both 1 and 2, 4=blast not a serious problem 5= no effective management method

F: Training on disease management

(i) Have you ever received any training related to rice production? 1=Yes 2=No

(ii) If yes, how many times have you received IPM training?

(iii) From whom did you learn about rice production? __________ 1) Extension staff, 2) ARI, 3) MoA, 4) fellow farmers, 5) TV, 6) Radio, 7) other (specify)

(iv) Have you received any training related to IPM? 1=Yes 2=No

(v) If yes, how many times have you received IPM training?

(vi) From whom did you learn about rice production? __________ 1) Extension staff, 2) ARI, 3) MoA, 4) fellow farmers, 5) TV, 6) Radio, 7) other (specify)