

**Effect of Nitrogen, Silicon, Rice By-product and Fungicide for
Management of Rice Blast Disease in Mwea Irrigation Scheme of
Kenya**

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my beloved husband Macharia, lovely son Alex and my loving daughter Anita, my mother as well as my sister and brothers.

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LIST OF ACRONYMS

AUDPC	Area under the disease progress curves
BTH	Benzothiadiazole
CIAT	International Centre for Tropical Agriculture
CIBK	Center for Business Information in Kenya
DHP-	Potassium hydrogen phosphate
EC	Emulsifiable concentrate
EPZ	Export Processing Zone
ERSWEC	Economic Recovery Strategy for Wealth and Employment Creation
FAO	Food and Agricultural Organization of United Nation
GOK	Government of Kenya
IRRI	International Rice Research Institute
K	Potassium
KCL	Potassium chloride
Me	Mill equivalent
MEA	Malt Extract Agar
MIAD	Mwea Irrigation Agricultural Development centre
MOA	Ministry of Agriculture
N	Nitrogen
NARL	National Research Agricultural Laboratories
NCPB	National cereals and produce board
NIB	National Irrigation Board
P	Phosphorous

PBZ	Probenazole
PPM	Parts per million
SAR	Systemic acquired resistance
SC	Soluble concentrate
Si	Silicon
USDA	United State Department of Agriculture
WASDE	Word Agricultural Supply and Demand Estimate
WE	Wetable Emulsifiable
WP	Wetable powder

ABSTRACT

Rice (*Oryza sativa* L) is a staple food for nearly-half of the world population contributing over 20% of total world calorie intake. In Kenya it is the third most important cereal after maize and wheat. Basmati 370 is the most preferred variety but highly susceptible to rice blast disease. Rice blast, caused by *Pyricularia oryzae* is one of the most important rice disease causing yield loss of 70-80%. A study was carried out in Mwea Agricultural Development Centre (MIAD) to determine the effect of fertilization with nitrogen, and silicon, application of rice by-product and fungicide for the management of rice blast. Soil and plant samples were collected from field that had infected and non-infected plants and analyzed for silicon and nitrogen levels. The effect of nitrogen and silicon on disease infection and yield of rice was tested using four levels of silicon fertilizer (0, 500, 1000, 1500kg Si/ha) and nitrogen fertilizers (0, 40, 80 120kg N/ha). Each treatment was replicated four times. The experiment was laid out in split plot design with fertilizer type as main plot and fertilizer levels as sub-plot factor. Data collected comprised of disease severity score, productive tillers, number of panicles, number of filled grains, seed weight and biomass. To determine the effect of different sources of silicon from rice by-products and commercial silicate on infection of rice by *Pyricularia oryzae*, calcium silicate (5000Kg/ha), rice straw (2ton/ha), rice husk ash (0.7tons/ha) and straw ash (0.6tons/ha). Basal fertilizers were added as recommended at 30 Kg K₂O/ha, 58 KgP₂O₅ /ha and 80 KgN which was applied as a top dressing in two splits. Inoculums' of *Pyricularia oryzae* (4×10^5 conidia/ ml) was used to infect the rice plants and panicle blast infection was assessed using the IRRI standard. This

experiment was laid in a Complete Randomized Design (CRD). To determine the effect of selected chemical fungicides on in-vitro mycelial inhibition of *Pyricularia oryzae*, four chemical fungicides (Azoxystrobin 250 CS, Thiophanatemethyl, Hexaconazole and Carbendazim) commonly used by farmers were tested. Each fungicide was tested at three concentrations to determine the effectiveness of chemical fungicides in control of rice blast. The assessment was carried out by *in-vitro* bioassays at ambient temperature (25⁰C). The efficacy of the fungicide was assessed by measuring the inhibition zones across a nine-mm Petri dish. Data in all the experiments was analyzed by Analysis of variance (ANOVA) using Statistical Analysis System (SAS) statistical package version 8.1 and means separations using least significance difference (LSD) at 5%. Results indicated significance differences among the infected and non-infected fields with nitrogen level being high in the rice blast infected field than in non-infected field in both 0-15 and 15-30cm depth. Silicon level was lower in the rice blast infected field at the two depth (0-15 and 15-30cm) than in non-infected. However highest concentration of silicon and nitrogen was at 0-15cm depth. Similarly infected plants showed higher nitrogen concentration while silicon was higher in the non-infected tissues. There was significant differences (P<0.05) in response of nitrogen and silicon in both yield and control of rice blast disease. The interaction between nitrogen and silicon was effective at 80KgN/ha and 1000 Si Kg/ha in both yield and in management of rice blast disease. Calcium silicate and the rice by products were significantly different in disease management but it was not different rice yield from the husk ash. There were significant differences (P<0.05) in mycelial inhibition of *P. oryzae* among the four fungicides. Carbendazim

and Thiophanate Methyl 50% w/v were effective at the highest and intermediate concentrations (2 and 3mls/lit) while Hexacanazole was effective at concentration (18.75mls/lit). The study has revealed that nitrogen at 80kg/ha and silicon at 1000kg/ha were effective in enhancing rice yield and management of rice blast. Carbendazim and Thiophanate Methyl 50% w/v were the most effective fungicides in the control of rice blast at 2mls/lit.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Rice (*Oryza sativa* L) is a unique crop that grows well in waterlogged condition. It is a staple food for nearly one-half of world's population, contributing to over 20% of the total calorie intake to humans (MOA, 2009). It is the third most important food crop in Kenya after maize and wheat. It is mainly grown by small scale farmers as commercial as well as food crop (MOA, 2009). It is an important diet for the urban as well as the rural people. The optimal growth of rice requires high temperature of 28⁰C that occur below 1200m above sea level and in vertisols soil type with a higher water holding capacity to hold the flooded water (Acland, 1971). In Kenya rice is mainly grown in irrigation scheme where paddy schemes are managed by National Irrigation Board (NIB)

The remaining 5% of the rice is rain fed by small-scale holders who accounts for 95% of the rice grown in Kenya (Export Processing Zone (EPZ), 2005. Most of the rain fed rice is grown in Kwale, Kilifi, and Tana River districts in Coast Province, and the Bunyala and Teso districts in Western Kenya. Rice milling in the country is carried out by small-scale milling enterprises, which are mainly privately owned.

Mwea irrigation scheme in Central Province accounts for 60% of all the rice production. The rest 40% is shared among other schemes in other provinces Nyanza (West Kano, Ahero Kuria and Migori), Coast (Tana delta, Msambweni) and Western

(Bunyala) (MOA, 2009). The average rice yield under irrigation is 5.5 tons/ha for the aromatic variety, and 7 tons/ha for the non-aromatic varieties (EPZ, 2005). Yield under rain fed conditions is slightly below 2 tons/ha (EPZ, 2005).

The local annual production of rice in the country is about 45,000-80,000 metric tons while the consumption estimated to be 300,000 metric tons. The huge deficit (83.9%) is met through importation valued at Ksh 7 billion (MOA, 2009). The annual consumption of rice has been on increase at a rate of 12 % as compared to 4 % and 1 % for wheat and maize respectively (MOA, 2009). This increase has been attributed to the change of eating habits. The per capita farmer income earned from rice production in central Kenya is USD\$3,500, which is considered a decent living by national standards (Oxfam, 2005). A case study carried out in Mwea, by Nguyo and Bezunchi, (2002) showed that 80% of the income in these areas generated is from rice. There is therefore a need to promote the crop in order to improve food security, increase small holder income, contribute to employment creation in the rural areas and most of all to reduce the rice import bill. To address some of these issues, the Government has drafted the National Rice Strategic Plan (2000-2013) that will guide the present and future effort of the Ministry of Agriculture and other stakeholders in providing technologies that will help in the improvement of rice productivity and enhancement of livelihoods of rice growing farmers. It is expected that with the continued expansion of rice irrigation scheme and opening up of new rice production areas production will continue to rise even higher. Table 1 shows Kenyan rice production, consumption and the importation in the period 2001-2007.

The most common rice varieties grown in Kenya are ITA 330, IR2793-80-1, BW 196

and Basmati/Pishori 217 and 370. Basmati 370 is the most preferred rice variety by most growers particularly in Mwea irrigation scheme (Wanjogu *et al.*, 1995). It was introduced by the National Irrigation Board (NIB) in 1990s and released for commercial production in 1998. The variety fetches much higher prices than other varieties due to its superior utilization characteristics. It has extra-long super fine slender grains with chalky endosperm (FAO, 2001). It also has a pleasant exquisite aroma, sweet taste, dry, fluffy and soft texture when cooked (FAO, 2001). According to Wanjogu *et al.* (1995) there was a marked increase of area under Basmati in 1995 that was attributed to its economic value as compared to other commercial varieties. It is estimated that the demand for Basmati rice cannot be met locally and internationally. (FAO, 2001; GOK, 2005)

Table 1. Rice productions, consumption and importation in Kenya in 2001 – 2007

	2001	2002	2003	2004	2005	2006	2007
Area (ha)	13,200	13,000	10,781	23,322	15,940	23,106	16,457
Production (Tonnes)	44,996	44,996	40,498	49,290	57,941	64,840	47,256
Unit price/tonnes	26,250	16,060	58,000	65,000	68,000	70,000	53,000
Average yield (Tonnes /ha)	3.4	3.4	3.7	3.6	3.6	2.8	2.8
Consumption (Tonnes)	238,600	247,560	258,600	270,200	279,800	286,000	293,722
Imports (Tonnes)	201,402	208,944	213,342	223,190	228,206	NA	NA
Total value (Billion KES)	1.2	0.7	0.7	1.3	0.9	3.3	2.7
World production (Million tonnes)	NA	378	391	401	416	415	421
World consumption (Million tonnes)	NA	107	86	78	81	78	72

Source: National cereals and produce board (NCPB) and Department of Land, Crop Development and Management, USDA-WASDE (2007)

Although Basmati rice variety fetches a premium price and is most preferred all over the world market, it is more susceptible to rice diseases mainly brown spot caused by *Bipolaris oryzae*, blast caused by *Pyricularia oryzae*, and sheath blight (*Thanatephorus cucumeris*) (Couch and Kohn, 2005). Rice blast caused by, is the most important fungal disease of rice (Couch and Kohn, 2005). In western Kenya the disease is known to cause yield losses of up to 50 percent in farmers' fields (Anon, 1992). It is also reported to cause losses of more than 70-80 percent in infected fields (Awodero, 1990).

In Mwea irrigation scheme the disease was first detected in the year 2006 while a more recent outbreak occurred in 2009 cite. This called for an intervention since the disease is a threat to food security. The disease is managed through various strategies like the use of resistant cultivars, water level management, proper fertilization, cultural practices, and treatment with fungicides (Cloud and Lee, 1993) since a single control method may not be effective. According to Magdoff *et al.* (2000), resistant plant is the most effective disease control strategy in high productive systems. Loung, (2003) showed that the plant's ability to resist disease is mainly influenced by soil properties and nutrients. Chemical control has been used effectively in India, Japan and Philippines and a number of fungicides have been found to curb the spread of the disease (Kumbhar, 2005).

1.2 Problem Statement

Rice is an important cereal in the country ranking third from maize and wheat hence ensuring food security among the Kenyans. Basmati varieties are widely grown and

preferred. However, they are the most susceptible to diseases that attack rice. The variety Basmati 370 was chosen as the test crop for the study since it fetches the premium price among rice varieties grown in Kenya. Rice blast is one of the most important diseases in rice causing an economic loss of over 70-80% and therefore causing a threat to food security in the country. Recent outbreak of the disease coupled with lack of knowledge on how to reduce the disease has resulted to increased rice blast incidence leading to yield drop.

Attempts to control rice blast using various fungicides in Kenyan have been done but not successful as the efficacy of these fungicides has not been determined on rice. An integration of more than one control measure is necessary since chemicals are expensive and un-friendly to the environment. For instance, use of organic materials as a source of silicon, and appropriate rate of fertilizer application. This study was initiated to investigate the role of plant nutrition and chemical fungicide application in the management of rice blast in Mwea irrigation scheme of Central Kenya.

1.3 OBJECTIVES

1.3.1 The Broad Objective

The main objective of the study was to determine the role of plant nutrition and selected fungicides application in the management of rice blast in Mwea irrigation scheme of Kenya.

Specific Objectives

- i) Determine the effect of different nitrogen and silicon levels in the soil and in plant tissues in the management of rice blast.
- ii) Determine the effect of silicon and nitrogen fertilizer on severity of rice by *Pyricularia oryzae* and their effect on yields
- iii) Determine the effect of silicon sourced from rice by-products and chemical in management of *Pyricularia oryzae*
- iv) Determine the effect of selected chemical fungicides on in-vitro mycelial inhibition of *Pyricularia oryzae*

1.4 Justification

Rice blast is a threat to food security in Kenya requiring mitigation against this disease. Ideally use of resistant cultivars is the most effective strategy in control of rice blast but it may take sometime before a desired variety is developed. However, as indicated by Magdoff *et al.*, (2000) nutrition management offers one of the most important practices for high production system that may affect response of rice to diseases. The use of plant nutrition is environmentally friendly and it's easily compatible with other disease management strategies such as cultural methods, water management and use of chemical treatment. Rice blast has been controlled by use of fungicide in other countries like United States of America (USA), Japan and India. In Kenya, there is no registered fungicide to control rice blast leaving farmers to use several fungicides of their choice. Fungicides are fast acting particularly where the disease is severe. According to Filippi and Prabhu (1997) there has been an emphasis

on the integration of fungicides as one of the inputs to keep the disease in tolerable levels. Rice blast being the most important disease in rice can lead to a considerable yield loss and in Kenya it has been found to cause a yield loss of about 50 percent.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rice Crop

There are two species cultivated *Oryzae sativa* L (Asian rice) and *Oryzae glaberrima* steud (African rice). *Oryzae glaberrima* is traditionally found in diverse West African agro ecosystems but it is largely abandoned in favor of high yielding *Oryzae Sativa* cultivar that has higher agronomic performance. However, *Oryzae sativa* cultivars are often not sufficiently adapted to various abiotic and biotic conditions in Africa. *Oryza glaberrima* has been found to have several useful traits like being moderate to high level of resistance to blast (Silue and Notteghem, 1991), rice yellow mottle virus (Attere and Fatokun, 1983); (John *et al.*, 1985), rice gall midge, insects (Alam, 1988) and nematodes (Reversat and Destombes, 1995). The variety has also been found to be tolerant to abiotic stresses such as acidity, iron toxicity, drought, and weed competition (Sano *et al.* 1984; Jones *et al.* 1994).

2.2. The casual agent of rice blast

Rice blast is said to be widespread in all the rice growing regions of the world and has been reported in more than eighty five countries (Rao, 1994). It is the most important fungal disease causing yield loss in both upland and lowland rice (Bonman *et al.* 1991; Lee, 1994). The disease is caused by a fungus *Pyricularia oryzae* (Cavara). Other synonyms are *Magnaporthe grisea* (Hebert) Barr, *Pyricularia grisea* (Cook) Sacc.) Webster and Gunnell, (1992); Couch and Konn (2002), Zhou *et al.* (2007);

Pyricularia oryzae is filamentous ascomycetes that can reproduce both sexually and asexually. On germination, the spore produce specialized infectious structure called appressorium that infect aerial tissues (Webster and Gunnell, 1992).

The asexual life cycle begins when the hyphae of the fungus produces fruiting structures and sporulates to give conidia. The conidia measure 20-22 x 10-12 μm , 2-septate, translucent, and slightly darkened. According to Webster and Gunnell, (1992) the conidia are obclavate and tapering at the apex or truncate or extended into a short tooth at the base. When the spores land on leaves and other aerials tissues of susceptible plant, they germinate and develop the appressorium which penetrate the plant cell by producing a penetration peg. Pressure in the appressorium increases and the structure explodes forcing the penetration through the cell wall and into the cell (Dean *et al.* 2005). The fungus grows hyphae inter or intracellular within the leaf and form lesions. Once it is established in the host plant the fungal hyphae sporulates and produce asexual spores (Kim, 1992). The pathogen completes its life cycle within one week. Each of the phases (sporulation, releases, germination and the penetration) play an important role during the blast epidemic and requires different environments. Under natural conditions sporulation is greatly affected by the age of the crop and the size of the lesion together with the variety of rice (Kim, 1992). Sporulation phase is the first step that facilitate in building up the leaf blast epidemic as it provides the inoculum population (Webster and Gunnell 1992; Kim, 1994). Sporulation capacity as termed by Chang, (1995) refers to the total amount of conidia produced on the natural leaf blast lesion under the field conditions. It is highest with increased relative

humidity of 90% or more with optimal temperatures of 25-28°C, and a minimum of 4 hours of leaf wetness (Webster and Gunnell 1992; Kim, 1994).

Sexual reproduction occurs when two strains of opposite mating types meet to form a fruiting structure known as perithecium in which ascospores is formed (Dean *et al.* 2005). The pathogen can continue to live in plants from one crop season to another in the tropics, or survive in the temperate zone on crop residues of diseased plants, or on ratoons (Zeigler, 1994). Seed are secondary hosts also have been reported as possible sources of primary inoculum (Lee and Dean, 1993).

Apart from rice, the pathogen has a wide range of hosts that includes *Agropyron repens* (L.) *Gould*, *Agrstics palustris*, *Atenuis*, *Sorghum vulgare*, *Triticum aestivum*, *Zea mays*, *Saccharum officinarum* *Setaria italica* (L) among others.

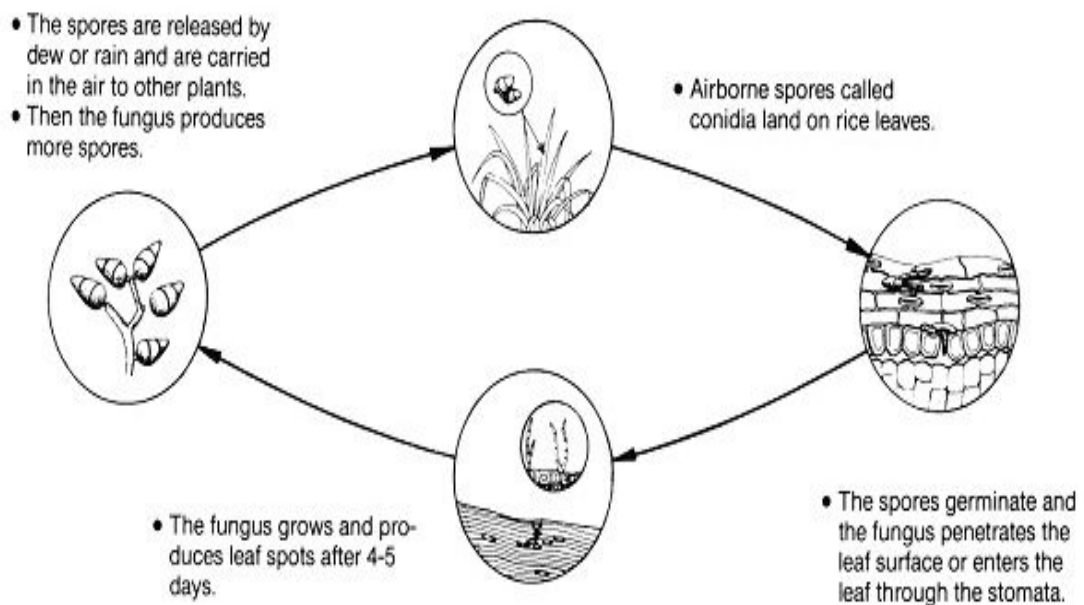


Figure 1 Rice blast life cycle Source: (Webster and Gunnell, 1992)

2.2.1 Disease cycle

A disease cycle begins when a blast spore infects and produces a lesion on the rice plant and ends when the fungus sporulates repeatedly for about 20 days and dispersing many new airborne spores produced in the lesions Zeigler *et al.* (1994). With suitable environment the conidia land on a leaf surface, germinate, and penetrate leaf and other plant tissues particularly when the plant surfaces remain moist, to cause an infection. It also requires high humidity, little or no wind at night and night temperatures between 12– 32 °C for the infection cycle to continue (Zeigler *et al.* 1994).

In the canopy of rice plants, newly developed leaves act as receptors for the spores. The rice blast disease is a typical polycyclic with many cycles of infection occurring within a growing season. In favourable conditions a single lesion can produce hundreds to thousands of spore in one night (Webster and Gunnell, 1992). Direct penetration by the conidia spores is facilitated by the accumulation of high concentration of glycerol within the appressorium (Jong *et al.*1997). An influx of water molecules moves into the infection structure (a specialized cell produced from the germ tube of the conidium) to generate a hydrostatic turgor, which is focused and translated into a mechanical force, allowing a narrow penetration peg to rupture the cuticle. The formation of this infection structure on the host surface marks the onset of the disease (Talbot, 2003). Once inside the epidermal cell, fungal hyphae differentiate from a vesicle produced by the penetration peg and ramify intra and intercellularly. The incubation period (the period between the infection and the first

symptoms of a disease) is affected by temperature (25-28⁰c) and relative humidity of 85% and above (Jong *et al.* 1997).

Development of the disease is characterized by a necrotic lesion that becomes visible on the leaf blade around 36-72 hours after infection. Aerial conidiophores differentiate within the lesion around 5 to 7 days after infection takes place to produce conidia, which initiate new blast cycles (Ou, 1985). The mechanism of pathogenesis of *Pyricularia oryzae* on rice includes elaboration of an appressorium that supports direct penetration through the cuticle of the host plant (Valent and Chumley, 1991).

2.2.2 Disease symptoms

The disease symptoms may include lesions on all parts of the shoot, stem and panicle. When nodes are infected, all plant parts above the infection point die and yield losses are severe. On the leaves it causes elongated diamond-shaped white to grey lesions with dark green to brown borders surrounded by a yellowish halo. The disease also kills the leaf collar, the stem, and occasionally the internodes (Long, 1996). If attack is at heading stage the disease may result in production of white panicle or breakage of the stem at the infected node. This is the most destructive symptoms and is referred to as neck rot, neck blast, or panicle blast stage of the disease (IRRI, 1983). If infection occurs early, the grains do not fill and the panicle remains erect. However late infection results in partial filling of the grains and because of weight, the base of the panicle breaks and droops.

2.3 Management of rice blast

There are several control strategies that may be undertaken in management of rice blast, these may include chemical control, nutrition management, cultural practices and use of resistant varieties.

2.3.1 Cultural practices

This is an important control measure but will not provide complete eradication of the disease. Removal of crop residues through burning reduces the over wintering inocula in the field but this may not prevent the inoculums coming from other sources (Zeigler *et al.* 1994). Use of treated seed may reduce inoculums load since seed have also been reported as possible source of primary inoculum (Lee, 1994). Water seeding (planting on very wet soil) is recommended as this will reduce the transmission of disease from the seed to the seedling. As reported by Manandhar (1998), water management through flooding is also recommended to reduce rice blast unlike when there is water stress. Draining of water allows the formation of nitrates resulting to drought stress. According to Austin, (1989); Kato, (2004) rice is more susceptible to drought than other cereals due to its inability to regulate its transpirational water loss a weakness that may be accelerated by rice blast attack.

2.3.2 Use of resistance varieties

After an extensive breeding effort to classify the various sources of resistance, International Centre for Tropical Agriculture (CIAT) released the variety *Oryzica Llanos 5* in 1989. This variety is resistant to rice blast not only in Colombian fields

where the disease is still very virulent but also in rice blast-resistant nurseries in Brazil, the Philippines, Thailand, Indonesia and Korea. In Colombia (CIAT) this variety was inoculated with 172 isolates from 13 genetic lines of the fungus; in IRRI it was inoculated with 202 isolates from six lines, and all showed resistance (Correa and Zeigler, 1995). The study of the variety components indicated that resistance durability was due to the multigenic combination of varieties expressing complementary resistance to a wide range of fungal pathogen.

Use of resistant varieties would offer a better management compared to other control strategies, as it is inexpensive. However, it may take a long time to develop a variety of a desired type that is resistant to rice blast (Zeigler *et al.* 1994; Webster and Gunell, 1992). Inducing the resistance to rice plant is also an eco-friendly strategy for rice blast control. Resistance in the host-pathogen was described by Graham and Webb, (1991) as the ability of plants to limit the penetration, development and/or reproduction of invading pathogens while tolerance of host plants is measured in terms of the ability to maintain growth and yield production in spite of infection or invasion of pathogens. Although both resistance and tolerance are genetically controlled, the environmental conditions for example levels of nutrients of the host plant can be modified to a certain extent its expression, especially in moderately susceptible or resistant genotypes/varieties.

2.3.3 Chemical control

Rice blast has been observed in every country where rice is grown and fungicides applications have been done particularly where conditions are favourable for severe

epidemics (Kim, 1994; Couch and Kohn, 2002). Many fungicides developed to control rice blast are often systemic although prolonged use of chemical with similar mode of action may result in development of resistance in the pathogen (Kim *et al.* 1994).

In California, Azoxystrobin (Quadris 2.08 SC) was full registered in 1999 for the management of rice blast disease. Efficacy results of various fungicides have been reported by many researchers in the world as reported by Kumbhar, (2005). Varier *et al.* (1993) tested and found tricyclazole at 4 kg/ha to be effective in rice blast control forty days after sowing. Field trials conducted by Dubey, (1995) showed Topsin M+Indofil (M-45 (thiophanate methyl) proved to be effective on management of rice blast. Carbendazim Pyroquilon thiophanate methyl and chlorbenthiazone as well as tricyclazole also proved to effectively control rice blast (Gouramanis, 1995). Di-potassium hydrogen phosphate (DHP) has also been used to reduce rice blast (Manandar *et al.* 1998).

Acibenzolar-s-methyl (BTH) was also reported to induce SAR in rice plants (Yamaguchi, 1998). The chemical structure of BTH looks similar to PBZ but it was found that they have different site of action; plants probenazole (PBZ) and its active metabolite (1,2-benzisothiazole-1,1 dioxide; BIT) act in the step prior to the salicylic acid but BTH induces systemic acquired resistance (SAR) by acting downstream of salicylic acid (Nakashita *et al.* 2002)

Tiadin (thifluzamide) was developed and registered as anti-blast (Umetani *et al.* 2003) while benomyl is registered in united state of America where two applications are made just before and after heading. Zeneca a British multinational pharmaceutical

company was also found to manufacture fungicides effective in controlling rice blast in the Southern US (Zeigler *et al.*, 1994). Application of pyroquilon granules or wettable powder at two kilogram per hectare effectively controlled rice blast (Moletti *et al.* 1998). Opus 15.5 SC and Ocatve 50 WP has also been found effective in eliminating rice blast (Tirmali and Patil, 2000) particularly at when sprayed at tillering, booting and heading stages. Other chemicals known to successfully control neck blast on susceptible cultivars are 30SC cupropamid, Folicur 250 (tebuconazole 250 + triadimenol 125); WE swing 250 EC and 75 WP at panicle initiation, and heading stage blast (Tirmali and Patil, 2000).

2.3.4 Nutrition management

The understanding of impacts of nutrition management on interactions between rice and pests and diseases is a base to stimulate high yield production system (Luong *et al.*, 2003). In this view Magdoff *et al.* (2000) indicated that nutrition management is one of the most important practices for high production system that may affect response of rice to diseases, as well as developmental pattern of the disease populations due to the change of environments. In deed most pest and disease management methods used by farmers can be considered as soil fertility management strategies (Magdoff *et al.* 2000). Increasingly, recent research is showing that the ability of a crop plant to resist or tolerate insect pests and diseases is tied to optimal physical, chemical and mainly biological properties of soils (Luong *et al.* 2003). Soils with high organic matter and high biological activity generally exhibit good soil

fertility as well as complex food webs and beneficial organisms that prevent infection (Luong *et al.* 2003). With this in mind it indicated that some fertilizer application may have a negative or positive response of plants toward the disease. Excess nitrogen encourages disease hence overlap must be avoided since this enhances the increase of inoculum levels

Despite the positive role played by nutrition in control of diseases, some farming practices may cause nutrition imbalances resulting to pest and disease resistance (Magdoff *et al.* 2000). Meyer, (2000) also indicated that soil fertility practices have impact on the physiological susceptibility of crop plants to insect pests and diseases either affecting the resistance of individual plant positively or negatively. Excess nitrogen encourages disease and this enhances the increase of inoculums' levels (Webster and Gunell, 1992). However, split application of nitrogen (N) in upland rice was found to decrease the rice blast as compared to a single application in furrow at planting. Kurschner *et al.* (1992) found that rice blast was not consistently reduced by timing and splitting of N applications.

2.3.4.1 Silicon

Silicon (Si) is known as a “beneficial element” for plants. However, it is not an essential nutrient. It is assimilated by plant roots as monosilicic acid (H_4SiO_4) where it accumulates in leaves and other plant tissue primarily as amorphous silicates or phytolithic opal (Epstein, 1994). Once deposited in this form, Si is immobile and is not redistributed within the plant (Ma *et al.* 1989; Epstein, 1999).

The direct and indirect benefits of the element for crops especially grasses are related to resistance to diseases, pests, and drought. Plant species are considered Si accumulators when the concentration of Si (in dry weight basis) is greater than 1 g/kg (Epstein, 1999). Compared to monocots, dicots such as tomato, cucumber, and soybean are considered to be poor accumulators of Si with values less than 1 g/kg in their biomass. Belanger *et al.*, (1995) found that application of silicon suppressed diseases in cucurbits caused by foliar and soil borne pathogens. Dry land grasses such as wheat, oat, rye, barley, sorghum, corn, and sugarcane contain about 10 g/kg in their biomass, while aquatic grasses have Si contents of up to 50 g/kg (Korndorfer *et al.* 2001). In rice, Si accumulation is about 108% greater than that of nitrogen while the concentrations between 3 and 5% may be the minimum tissue levels needed for disease control (Datnoff, 1997). It is estimated that a rice crop producing a total grain yield of 5000 kg/ha will remove Si at 230 to 470 kg/ha from the soil (Savant, 1997). In the absence of adequate silica, brown spot disease (*Bipolaris oryzae*) was often found to be very severe giving rice an over all brownish appearance. Neck rot (*Pyricularia oryzae*) too increased in the rice field that contained inadequate silicon (Datnoff *et al.* 1990; Datnoff *et al.* 2001). Low Si uptake was also seen to increase the susceptibility of rice to blast (*Magnaporthe grisea* (Hebert) Barr), leaf blight (*Xanthomonas oryzae* pv. *Oryzae*) brown spot (*Cochliobolus miyabeanus*, stem rot (*Magnaporthe salvinii* Catt.), scald (*Monographella albescens* Theum), and grain discoloration (Mathai *et al.* 1977; Winslow, 1992; Rafi *et al.* 1997; Savant *et al.* 1997; Kobayashi *et al.* 2001; Rodrigues *et al.* 2001; Massey and Hartley, 2006). The leaves and culms of rice plants, grown in the presence of silicon showed an erect

growth that greatly improved the distribution of light within the canopy. This avoided the shading that would otherwise encourage a suitable environment for survival of the pathogens (Elawad and Green, 1979; Ma and Takahashi, 1991).

For plants disease resistance, epidermal cell walls of silicon accumulators are impregnated with a firm layer of silica and become effective barriers against water loss and fungal growth thereby preventing formation of haustoria and hyphal penetration (Marschner, 1995). This hypothesis is further strengthened by the findings of Kim *et al.* (2002) who suggested that silicon created a mechanical barrier to penetration which seemed logical given that Blaich and Grundhorfer, (1998) had also found the accumulation of silicon in the sites of attempted but failed pathogen penetration. Seebold, *et al.* (2001) noted a reduction in number of the sporulating lesions on partially resistant and susceptible rice cultivars fertilized with calcium silicate indicating fewer successful infections per unit of inoculum. Similarly Prabhu, *et al.* (2001) found that rice cultivar that accumulated more silicon on the shoots showed less incidence of rice blast. The function of silicon deposition in the defense mechanism may be similar to that of enhanced synthesis of polyphenols and lignin at the site of infection (Vance *et al.* 1980). The phenolics play a role as either phytoalexins or as precursors of lignin and suberin biosynthesis. Silicon can also be associated with lignin-carbohydrate complexes in the cell wall of rice epidermal cells (Inanaga, 1995).

2.3.4.2 Nitrogen

Nitrogen is essential for plant growth and development and is usually a limiting factor for high productivity. The crop plant obtains nitrogen from the soil as nitrates or ammonia while legumes get through nitrogen fixation (Lea *et al.* 2007). Work done by Robert *et al.* (2005) indicated that when nitrogen in the wheat and rice plant was applied in limited quantities, production of spore of leaf rust was reduced. Jensen and Munk (1997) and Long *et al.* (2000) found an increase in blast lesion when the level of nitrogen was applied above the recommended rate. Similar findings were found by Kurschner *et al.* (1992) who reported that while nitrogen was essential for productivity, the severity of blast also increased with higher rate of application which increases metabolism in rice plant resulting to tissue susceptibility to rice blast. Some of this physiological plant metabolism includes chlorophyll enzymes, alkaloids, nucleotides, proteins, hormones and vitamins (Marschner, 1995). Nitrogen influences the branching, tillering and in leaf expansion which determines the size of canopy produced. Other authors (Kim and Kim, 1990; Cloud and Lee, 1993; Ishiguro, 1994) also found that excessive growth due to unbalanced nitrogen supply creates microclimate conditions favourable to fungal diseases. This is accelerated by lodging of cereals that are over-supplied with nitrogen and have inadequate potash. Humidity remains higher in lodged crops creating a microclimatic that provides ideal conditions for spores germination and survival of the fungi. According to Leitch and Jenkins, 1995; Tiedemann 1996, and Solomon *et al.* 2003), high leaf nitrogen concentration increases the growth of fungus. On the contrary Hoffland *et al.* 1999; Snoeijers *et al.* 2000) observed that low nitrogen also led to disease increase resulting from weak

plants that lacked sufficient defense against disease.

At heading and during fillings stage of the rice plant nitrogenous compounds may increase, decrease or cease, depending on the environmental conditions, cultivar, and/or nitrogen fertilizer rate of which may influence rice blast incidence (Wilson *et al.* 1990; Norman *et al.* 1992) . This lead to grain nitrogen as a result of translocation from vegetative organs after heading which is positively correlated with grain yield and protein concentration (Samonte *et al.* 2006).

This remobilized nitrogen accounts for 70 to 90 % of the total panicle nitrogen (Mae, 1997). Leaf blades, particularly the three leaves at the top of the plant, are the major source of the remobilized nitrogen, followed by leaf sheaths and stems (Mae, 1997; Zhang *et al.* 2003). Bastiaans, (1993) reported that rice blast disease reduced nitrogen uptake before flowering and increased relative contribution of the stem to the overall nitrogen reallocation during the grain filling stage. Recent studies by Koutroubas *et al.* (2008) showed that when rice plant is inoculated with blast fungus, nitrogen utilization efficiency for both yield and biomass production is reduced.

2.3.4.3 Role of potassium

Potassium is involved in numerous functions in the plant. These functions includes enzyme activation, cation and anion balance, stomatal movement, phloem loading, assimilate translocation and turgor regulation. observed that foliar spray of potassium as a stimulated dose had a significant effect on the dry weight of leaves and N % as

well as K % in leaves tissues and significantly increased total yield, fruit quality Fawzy *et al.* (2007), resistance against pest and diseases, drought as well as frost stresses (Marschner, 1995). Peoples and Koch, (1979); Haerdter, (1997) summarized the effect of K on crop specific host or pathogen relationships for rice in Asia giving an example of stem rot, *Helminthosporium sigmoideum*, that generally occurs at high nitrogen (N) supply in soils poor in K. A similar inverse relationship between disease incidence and plant nutrition with K was quoted for brown leaf spot in rice (*Helminthosporium oryzae*), rice blast (*Piricularia oryzae*) or sheath blight of rice (*Thanatephorus cucumeris*). A curative effect from applying K was also seen for bacterial diseases in rice like bacterial leaf blight, *Xanthomonas oryzae*. Mondal *et al.* (2001) found a negative correlation between K content in soybean and sesame with disease incidence and a positive correlation with their respective yield. Sweeney *et al.* (2000) reported that K fertilization reduced leaf rust (*Puccinia triticina*) severity and improved yield.

CHAPTER THREE

3.0 A SURVEY OF NITROGEN, SILICON, PHOSPHOROUS AND POTASIAM LEVELS IN RICE INFECTED AND NON-INFECTED FIELDS

3.1. INTRODUCTION

Plant health is an important factor for plant growth and development. It possesses several roles in formation, partitioning and utilization of photosynthates. A crop that is deficient of mineral nutrients experience limitation in flower initiation and development affecting the general crop yield and quality. Sharma *et al.* (1991) pointed out that mineral deficiency may bring variability of pollen grain affecting the crop yield. Silicon is said to be the second most prevalent element within the soil and though regarded as non-essential for most plants, its uptake usually provide many benefits. It is referred to as a “beneficial element” for plants. Some benefit accrued to the use of silicon includes improved yield and quality (Korndorfer *et al.*, 2001), drought tolerance (Neumann, 2001), resistance to diseases, pests, and drought. Plant species are considered silicon accumulators when the concentration (in dry weight basis) is greater than 1 g/kg (Epstein, 1999). The direct and indirect benefits of the element for crops especially grasses are related to resistance to diseases, pests, and drought. Concentrations between 3 and 5% may be the minimum tissue levels needed for disease control (Datnoff, *et al.* 1997).

Marschner, (1986) observed that silicon accumulates in the epidermal cell at sites of penetration to create a physical barrier to fungal growth by preventing formation of

haustoria and hyphal penetration. The element nitrogen is essential for the normal growth of plants. All vital biological processes are related to the existence of functional plasma of which nitrogen is a basic constituent of protein and nucleic acids. Nitrogen is also responsible for the physiological importance to plant metabolism such as chlorophyll enzymes alkaloids, nucleotides, proteins, hormones and vitamins (Marschner, 1995). Nitrogen is one nutrient that is usually required by plants in comparatively large quantities than other soil-borne elements. However, both high and low supply of nitrogen has an influence on disease infection on plants. Potassium is involved in some functions like enzyme activation, cation and anion balance, stomatal movement, phloem loading, assimilate translocation and turgor regulation Fawzy *et al*, (2007). According to Mondal *et al*. (2001) there is inverse relationship between disease incidence and the potassium nutrition for an example *Pyricularia Oryza* in rice. Phosphorous content plays a role in plant nutrition in that at low application brown spot disease in rice increased (Singh *et al*.2005).

A nutritional survey of rice blast infected and non-infected field was conducted in order to determine the relationship between the rice blast infection and the level of nitrogen, phosphorous potassium and silicon.

3.2 MATERIALS AND METHODS

3.2.1 Nitrogen, phosphorus, potassium and silicon analysis in soil and plant

Soil sampling and analysis

Soil sampling was done in May 2008 at Mwea Irrigation Agricultural Development

(MIAD) on block J6 (0.25 ha) that had diseased plants and block B (0.25 ha) that had healthy plants to determine concentrations of nitrogen (N), phosphorus (P), potassium (K) and silicon (Si). The soil in these blocks is classified as pellic vertisols (Sombroek *et al.*, 1980). In both blocks, soil samples were taken using the zig zag method described by Okalebo *et al.* (1992) from 0-15 and 15-30 cm depths. Each sample was a composite of four cores since the field was uniform in terms of topography, soil colour, and texture. Eight composite soil samples were taken from each block at 0-15 cm and 15-30 cm making a total of 32 samples.

The samples were air-dried for four days in a fibreglass house. The samples were then ground and sieved through a 2-mm sieve.

Diseased and healthy plants at heading stage were randomly taken from the field for two seasons. Plants were oven dried at a temperature of 70⁰c, and then ground. There were sixteen samples that were diseased and sixteen healthy plants.

3.2.1.1 Silicon in the soil

Soil samples were collected from MIAD fields (infected and non-infected fields) and air dried for four days in a fibreglass house. To prepare the reagent A that was used for extraction 24.5g sodium acetate was dissolved in 80ml water, 49.2ml glacial acetate was added and pH adjusted to 4 with acetic acid. Distilled water was added to 1 litre and stored at room temperature as described by Imaizumi, and Yoshida. (1958).

Reagent B that was used for colouring was prepared by dissolving 50gm ammonium molybdate in 200mls of hot water. The mixture was allowed to cool. Distilled water

was added to make 250mls. Fifty ml of water was added to 100 ml concentrated hydrochloric acid (HCL) and allowed to cool then mixed with the 250ml solution. Reagent C for colouring was prepared by dissolving 40gm of tartaric acid in 100mls of distilled water and kept at room temperature. Reagent D (Colouring) was prepared by adding 70gm of anhydrous sodium sulfite and 40gm potassium sulphate and stored at room temperature.

The dried soil was ground and sieved through the 0.5 mm sieve. With the use of weighing balance 2gm of soil was measured and put in a shaker tube. Ten mls of reagent A was added covered with a cap and the test tube heated in a boiling water bath for 10 minutes. The boiling water was kept at 100°C. The caps were removed and the shaker tubes covered with a plastic stopper, placed in the shaker tube for 20minutes. A filter paper (Whatman number 42 paper) was placed to drain the filtrate into a test tube indicated clear colour and therefore used as the blank. To prepare a sample solution 1ml of the of the filtrate was collected and transferred to another test tube, distilled water was added up to a 5 graduation mark of the test tube with the use of micropipette. Zero point five mls of the reagent B was collected added to the sample and shaken well. It was allowed to stand for three minutes then added 0.5ml of the reagent C shaken well and allowed to stand for three minutes. One mls of reagent D was added and immediately sealed with a rubber stopper and shaken well to dissolve it to prevent it from solidifying in the test tube. It was allowed to stand for 20 minutes together with the blank. A filter and No1 graduation plate was fitted in the colorimeter and blank and the sample readings were taken. The results obtained were converted mg/kg as the formula shows.

Reading X % (0.46)/molecular weight * valency of the element. Conversion to mg/Kg the formula used was as follows

$$\text{Me}/100\text{gX relative atomic weight}/\text{valency} /1000 * 10 *1000$$

Where X is the reading of the sample and Me as mill equivalent

3.2.1.2 Determination of soil phosphorous

The Olsen method was used in phosphorous determination due to its suitability for a wide range of pH values (Okalebo *et al.*, 2002). Soil was air-dried for four days in fibreglass and later ground to pass through a 2mm sieve. Two point five grams was put into a 150g bottles and shaken for thirty minutes and then opened. 50 ml of Olsen extraction (0.5M NaHCO₃ pH 8.5) 1g of activated charcoal was added to obtain a clear filtrate. The mixture was shaken for 30 minutes before filtering with number 42 Whatman filter papers.

Phosphate standards solution stock solution 250ppm Phosphorous

1.0984 g of oven dried potassium dihydrogen phosphate (KH₂P0₄ AR) was weighed dissolved in distilled water and filled to 1000 ml mark.

Phosphate standards solution

From the prepared stock solutions (250ppm), 0, 1,2,5,10,20 and 25ml were pipetted into clean 500ml volumetric flask. A 100ml of the Olsen's extracting solution was added and filled to the 500ml mark with distilled water. These solution contained 0, 0.5, 1, 2.5, 5, 7.5 10, and 12.5ppm Phosphorous

Colorimetric measurements

Ten (10ml) of each phosphorus standard and 10ml of sample filtrate and a reagent blank were pipetted into 50 volumetric flasks. 5ml of 0.8M boric acid was added to each flask. Beginning with the standard 10 ml of ascorbic acid reagent was added into each flask and filled to 50ml mark. After one hour the absorbance of the solution was measured at a wave length set at 880nm. The Phosphorus (ppm) in the solution of the standards phosphorus was obtained from a calibration curve. Correction for reagents blank Phosphorus concentration were made

Calculations

The concentration of P in the sample expressed in mg/kg was calculated using the formula.

$$P \text{ mg kg}^{-1} = (a-b)*v*f*1000/1000*w$$

Where a = the concentration of P in the sample

B = Concentration of p in the blank

V = Volume of the extracting solution

F = Dilution factor

W = Weight of the sample

3.2.1.3 Determination of soil potassium in the soil

The potassium was extracted from the dried soil in a ratio 1:5 (w/v) with a mixture of 0.1N HCL and 0.025N H₂S₀₄ solution. 500g of dried soil was weighed in a 50ml polythene bottle. A scoop of (0.5g) of activated charcoal and 25ml extracting solution was added. The solution was mechanically well mixed for one hour at room

temperature and later filtered as described by Okalebo *et al.*, (2002)

Standard stock solution 0.005N

Zero point three seven three (0.373g) of potassium chloride was and dissolved in 800ml distilled water 25ml of extracting solution was added then filled up to the one liter mark and mixed well

Standard series

A series of 0,20,40,60 and 80ml of standard stock solution was pipetted into 100 ml volumetric flask and filled up to the mark with extracting solution.

Two millilitres of working standard series, soil extract and blank were pipetted into 25ml test tubes. 20mls of distilled water was added and well shaken. The working standard series soil extract and blank solution were aspirated into flame photometer and the transmission recorded. Calibration graph of transmission of working standard series against potassium concentration (in me/100g soil) were obtained. The concentrations in samples were obtained from the graph. Further conversion was done from me/100g to centimol per kg (Cmol/kg) by multiplying the number with a factor of 1.

3.2.1.4 Determination of Nitrogen

Zero point five gram (0.5g) of soil was weighed and put into the digestion tube. 10ml of concentrated sulphuric acid was added and mixed well with (CUSO₄ + K₂SO₄ + Selenium) as described in Kjeldahl digestion method (Okalebo *et al.*, 2002).

The mixture was put in the digestion block heated at a temperature ranging between 250⁰C - 350⁰C for 2-6 hours until the solution turned cream yellow. Two (2mls) of hydrogen peroxide (H₂O₂) was added in the digestion tube after every one to one and half hour (1-1/2) in well aerated area until the mixture became colourless. By the end of the digestion the solution was light yellow and the remaining sand had turned to white colour. The mixture was allowed to cool and transferred to a 100mls volumetric flask and filled to the mark with distilled water to make 100mls of the solution. At this point the digest solution contained the nitrogen in form of (NH₄SO₄)

In the distillation process 100mls of the solution, 25mls was collected and 10mls of sodium hydroxide added into it. This was distilled for seven minutes. The ammonium liberated was collected in 10 mls boric acid indicator solution (H₃BO₃) contained in 50ml conical flask. Distillation continued until the colour of the solution changed from pink to green. To calculate the nitrogen percentage the shown formula was used.

$\% N = (V_s - V_b) \times 0.28 \times 100 / V \times 100 / S$ where;

% N = Nitrogen content in the sample

V_s - Volume of the titre ml 0.01N H₂SO₄ for sample

V_b - Volume of the titre ml 0.01N H₂SO₄ for the blank

V = Volume (ml) of digest solution used for distillation (Morality of the H₂SO₄)

S = Weight of the sample in mg

3.2.2 Plant tissue sampling and analysis

3.2.2.1 Nitrogen in the rice plant

Sixteen plant samples were randomly collected from MIAD fields using the zig zag method as described by Okalebo *et al.*, (2002). Diseased and healthy plants were oven dried for three days at 70 °c and grinded. The plants were analyzed for nitrogen which was determined by use of Kjeldahl nitrogen method as described by Okalebo *et al.*, (2002). Zero point three (0.3g) of oven dried plant samples were weighed and put into the digestion tube. Two point five millilitres of digestion mixture (Selenium powder, salicylic acid and sulphuric acid) and added to each tube and the reagent blanks. The mixture was digested at 110⁰C for one hour then removed and cooled. Three successive 1ml portions of hydrogen peroxide (H₂O₂) was added to the mixture, temperatures raised to 330⁰C and heating continued for two hours. By the end of the digestion, the solution was light yellow to clear in colour. It was allowed to cool and transferred to a 50mls volumetric flask and filled to the 50mls mark with distilled water.

Distillation and titration

Blank was first run through the processes followed by the samples where a 5ml of the sample (digest) was transferred to reaction chamber. Ten millilitres of 40% sodium hydroxide added into it and the steam distilled immediately into a 10ml boric acid indicator solution containing 4 drops of mixed indicator. Distillation continued for two minutes until the indicator turned to green then removed for titration. Titration was done with 0.01N HCL through an automatic burette until the

colour of the indicator changed to pink. In order to calculate the percentage of the nitrogen content the formula below was used.

$\% N = (V_s - V_b) * 0.14 * 14 * V * 100 / 1000 * w * al$ where;

% N = Nitrogen content in the sample

V_s - Volume of the titre ml 0.01N HCL for the sample

V_b - Volume of the titre ml 0.01N HCL for the blank

V = Final volume (ml) of digest solution.

S = Weight of the sample taken in g

3.2.2.2 Phosphorus in rice plant.

Sixteen plant samples were randomly collected from MIAD fields using the zig zag methods as described by Okalebo *et al.* (2002). Diseased and healthy plants were oven dried for three days at 70 °c and grinded. The plants were first taken through the digestion process using the Kjeldahl method as described by Okalebo *et al.* (2002)

The standards were prepared through a phosphorous stock solution of 1000ppm where 1.0982g of oven dried KH₂PO₄ was dissolved in distilled water and filled to 250ml mark. 10mls of the stock solution was diluted to 1 litre with distilled water. A series of 0,1,2,3,4,5 and 6 of the 10mls was pipetted into 50ml volumetric flask. Ten (10 ml) of ascorbic acid reducing agent was added into each flask and filled up to 50ml with distilled water and was let to stand for one hour and absorbance read through the colorimeter. By then the standard contained 0.0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ppm respectively. Five millilitre of the supernatant clear wet-ashed digest was pipetted into 50ml volumetric flask and 20ml of distilled water added into each flask.

Ten millilitres of ascorbic acid reducing agent was added into each flask and filled up to 50ml with distilled water, stoppered and shaken well. The solution was let to stand for one hour to allow full colour development. The absorbance of both the standards and the samples were read in a calorimeter at 880nm. The amount of phosphorous present in the solution was read from the calibrated curve prepared plotting absorbance readings against phosphorous concentration in the standards series. Corrections of reagent blank were made by subtracting the blank value from the sample concentration value.

3.2.2.3 Determination of potassium in rice plant

Sixteen plant samples were collected from different fields in MIAD that had infected and non-infected plants. The plants were oven dried for three days at 70 °c and grinded. The plants taken through the digestion process using the Kjeldahl method as described by Okalebo *et al.* (2002)

The standards were prepared through a stock potassium solution of 1000ppm. A 1.907 g potassium chloride was dissolved in one litre of distilled water. A 10ml of the stock solution was diluted to 100ml while the standard series of 0,1,2,3,4 and 5ml of a 100ppm potassium was were pipetted into 100ml flask and filled to the mark with distilled water. These solutions had concentrations of 1, 10,20,30,40 and 50ppm respectively. A 2ml of the digest sample solution was pipetted into 50ml volumetric flask and filled to 50ml mark with distilled water and thoroughly mixed. The working standard series, plant extract and the blank solution were aspirated into a

flame photometer at a wave length of 766.5nm. The amount of potassium present in the solution was read from the calibrated curve prepared by plotting absorbance readings against potassium concentration in the standards series. Corrections of reagent blank were made by subtracting the blank value from the sample concentration value.

3.2.2.4 Silicon in rice plant

Plant samples were collected from MIAD fields (diseased and healthy plants). The analysis was done on nitrogen, phosphorus, potassium and silicon nutrient of which the nutrients of interest were mainly silicon and nitrogen. Nitrogen was determined by use of Kjeldahl nitrogen procedure which involved digestion with NaOH and dissolved in distilled water. The procedure followed was similar to that of the soil analysis with a difference of initial measurement of the sample which was 0.5g. Similarly the silicon concentration in the plant tissue followed the same procedure of colorimetric molybdenum blue method as for the soil however; the digestion was done by use of Kjeldahl method was determined by the (Elliot and Snyder, 1991; Snyder, 2001). Silicon content in rice was measured as the amount of silicon in milligrams per 100mg weight in the plant. The data was subjected to the analysis of variance using SAS was done between the infected and the non-infected tissues. The same was done between the infected and non-infected soils.

One gram of ground plant tissue sample was weighed into a crucible. Sample was fused into a muffle furnace and temperatures were slowly raised to 550⁰C and ashed

for five hours until no black ash remained as described by Gupta, (1999). The sample were removed from the furnace and allowed to cool and weighed. 3mls of hydrochloric acid was added and heated for four half an hour in a steam bath. The mixture was cooled and transferred into 100mls volumetric flasks and mixed immediately into a polythene bottle. 2mls of the aliquot samples were pippered into 50mls and neutralized by adding a few drops of sodium hydroxide using phenothalein as indicator. The solution was neutralized by adding 1% hydrochloric acid. 1.25ml molybdate reagents was added, mixed and left for 10 minutes, 1.25ml of tartaric was added and left for 5 minutes. 1ml of reducing solution was added and the flask allowed to settle for 15 min to allow colour development. The sample and the standard were read using spectrophotometer (model UV-mini 1240) at 810nm. Calibration curve for the standard was constructed and from it obtained the ppm silicon in the sample aliquots.

The Standard was prepared by using 0.41g calcium silicate and 2gms of sodium carbonate that was dissolved in 1000mls distilled water. The mixture was boiled until it became clear. Standard of 0, 10,15,20,25 were prepare and filled to 100mls volumetric flask,

3.4 RESULTS AND DISCUSSION

Results

3.4.1. Nutrients levels in rice blast infected and non- infected fields

The results indicated that there were significant differences among the infected and

non-infected fields in total nitrogen, silicon, phosphorous and potassium levels ($P < 0.05$) respectively (Figure 2, 3, 4 and 5). Nitrogen level was high in the field that had high disease incidence than in the healthy field in both 0-15 and 15-30cm depth.

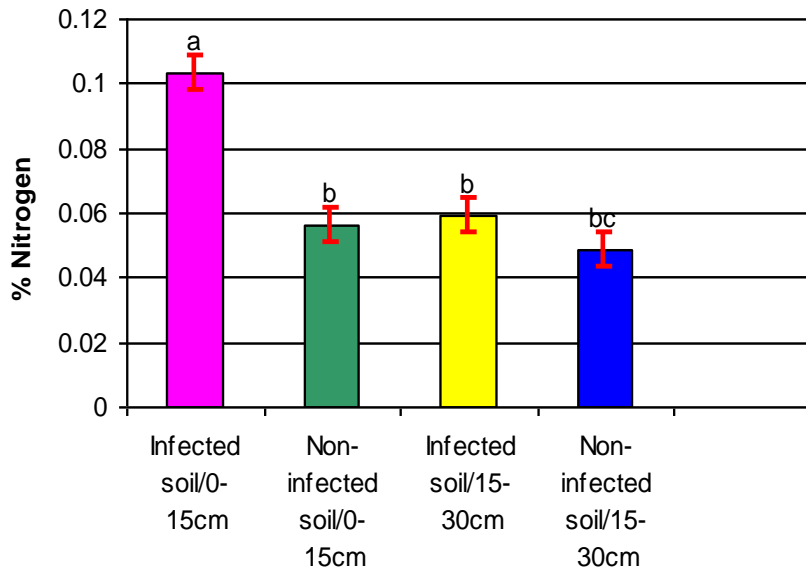


Figure 2 Nitrogen level in the field with diseased and disease free field

Available nitrogen was compared among the infected and non-infected as shown in Table 2, the results indicated significance difference ($P < 0.05$) in the two fields (infected and non-infected). Nitrogen concentration was higher in rice infected field than in non-infected for the two seasons the analysis was carried out. In the second season the percentage of available nitrogen was higher than the first season which may have resulted from the additional organic mineral from the previous season crop residues.

Table 2. The level of Available nitrogen in infected and non-infected fields

Treatments	Available Nitrogen (%)	
	Season one	Season two
Field with diseased plants	2.22 a	2.33 a
Field with healthy plants	1.51 b	1.89 b

NB: Means followed by the same letter within the column are not significantly different

Results showed that silicon level was lower in the field that had higher disease incidence at the two depth (0-15 and 15-30cm) than in the field that had healthy plants. The highest concentration of silicon was at 0-15cm depths (Figure 3). The lowest silicon concentration was at 0-15cm depth though not significantly different from 15-50cm.

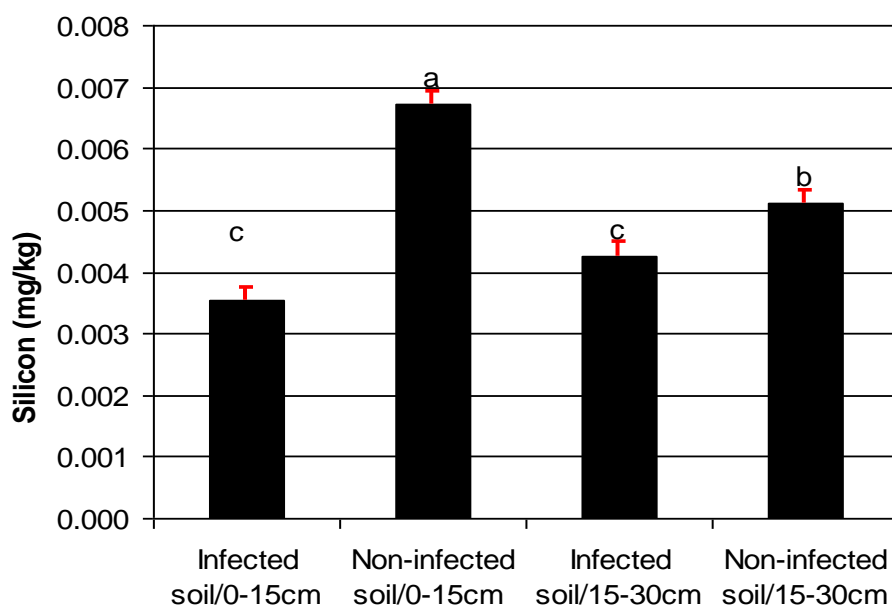


Figure 3. Silicon level in the field with diseased and disease free field

The result in figure 6 showed that potassium level was significantly different ($P<0.05$) in the two fields but only in the depth of 15-30cm. The field that had diseased plants had lower level of potassium compared to the field with healthy plants.

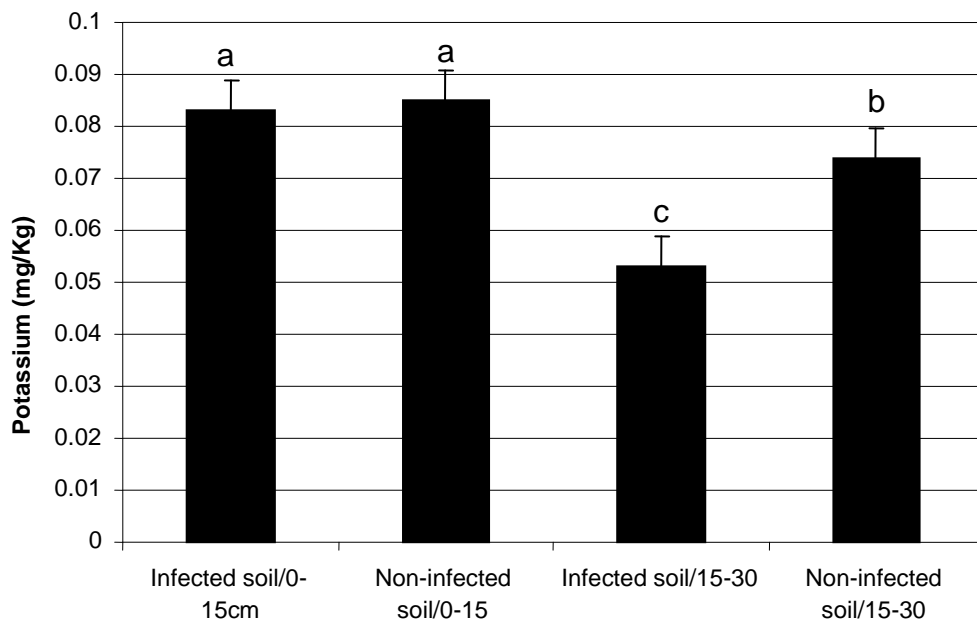


Figure 4. Potassium level in the field with diseased and disease free field

Phosphorous concentration was higher in the field that had healthy plants at 0-15cm and 15-30cm than in the field with disease crop at the same soil depth (Figure 5). The depth of 15-30cm in the field that had diseased plants showed the least concentration of phosphorous. There was also significance difference between the field with diseased plants and the field with healthy plants. The field with diseased plants had less phosphorous than in the field with healthy plants at the two depths (0-15 and 15-

30cm). On contrary the plants analyzed for the phosphorous did not indicate and difference in the diseased and the healthy plants (Table 3).

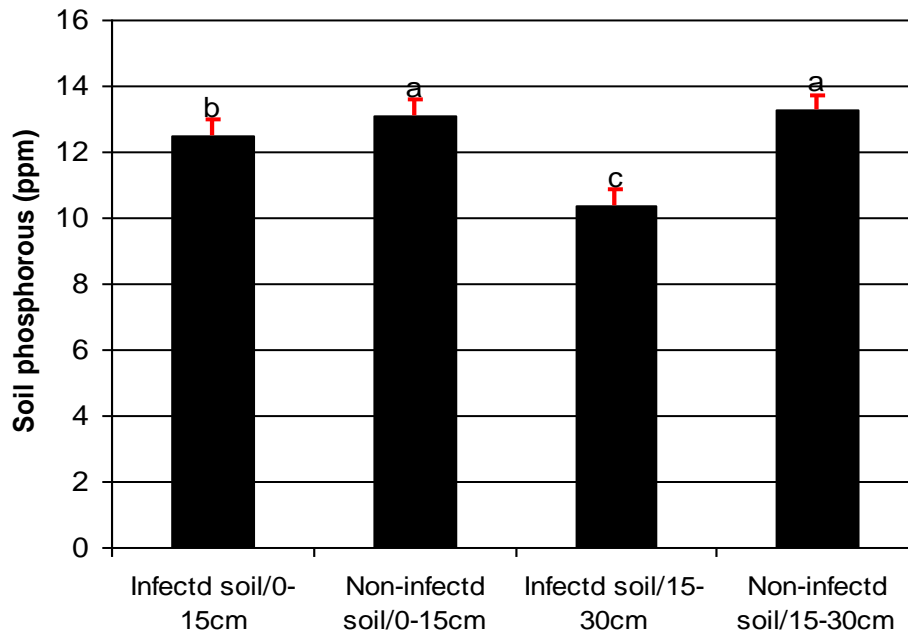


Figure 5. Phosphorous level in the field with diseased and disease free field

The results in Table 3 showed that there were significant differences ($P < 0.05$) among the plants that had disease incidence and the healthy plants in nitrogen, silicon and potassium levels. Phosphorus however, did not show significance difference among treatments ($P > 0.05$). The plant tissues showed higher nitrogen concentration in plants that had disease incidence while it was lower in the healthy plants.

Results in table 3 showed that potassium in plant tissues was significantly higher in the healthy plants than in the diseased plants.

Table 3: Levels of nutrients (P, K and Silicon) in rice blast infected, non-infected plants.

Treatments	N (%)	P (mg/kg)	K (mg/kg)	Si (mg/Kg)
Rice blast infected plants	4.2a	1890a	3123.9b	1.98b
Non-infected plants	1.8b	1660a	3354a	2.93a

Means in the same column followed by the same letter are not significantly different (P<0.05) according to LSD test.

Discussion

The results showed significance difference in the level of nitrogen in both soils and plant tissues from the two fields, the level of nitrogen was higher in 0-15 cm depth indicating higher nitrogen level in the rice plant rooting zone that is usually at 20 cm (Figure 2). The higher nitrogen in this zone may also have been contributed by the decomposed plant residues and the fertilizer previously applied. Findings from Kurschner *et al.* (1992) reported that the severity of rice blast increased with high rate of fertilizer applied. The findings also agreed with results in (IRRI, 1996) which showed that application of high nitrogen resulted in increased attack of rice blast. High nitrogen levels in the soil resulted to higher disease pressure which may have resulted from the creation of canopy that became favourable to the fungus to thrive. It is also likely that the pathogen easily penetrated the tender tissue without much resistance due to higher nitrogen (Table 3).

The depth of 0-15cm was found to have more silicon than 15-30cm depth. The

higher level could have resulted from the decomposed straws which from the analysis done by Muriithi, *et al.* (2010) showed to contain some levels of silicon. A study by Korndorfer *et al.* (2001) indicated that grasses such as wheat, oat, rye, barley, sorghum, corn, and sugarcane contain about 10 g/kg in their biomass (figure 3).

Disease incidence was lower in the field with high level of potassium was an indication that potassium may have played a role in prevention of disease occurrence, it may also have implied that it was in available form to the plant. Healthy plants had higher level of silicon which show a correlation between silicon and rice blast incidence. Increased level of silicon in the plant may have increased plant resistance (Table 3). These results support the findings of Bringezu *et al.* (1999) who found that silicon cause resistance of disease to the plant as it is localized in the cell wall. It is also likely that it might have accumulated in external layers below and above the cuticle of leaves preventing the pathogen from penetration

Potassium was found to be higher in the soil that had healthy plants in 15-30cm depth (figure 4) as well as in the healthy plant tissues' in table 3. These results showed that potassium level at 15-30cm depth may have been available to the plants. The rice plant rooting zone ranges in this depth and therefore benefiting from the fertilizer leached at this depth. Results by Haerdter, (1997) indicated decrease in rice disease incidence with increased application of potassium.

Phosphorous showed significance difference in soil that had diseased plants and the

healthy plants which may imply that it might have contributed in disease severity. Study done by Singh, (2005) showed that phosphorus contents in the soil had contribution on disease infection. The plants analysis did not show significance difference in the levels of diseased and the healthy plants which may imply that phosphorous did not have any effect on the occurrence of rice blast disease. Similar results were found by Luong *et al.*, (2003) who reported that phosphorous does not affect the outbreak of insect pest and diseases. Due to this discrepancy further work is required to find out the relationship between the phosphorous and the rice blast disease.

3.5 CONCLUSION

From the analysis of soil and plants tissues it can be concluded that high nitrogen concentration leads to rice disease infection. Soil with high silicon levels exhibit reduced rice blast disease. It is also revealed that more nitrogen and silicon levels are concentrated in 0-15cm depth. Basing on these findings it was necessary to determine the optimal nitrogen and silicon on the management of rice blast.

CHAPTER FOUR

THE EFFECT OF NITROGEN AND SILICON ON RICE YIELD AND RICE BLAST DISEASE MANAGEMENT

4.1 INTRODUCTION

The interaction between mineral nutrients is an important factor that affects the growth and the production of the field crops as well as foliar pathogens. This implies that sufficient supply of nutrient in rice plant is required for healthy growth and higher productivity. Interactions of silicon with other nutrients particularly nitrogen, phosphorous and potassium offers a potential to improve efficiency in terms of yield response. According to Servant *et al.*, (1997) silicon helps the rice plant to resist both the biotic and abiotic stresses. It also helps to reduce cuticular transpiration and to some extent lodging caused by excess nitrogen. It has also been found to increase dry matter production and grain yield in rice (Marschner, 1995). Besides improvement of yield and yield component large amount of silicon has been seen to accumulate in some crops like rice and other grasses. Rice containing inadequate silicon is severely infected by rice blast (Datnoff, *et al.* 1990; Datnoff *et al.* 2001)

Nitrogen on the other hand is an important element for normal and health growth of plants. It is the element responsible for vital biological processes however, as reported by Kurschner *et al.* (1992) high nitrogen concentration in plant increase the severity of infection by parasites. Nitrogen supplies influences branching and leaf expansion which together determines the canopy size. A large canopy is conducive

for spore transfer and pathogen infection than a sparse one. The minerals may either increase or decrease the plants resistance/tolerance to pathogens by affecting changes in plant morphology, growth pattern and also chemical composition as stated by Marschner, (1995). Rice in Kenya is affected by rice blast caused by *Pyricularia oryzae* (synonym *P. grisea*, teleomorph *Magnaporthe grisea*). Rice blast has been identified and reported as the most important fungal disease in rice as (Bonman *et al.* 1991; Lee, 1994). Two experiments were conducted to determine the effect of silicon and nitrogen on yields and management of rice blast caused by *Pyricularia oryzae*.

4.2 THE EFFECT OF NITROGEN AND SILICON ON RICE YIELD

4.2.1 MATERIALS AND METHODS

An experiment was conducted in MIAD for two seasons (Oct 2008-March 2009 and April-August 2009). Vertisols soil type was obtained from Mwea irrigation Agricultural Development (MIAD) fields. It was analyzed for nitrogen, phosphorus, potassium and silicon to determine the contents in the soil. Certified seed was sown in the nursery which was preceded by seed treatment through soaking and incubation (pre-germination). Soaking was done for 24 hours while pre-germination took 3 days before sowing. Seedlings were later transplanted into plastic bags after twenty eight days.

Six kilograms of soil was filled in plastic pots (25*37cm) with different levels of silicon and nitrogen which were randomly assigned into experimental units. These experiment units were laid in a Complete Randomized Design (CRD). The treatments included four levels of silicon (0, 500, 1000 and 1500kgSi/ha), four levels

of nitrogen 0, 40, 80 and 120NKg/ha. This translated to 0, 2500, 5000 and 7500kgSi/ha of calcium silicates (0, 1.8, 3.6, and 5.4gm/6kg pot) respectively. Sulphate of ammonia was the source of nitrogen applied at 0, 0.46, 0.91 and 1.37gm per pot as a top dress into two splits. The first split was applied two weeks after transplanting and at maximum tillering (35days after transplanting). Potassium and phosphorus was applied at recommended rates of 30Kg K₂O/ha and 58kgP₂O₅/ha. The two fertilizers were obtained from Muriate of Potash and Triple Super Phosphate. Calcium element was added in all the treatments that had not received calcium silicate at 257,515,772kg/ha (0, 0.62, 1.24 and 1.85g/pot) in order to get similar effect of calcium in all the treatments. The media was well-irrigated four days before the seedlings were transplanted. The seedlings that were of the same height and thickness were selected for transplanting.

4.2.2 Data collected

Data collection started two weeks after the seedlings were transplanted. It included plant height, number of tillers both productive and non-productive, panicles size, plant biomass, grain yield adjusted to 13 % moisture content. The leaf angle was scored according to the IRRI standard using a score of 1-7 where 1 stands for erect, 3-intermediate, 5-horizontal and 7 for descending leaves

4.2.3 Data analysis

Data was analyzed by analysis of variance (ANOVA) using Statistical Analysis System (SAS) statistical package version 8.1 at 5% and means separations using LSD.

4.2.4 RESULT AND DISCUSSIONS

Results

The result of interaction of silicon and nitrogen (Table 4) showed that the highest number of productive tillers were produced at 120KgN/ha, 1000KgSi/ha (N₃S₂), 80KgN/ha, 1500kgSi/ha (N₂S₃) and 120KgN/ha, 1500kgSi/ha (N₃S₃). These were followed by 80KgN/ha, 1000kgSi/ha (N₂S₂) which was not significantly different from (N₃S₃). However, the least was produced at 0KgN, 0Kg Si /ha (N₀S₀), 0KgN, 500Kg Si/ha (N₀S₁), 120KgN/ha, 0kgSi/ha (N₃S₀) and N₂S₀ 80KgN/ha, 0kgSi/ha

On the contrary as nitrogen level increased from 80Kg/ha to 120Kg/ha (N₃S₀) without any silicon the number of productive tillers decreased.

The mean panicle filled grain showed similar trend as those of productive tillers as indicated in Table 4. The highest number of grain was obtained at 120KgN/ha, 1500kgSi/ha (N₃S₃) 120KgN/ha, 1000KgSi/ha (N₃S₂), 80KgN/ha, 1500kgSi/ha (N₂S₃) and 80KgN/ha, 1000kgSi/ha (N₂S₂). It was least at 0N kg/ha, Si0kg/ha (N₀S₀) and 40Kg N/ha, Si0kg/ha (N₁S₀). .

Leaf angle was highly influenced by nitrogen/silicon interaction (P<0.05). The erect leaves were realized from the pots that had 40KgN/ha, 1000KgSi/ha (N₁S₂), 0KgN, 80KgN/ha (N₀S₂), 0KgN, 1500kgSi/ha (N₀S₃), 40KgN/ha, 1500kgSi/ha (N₁S₃) 80KgN/ha, 1000kgSi/ha (N₂S₂), 80KgN/ha, 1500kgSi/ha (N₂S₃), 120KgN/ha, and 1500kgSi/ha (N₃S₃). Pots that had 120KgN/ha, Si0kg/ha (N₃S₀) and those that had no nitrogen (0KgN) or silicon (0KgSi/ha) (N₀S₀) added produced descended leaves.

Although the general trend showed that the pots with silicon produced erect leaves, this did not always contribute to high yield. The weight of a 1000 seed (Table 5) was highest at 80KgN/ha, 1000kgSi/ha (N₂S₂) and lowest at 120KgN/ha, 0Kg Si/ha (N₃S₀).

Table 4 The effect of silicon nitrogen interaction on rice growth and yields season one(Oct 2008-March 2009)

Nitrogen level	Silicon level	Productive Tillers	Total Tillers	Leaf angle	Mean plant height	Wt biomass T/ha	Mean Panicle length	Mean Panicle filled grain	Weight of 1000seed	Yield T/ha
N0	S0	10.3h	14.0g	6.0ab	103.0bc	0.8h	21.0defg	33.8g	16.6f	0.9f
N0	S1	11.3gh	15.0fg	3.0e	96.0c	0.9h	22.5bcdef	39.9f	16.6f	1.0ef
N0	S2	13.3ef	14.8fg	1.3f	99.8bc	0.9fgh	20.5fg	39.2cd	17.1def	1.3c
N0	S3	14.8ed	15.8efg	1.0f	94.750c	0.9gh	19.5g	59.5e	18.3cd	1.3cdef
N1	S0	11.0g	15.8efg	3.5cd	94.8c	1.1defg	22.0cdef	38.5fg	16.5f	1.1cdef
N1	S1	15.3d	19.0def	3.0e	109.8abc	1.2cdefgh	21.9cdefg	59.6cd	18.0cde	1.6b
N1	S2	17.3c	18.8def	1.0f	106.8abc	1.1cdefgh	22.8bcdef	63.4bc	18.2cd	1.7b
N1	S3	17.3c	21.3cd	1.0f	109.0abc	1.1efgh	23.3abcd	65.8b	18.2cd	1.7b
N2	S0	11.0gh	22.5cd	4.5a	112.0ab	1.5bcd	20.8efg	57.3d	18.3def	1.2 cde
N2	S1	14.0def	20.0de	5.0bc	101.5bc	1.4bcde	23.6abc	61.1bcd	18.4cd	1.5c
N2	S2	19.3b	23.0cd	1.0f	112.0ab	1.3bcdef	24.0abc	75.2a	23.1a	2.4a
N2	S3	21.5a	23.3bcd	1.0f	112.3ab	1.4bcde	22.7abcdef	74.5a	20.7b	2.5a
N3	S0	10.3h	30.8a	7.0a	105.3abc	1.6b	22.5bcdef	51.1e	13.0g	1.5def
N3	S1	12.3fg	24.8bc	5.0bc	119.0a	1.3bcdefg	23.2abcde	58.8cd	18.7c	1.6cd
N3	S2	21.5a	31.3a	3.0e	112.5ab	1.5bc	24.8ab	73.0a	21.1b	2.4a
N3	S3	20.8ab	27.8ab	6.0f	106.8abc	2.4a	25.3a	72.7a	21.6 b	2.5a
CV (%)		15.64	21.1	27.5	106.5	21.7	22.53	5.8	18.9	1.64
Means		10	15.4	3.3	9.9	1.27	7.61	7.27	5.03	8.5

KEY: (N0S0) 0KgN,0KgSi/ha (N0S1) 0KgN,500KgSi/ha (N0S2) 0KgN,1000KgSi/ha, (N0S3) 0KgN,1500KgSi/ha (N1S0)40KgN,0KgSi/ha, (N1S1) 40KgN,500KgSi/ha,(N1S2)40KgN,1000KgSi/ha,(N1S3)40KgN,1500KgSi/ha,(N2S0)80KgN,0KgSi/ha,(N2S1)80KgN,500KgSi/ha,(N2S2)80KgN,1000KgSi/ha, (N2S3)80KgN,1500KgSi/ha,(N3S0)120KgN,0KgSi/ha,(N3S1)120KgN,500KgSi/ha,(N3S2)120KgN,1000KgSi/ha,(N3S3)120KgN,1500KgSi/ha

The crop yield was influenced by nitrogen, silicon interaction. The interaction of silicon and nitrogen at 120KgN/ha, 1500kgSi/ha (N3S3) gave the highest yield. However, this was not significantly different from 80KgN/ha, 1000kgSi/ha (N2S2), 120KgN/ha, 1000KgSi/ha (N3S2), and 80KgN/ha, 1500kgSi/ha (N2S3) Table 4. A lower yield was obtained from 0KgN, 0Kg Si /ha (N0S0), 0KgN, 500Kg Si/ha (N0S1), 40KgN, 0Kg Si /ha (N1S0) and 120KgN/ha, 0Kg Si/ha (N3S0). Although they were not significantly different their mean varied. The treatment that showed higher productive tillers similarly obtained a higher yield and increased weight of 1000 grain.

The results (Table 4) revealed that there was significant difference ($P < 0.05$) in weight of the biomass per hectare as influenced by nitrogen silicon interaction. Nitrogen and silicon applied at 120KgN/ha, 1500KgSi/ha (N3S3) gave the highest mean (2.4ton/ha) followed by 120KgN/ha, 0KgSi/ha (N3S0) while the least came from the treatment that had no nitrogen or silicon (0KgN, 0Kg Si/ha (N0S0). Higher number of tillers (23-25) were realized at an application of 120KgN/ha, 80KgSi/ha (N3S2) 120KgN/ha, 0Kg Si/ha (N3S0) and 120KgN/ha, 1500Kg Si/ha (N3S3) while the least number of tillers were produced in the pots that had no nitrogen nor silicon 0KgN, 0Kg Si/ha (N0S0) and in all others pots that did not receive nitrogen which included 0KgN, 500Kg Si/ha (N0S1) 0KgN, 1000Kg Si/ha(N0S2). However, the pots that had 80KgN/ha, 1000kgSi/ha (N2S2), 80KgN/ha, 1500kgSi/ha (N2S3) were not significantly different in the number of tillers.

The mean panicle length did not differ significantly among the treatments however

the highest mean was obtained from 120KgN/ha, 1500Kg Si/ha (N3S3) and the least was from 0KgN/ha, 1500Kg Si/ha (N0S3)

Plant height was significantly influenced by the treatments with mean height of 103cm. The highest response was at 120KgN/ha, 1500kgSi/ha (N3S3), 120KgN/ha, 1000kgSi/ha (N3S2), 120KgN/ha, 500kgSi/ha (N3S1) and 120KgN/ha, 0kgSi/ha (N3S0).

The result presented in Table 5 in the second season indicated that 120KgN/ha, 1500kgSi/ha (N3S3) and 80KgN/ha, 1000kgSi/ha (N2S2) had the highest response in production of the productive tillers (23 tillers) followed 80KgN/ha, 1500kgSi/ha (N2S3) 120KgN/ha and 1000KgSi/ha (N3S2) (22 tillers). The weight of a 1000 seed was also influenced by the different treatments, 80KgN/ha, 1500kgSi/ha (N2S3) obtained the highest means but it was not significantly different from 80KgN/ha, 1000kgSi/ha (N2S2), 40KgN/ha, 1000KgSi/ha (N1S2) and 120KgN/ha, 1500kgSi/ha (N3S3). Similar to the first season the highest yield was obtained in the pots that had 80KgN/ha, 1000kgSi/ha (N2S2), 120KgN/ha, 1500kgSi/ha (N3S3) and 80KgN/ha, 1500kgSi/ha (N2S3). These were followed by 120KgN/ha, 1000kgSi/ha (N3S2) while the least was obtained from 0KgN, 0Kg Si /ha (N0S0), 0KgN, 500Kg Si/ha (N0S1) and 40KgN, 0Kg Si /ha (N1S0).

Table 5. The effect of yields and its growth parameters in the interaction of nitrogen and silicon season two (April-August 2009)

Nitrogen	Silicon level	Prod tillers	Total Tillers	Leaf angle	Mean plant height	Wt biomass T/ha	Mean Panicle length	Weight of 1000 seed	Yield t/ha
N0	S0	12g	17ij	7a	88.8h	1.1def	19.4abc	15.3ef	1.0i
N0	S1	12g	16jk	3de	89.5h	1.2def	19bc	16.9de	1.1hi
N0	S2	14f	17ij	2.ef	100.40efg	1.2ef	17.9cd	19.4ab	1.4e
N0	S3	14.3fg	16j	1.f	94.7fgh	1.3 def	19.7abc	18.5bc	1.4e
N1	S0	10h	17hij	3.5cd	92gh	1.2cdef	18.8bc	18.9abc	1.0i
N1	S1	16de	18ghij	3.ed	94.8fgh	1.1def	18.9bc	15.2f	1.3fg
N1	S2	17cd	20fghi	1.f	101.8def	1.31bcdef	19.9abc	19.6ab	1.6d
N1	S3	18c	20fgh	1.f	101def	1.2 def	19.8abc	17.3cd	1.8c
N2	S0	14f	21fg	6a	104.5bcd	1.4bcde	19.8abc	14.8f	1.2gh
N2	S1	18b	22ef	5b	103cdef	1.4cdef	20.5ab	13.9f	1.4e
N2	S2	23a	25cde	1f	111.3abc	1.4bcd	21.0ab	19.7ab	2.5a
N2	S3	22a	25de	1f	111.0abcd	1.3def	17.8c	20.3a	2.5a
N3	S0	17bc	29b	7a	113ab	1.5ab	20.3abc	13.9f	1.3ef
N3	S1	18bc	28b	7a	118.5a	1.404bcde	20.8ab	15.2eg	1.2fg
N3	S2	22a	28bcd	4cb	114.50a	1.4bc	21.6a	18.6abc	2.4b
N3	S3	23a	36a	3.de	103.5cdef	1.7a	22.8a	19.6ab	2.5a
CV (%)		5	10	20	6.4	30	8.8	6.7	4.9
Means(p<0.05)		16.72	21.98	3.5	102.7	1.31	19.8	17.24	1.6

NB: Means followed by the same letter down the column are not significantly different (P>0.05) KEY: (N0S0) 0KgN,0KgSi/ha (N0S1) 0KgN,500KgSi/ha (N0S2) 0KgN,1000KgSi/ha, (N0S3) 0KgN,1500KgSi/ha (N1S0)40KgN,0KgSi/ha, (N1S1) 40KgN,500KgSi/ha, (N1S2) 40KgN,1000KgSi/ha, (N1S3) 40KgN,1500KgSi/ha, (N2S0) 80KgN,0KgSi/ha, (N2S1) 80KgN,500KgSi/ha, (N2S2) 80KgN,1000KgSi/ha, (N2S3) 80KgN,1500KgSi/ha, (N3S0)120KgN,0KgSi/ha, (N3S1)120KgN,500KgSi/ha, (N3S2) 120KgN,1000KgSi/ha, (N3S3)120KgN,1500KgSi/ha

Discussion

The results showed that the treatment that received 1500 Kg Si/ha (1.8g CaSiO_3 /pot) together with either 80KgN/ha (0.916gSA/pot) or 120KgN/ha (1.37gSA/pot) indicated higher number of productive tillers indicating that both silicon and nitrogen influenced the productive tillers. When nitrogen level was high (N3S0) and without any silicon there was lower number of productive tillers. Increased production of productive tillers may probably have resulted from increased nitrogen and silicon uptake efficiency. These results were in agreement with those found by Takahashi, (1995) who reported that silicon increased fertility of the spikelet which contributes to yield. When nitrogen level increased from 80KgN/ha to 120KgN/ha (N3S0) without any silicon the number of productive tillers decreased. This reduction may be due to increased level of nitrogen leading to increased number of total tillers which caused a minimal number of vegetative buds becoming productive. Study by Kim and Kim, (1990), Cloud and Lee, (1993) and Ishiguro, (1994;) found that high nitrogen application causes excessive growth of tillers due to unbalanced nitrogen supply that creates a microclimatic conditions (Table 4).

The increased filled panicle grains resulted from spikelet fertility as a result of increased silicon. With increased number of productive tillers the filled panicle grain was bound to increase meaning that silicon does not only increase the productive tillers but also contribute to the filling of the spikelets (Table 4). These results support the findings of Falla *et al.* (2004) who found that silicon significantly increased spikelet filling resulting to increased grain yield. On contrary the increased

level of nitrogen from 80 to 120Kg N decreased the number of filled panicle grains perhaps due to decreased amount of productive buds. As found by Kim and Kim high nitrogen increases higher number of tillers but not necessarily the productive tillers that contribute to filled grains

Leaf angle was highly influenced by nitrogen/silicon interaction the pots that 80KgN/ha, 1000kgSi/ha (N2S2), 80KgN/ha, 1500kgSi/ha (N2S3), 120KgN/ha, and 1500kgSi/ha (N3S3) obtained the highest yield indicating a relationship between the erectness of the leaves and the yield. The erect leaves may have resulted in capturing of more light hence more carbon assimilation leading to high yield. Similar results were found by Deren *et al.* (1994) who found that nitrogen silicon interaction improved plants architecture leading to smaller opening of the leaf angle.

The weight of a 1000 seed (Table 5) was highest at 80KgN/ha, 1000kgSi/ha (N2S2) and lowest at 120KgN/ha, 0Kg Si/ha (N3S0). These results showed that additional nitrogen from 80kg/ha (N2) to 120 kg/ha (N3) decreased the weight of 1000 grain indicating that 80kgN/ha as the optimal level of nitrogen. As silicon was added from 1000kgSi/ha to 1500kgSi/ha there was no increase in weight. Combination of 80KgN/ha and 1000kgSi/ha (N2S2) resulted as an effective rate in the increment of weight of a 1000 seed. High rate of nitrogen (120 kg/ha) may have led to reduced carbohydrates supplied to the seed due to shading resulting from high number of tillers. Since silicon transportation is mainly dependant upon transpiration, there was likelihood of the element being deposited during the grain filling stage contributing to a bigger weight of a 1000 seed. These results agree with what was found by

Balastra *et al.* (1989) who reported an increased mass of a 1000 seed resulting from silicon fertilizer which he attributed to the deposition of silicon on the paleae and lemmas.

Results showed that yield increased as the silicon level increased while it reduced with increased nitrogen in the presence of silicon. Application of silicon progressively increased all the yield parameters. Similar results were found by Datnoff *et al.* (1992) who found an increase in yield even in the absence of disease. Since the application of silicon at the 1500Sikg/ha was not significantly different from 1000kgSi/ha in yield, it was economically viable to use the lower rate of 1000Sikg/ha. However, no gross margin was done. The result showed a decreased yield as nitrogen level increased from 80 to 120kgN/ha (Table 4). Excess nitrogen (120KgN/ha) application limited the yield probably through promotion of shades and a lot of assimilate getting into the forages rather than in the grain. Similarly where there was no nitrogen (N0) or where the rate was low (N1) the yield were lower indicating that nitrogen is a vital nutrient in plant growth and development. It can reduce the yield if not applied to the plants or if the application is very little to meet the plant needs as was the case at rate of 40KgN/ha.

The results showed that biomass and total tillers production increased significantly progressively with increasing level of nitrogen in season one and two respectively. The increased number of tillers and higher biomass at high level of nitrogen 120KgN/ha, 0Kg Si/ha (N3S0) may have resulted due to high stimulation from

nitrogen leading to higher cell multiplication and formation of new tissues resulting to high biomass as was reported by (Marschner, 1995).

Unlike nitrogen, silicon at low or higher level did not seem to influence the number of tillers. These results are in agreement with the findings of Ma *et al.* (1989) and Deren *et al.* (1994) but contradicts those of Takahashi, (1995) who found a significance difference in the influence of silicon on biomass. It is therefore likely that silicon had little effect on the increase of the vegetative growth unlike nitrogen. The mean panicle length did not seem to be influenced neither by either nitrogen, silicon nor in their interaction.

The height attained by the plants fertilized with highest level of nitrogen and silicon at 120KgN/ha, 1500kgSi/ha (N3S3) could be due to the stimulation of cells as a result of high production of amino acids that may have led to higher plant height as reported by (Marschner, 1995). It may also indicate the efficiency of the nitrogen uptake by the plants which eventually lead to plant fast elongation. These results however contradicted the work done by Munir *et al.*, (2003) who observed that plant height decreased with increase of nitrogen. Increased silicon supply without nitrogen tended to have no or little effect on the plant height. This was in agreement with work done by Ghanbari-Malidareh, (2011). The result in the second season as presented in Table 5 did not differ with the first season in the various parameters studied as a result of the effect of silicon and nitrogen.

4.2.5 CONCLUSION

From the study it can be concluded that nutrient management is paramount to the growth and yield of rice plants. The interaction of silicon and nitrogen at 80KgN/ha, 1000kgSi/ha (N2S2) produced the highest yield. Silicon had a positive effect on almost all the yield attributes. Being an important element there is a necessity to supplement those soils that are deficient of silicon since it is one element that farmers in Mwea do not utilize. Although the trial was conducted under screen house condition further work is recommended to verify these results under field conditions.

4.3 EFFECT OF NITROGEN AND SILICON IN THE MANAGEMENT OF RICE BLAST

4.3.1 MATERIALS AND METHOD

4.3.2. Rice culture and treatment application

The vertisols soil was obtained from Mwea Irrigation Agricultural Development (MIAD) fields. It was analyzed for nitrogen, phosphorus, potassium and silicon to determine the contents in the soil. The soil was mixed to make it homogenous before the treatments were applied. Certified seed was obtained from MIAD and sown in the nursery. Before sowing, the seeds soaked for 24 hours and incubation (pre-germination). Soaking was done and pre-germination for three days before sowing.

The soil used in the experiment was thoroughly mixed with individual treatments in 200 gm of soil, filled in plastic pots (12x8cm) after randomization. Treatments included four levels of silicon (0, 500, 1000 and 1500Si kg/ha), four nitrogen levels 0, 40, 80 and 120NKg/ha (0, 0.015, 0.030,046 SAg/200gm soil) making a total of sixteen treatment. Calcium silicates was the source of silicon at 0, 2500, 5000 and 7500kg/ha. This translated to 0, 0.06,0.12,0.18gCasio3/ 200gsoil per pot respectively. The potassium and phosphorus were applied as basal fertilizer at recommended rates of 30Kg K₂O/ha (0.01g/pot) and 58kgP₂O₅ /ha (0.027g/pot) in form of muriate of potash and triple super phosphate (TSP) respectively. Due to the calcium contained in the calcium silicate the calcium element was added to the pots that did not have calcium silicate to ensure the effect of disease management was due to silicon influence. The mixture was well-irrigated four days before the seedlings

were transplanted after 28 days in the nursery. The seedlings transplanted were selected on the basis of uniformity.

The experimental design was split plot laid out in a complete randomized design (CRD) with four replications. Fertilizer was the main plot while fertilizer level was the subplot. Each treatment contained six transplants.

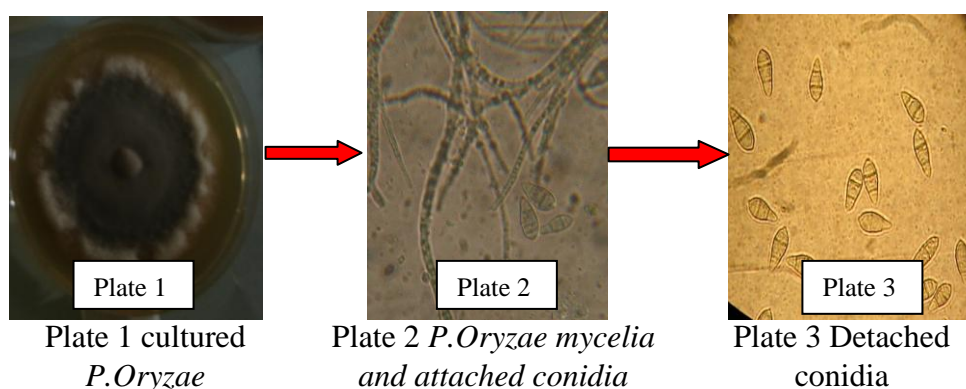
4.3.3 Media preparation for the growth of *Pyricularia oryzae*

The media was prepared by dissolving 35.5gm of malt extract agar (MEA) in one litre of distilled water and mixed homogeneously on hot plate/stirrer before autoclaving for 15 min at 121⁰c (15 pounds per sq. inch). The media was supplemented with 0.05g chlorotetracycline, 0.1g penicillin G and 0.2 g streptomycin-sulphate per litre to prevent bacterial contamination. The media was dispensed into culture plates after attaining a temperature of 30-40°C.

4.3.4 Preparation of inoculum

Inoculum was obtained from infected plant tissues collected from MIAD and the surrounding infected fields. The conidia were harvested from different plants parts (stems, panicles, sheaths) washed thoroughly with tap water. Under aseptic conditions, the infected plant tissue were cut in small section 5-10mm square from the margin of the infected lesion such that it contained both diseased and healthy looking tissue. The tissues were surface sterilized for two minutes in 70% ethanol, washed three times with sterile water and blotted dry on clean sterile paper and finally plated in 90mm Petri dishes containing Malt Extract Agar (MEA).

The cultured media was incubated under continuous light at 25°C for 24 hours, after which the light was put off and incubation continued for seven days to allow mycelia growth. Under sterile conditions a drop of sterile water was put on the slide and a small piece of mycelia placed on it, covered with a cover slip and placed under a light microscope for observation. After the identification of the fungus, the pathogen was sub-cultured in order to isolate rice blast fungus for accelerated sporulation. The Petri dishes were re-incubated in the laboratory for 36 hours during which growing fungi was viewed under a light microscope (x40 magnification). Further sub-culturing was done to obtain pure cultures. (Plate 1, 2 and 3)



4.3.5 Pathogenesis tests

Once the pathogen was identified and isolated, pathogenesis tests were carried out to verify the fungus was rice blast disease. This was done through inoculation of grown plants in the screen house. The method used was as described by Matsuo and Hoshikama, (1993). Initial soil collected from the field was heat sterilized (121°C) for 60 minutes and allowed to cool before potting in the plastic containers (200 gm). Using this soil, certified seed were raised and later transplanted into two different pots. Two weeks later one pot was inoculated while the other was left with healthy plants. Distilled water was used to flood the soil as required by rice plant. The

inoculum with conidia level of 4×10^4 /ml was foliar sprayed by use of a hand sprayer on the plants in one pot and covered with a polythene bag to maintain the high relative humidity for 24 hours at 25°C. Plants in the other pot were not inoculated. The plastic bags that covered the plants were removed after 24 hours. Disease incidence from the fungus was observed seven days after inoculation. The fungus was re-isolated, grown in MEA and found to be as the one isolated and observed after one week. The plant parts of the un-inoculated plant were surface sterilized in 70% ethanol for 2 minutes. Similarly the diseased plants parts too were surface sterilized the same way and placed on the malt extract agar media and incubated for 24 hours of light and observed after one week. The fungus was found to be present in the inoculated plants

4.3.6 Inoculation

Inoculation was done at the seventh leaf from the main tiller as described by Matsuo and Hoshikama, (1993). A conidial suspension of *Pyricularia oryzae* (4×10^4 conidia/ ml) was used. The sore concentration was achieved by placing a cover slip over the hemocytometer (Indian made) counting chambers. By use of a Pasteur pipette a drop of conidia suspension was placed at the edge of the V-shaped allowing the suspension to be drawn into the chamber by capillary action. Gelatin (1%, wt/vol) was added to the sterile water to aid conidial adhesion to the leaf blades. This suspension was applied as a fine mist to the upper leaf blades of six plants per pot until runoff with a hand sprayer. Immediately after inoculation, plants were covered with a plastic bag to increase humidity and placed in darkness for 24 hours. There

after the plants were kept at relative humidity of approximately 85% and above throughout the experiments.

4.3.7 Disease assessment

The data regarding the occurrence of the rice blast disease was collected one week after the inoculation using the modified disease rating scale of 0-9 developed by International Rice Research Institute (IRRI, 2002). The lesion score and the host responses is as indicates in the table 6 as a standard evaluation (IRRI, 2002). Four plants were randomly selected out of the six plants planted in a pot. The fourth leaf was tagged in each of the plant and the largest lesion measured in both width and length.

4.3.8 Tissue analysis

Plant tissue sampling and analysis

At the fifth week some plants tissue were cut at base, dried, ground and analyzed for nitrogen and silicon. The analysis for nitrogen was by Kjeldahl nitrogen method as described by Okalebo *et al.* (2002). Briefly, 0.3g of 'oven dried plant samples were digested in 2.5 ml of digestion mixture (Selenium powder, salicylic acid and sulphuric acid) at 110⁰C for one hour and cooled. Three successive 1ml potions of hydrogen peroxide (H₂O₂) were added. The digest was allowed to cool and transferred to a 50mls volumetric flask and filled to the 50mls mark. 5 ml of the digest was then transferred into a distillation apparatus.10mls of 40% sodium hydroxide added into it and the steam distilled immediately into a 10ml boric acid indicator solution containing 4 drops of mixed indicator. Distillation continued for

two minutes until the indicator turned to green then removed for titration. Titration was done with a standardised 0.01N HCL through an automatic burette until the colour of the indicator changed to pink. Nitrogen was calculated and expressed as a percentage.

Silicon was determined as per modified method of Reay and Bennett (2002). Briefly, one gram of ground plant tissue sample was weighed into a crucible. Sample was fused with sodium carbonate in a muffle furnace with gradual raising of temperatures to 550⁰C for 15 minutes. The sample was removed from the furnace and allowed to cool and weighed. 3mls of hydrochloric acid was added and heated four half an hour in a steam bath. The mixture was cooled and transferred into 100ml volumetric flasks and mixed immediately into a polythene bottle. 2mls of the aliquot samples were pippered into 50mls and neutralized by adding a few drops of sodium hydroxide using phenothalein as indicator. The solution was neutralized by adding 1% hydrochloric acid. 1.25ml molybdate reagents was added, mixed and left for 10 minutes, 1.25ml of tartaric was added and left for 5 minutes. 1ml of reducing solution was added and the flask allowed to settle for 15 min to allow colour development. The sample and the standards optical density were read using a spectrophotometer (Shimadzu model UV-mini 1240) at 810nm.

Table 6 Lesion score and host response

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

Sources: International Rice Research Institute (2002). *Standard evaluation systems for rice*

Data analysis

Data on the infection of plant by rice blast was analyzed using Statistical Analysis System (SAS) version 8.1. The analysis conducted were (ANOVA) and means separations using LSD.

4.4 RESULTS AND DISCUSSION

Results

The results indicated that the pots that had no silicon (0KgSi/ha) or nitrogen (0KgN) N0S0 experienced a higher rice blast disease incidence (Table 7). Similarly 80KgN and 0KgSi/ha (N2S0), 0KgN and 500KgSi/ha (N0S1), 40KgN and 500KgSi/ha (N1S1), 40KgN and 0KgSi/ha (N1S0), 80KgN and 500KgSi/ha (N2S1) also had higher disease increase compared to those with silicon.

Plants that were not supplied with nitrogen but had two levels of silicon (0KgN, 1000SiKg/ha) (N0S2) were moderately susceptible while those treated with 80KgN and 1000SiKg/ha (N2S2), 80KgN and 1500SiKg/ha (N2S3), 120KgN and 1000SiKg/ha (N3S2), 120KgN and 1500SiKg/ha (N3S3) showed the plants were moderately resistant to the pathogen. The disease was eradicated at 80KgN, 1000SiKg/ha (N2S2) and 80KgN and 1500SiKg/ha (N2S3) which were not significantly differently with a mean score of 2-3 (moderately resistant). The trend was a similar in the second season although the means in the second season were higher (Table 8)

Table 7. Nitrogen and silicon in management of rice blast at different sampling periods (season one)

Treatment	Week 1	Week 2	Week 3	Week 4
N0S0	4.06 b	5.00 bc	6.31 b	7.31 abc
N0S1	3.75 b	4.88 c	5.81 c	7.19 c
N0S2	2.81 d	3.56 e	4.00 e	4.63 f
N0S3	2.13 e	2.19 g	2.63 g	2.94 kl
N1S0	3.81 b	4.69 c	6.25 b	7.25 bc
N1S1	3.19 cd	4.19 d	5.13 d	6.25 e
N1S2	2.56 d	2.94 f	3.25 f	3.38 ij
N1S3	2.00 e	2.06 g	2.44 g	2.69 l
N2S0	4.63 a	5.38 b	6.69 ab	7.38 abc
N2S1	3.31 c	4.00 d	5.13 d	6.69 d
N2S2	2.06 e	2.25 g	2.63 g	2.88 l
N2S3	2.00 e	2.19 g	2.69 g	3.00 lk
N3S0	4.50 a	6.13 a	7.00 a	7.60 ab
N3S1	4.50 a	5.75 ab	6.81 a	7.63 a
N3S2	3.00 cd	3.50 e	3.69 eg	3.81 gh
N3S3	2.44 de	2.94 f	3.38 f	3.67 hi
Means	3.15	3.83	4.57	5.19
CV	17.34	15.55	12.93	9.12

NB: Means followed by the same letter down the column are not significantly different

KEY: (N0S0) 0KgN,0KgSi/ha (N0S1) 0KgN,500KgSi/ha (N0S2) 0KgN,1000KgSi/ha, (N0S3) 0KgN,1500KgSi/ha (N1S0)40KgN,0KgSi/ha, (N1S1) 40KgN,500KgSi/ha, (N1S2) 40KgN,1000KgSi/ha, (N1S3) 40KgN,1500KgSi/ha, (N2S0) 80KgN,0KgSi/ha, (N2S1) 80KgN,500KgSi/ha, (N2S2) 80KgN,1000KgSi/ha, (N2S3) 80KgN,1500KgSi/ha, (N3S0)120KgN,0KgSi/ha, (N3S1)120KgN,500KgSi/ha, (N3S2) 120KgN,1000KgSi/ha, (N3S3)120KgN,1500KgSi/ha

Table 8. Effect of nitrogen silicon interaction on infection of rice by rice blast disease at different sampling periods (season 2)

Treatment	Week 1	Week 2	Week 3	Week 4
N0S0	4.02 b	5.31 bc	6.50 b	7.19 c
N0S1	3.65 b	4.94 c	6.13 b	7.06 c
N0S2	2.81 d	3.56 d	4.19 d	4.69 e
N0S3	2.19 e	2.50 f	2.81 f	3.00 ih
N1S0	3.75 b	5.06 c	6.25 b	7.25 bc
N1S1	3.18 cd	4.50 c	5.31 c	6.05 d
N1S2	2.60 d	3.00 e	3.44 e	3.75 g
N1S3	2.00 e	2.19 f	2.63 f	2.75 i
N2S0	4.56 a	5.56 b	6.81 ab	7.55 ab
N2S1	3.31 c	4.00 d	5.19 c	6.56 d
N2S2	2.00 e	2.25 f	2.69 f	2.94 ih
N2S3	1.87 e	2.19 f	2.75 f	3.00 ih
N3S0	4.63 a	6.25 a	7.06 a	7.75 a
N3S1	4.50 a	5.81 ab	7.00 a	7.63 a
N3S2	3.00 cd	3.63 d	3.94 d	4.25 ih
N3S3	2.50 d	2.88 ef	3.25 e	3.56 h
Means	3.13	3.96	4.69	5.25
CV	17.29	16.73	12.06	9.02

NB: Means followed by the same letter down the column are not significantly different

(P>0.05)

End of the season tissue analysis results

Results on tissue analysis done at the end of the seasons (Table 9) showed there was significant difference ($P < 0.05$) in nitrogen and silicon level in the rice plants under different treatments. The treatments that had the highest nitrogen application without silicon (120KgN, 0KgSi/ha) showed the highest concentration of nitrogen and the same application resulted to the highest disease incidence. The disease was minimal at the rate of 80KgN, 1000KgSi/ha (N2S2) which was not however significantly different from 40KgN, 1500KgSi/ha (N1S3). It was followed by 120KgN, 500KgSi/ha (N3S1) 120KgN, 1500KgSi/ha (N3S3) 80KgN, 500KgSi/ha (N2S1) (Table 9).

Table 9 Plant tissue analysis on nitrogen and silicon from different treatments

Treatment	Nitrogen (%)	Silicon (mg/kg)	Rice blast (IRRI score)
0KgN,0KgSi/ha (N0S0)	1.973i	0.004i	7.31abc
0KgN,500KgSi/ha (N0S1)	2.044i	0.007gh	7.19c
0KgN,1000KgSi/ha (N0S2)	2.401ih	0.008ef	4.63f
0KgN,1500KgSi/ha (N0S3)	2.389h	0.010cd	2.94kl
40KgN,0KgSi/ha (N1S0)	2.919ef	0.004i	7.25bc
40KgN,500KgSi/ha (N1S1)	2.505gh	0.007fg	6.25e
40KgN,1000KgSi/ha (N1S2)	2.636g	0.009ef	3.38ij
40KgN,1500KgSi/ha (N1S3)	2.849f	0.012bc	2.69l
80KgN,0KgSi/ha (N2S0)	3.101de	0.005ih	7.38abc
80KgN,500KgSi/ha (N2S1)	3.584ab	0.007g	6.69d
80KgN,1000KgSi/ha (N2S2)	3.411bc	0.009de	2.88l
80KgN,1500KgSi/ha (N2S3)	3.301cd	0.011bcd	3.00lk
120KgN,0KgSi/ha (N3S0)	3.619a	0.005ih	7.60ab
120KgN,500KgSi/ha (N3S1)	3.472abc	0.008fg	7.63a
120KgN,1000KgSi/ha (N3S2)	3.570ab	0.012abc	3.81gh
120KgN,1500KgSi/ha (N3S3)	3.612ab	0.013a	3.67hi

NB: Means followed by the same letter within a column are not significantly different

(P>0.05)

The result in figure 6 showed that when the ratio of silicon to nitrogen was small the disease pressure was high. As the ratio increased the disease score was lower.

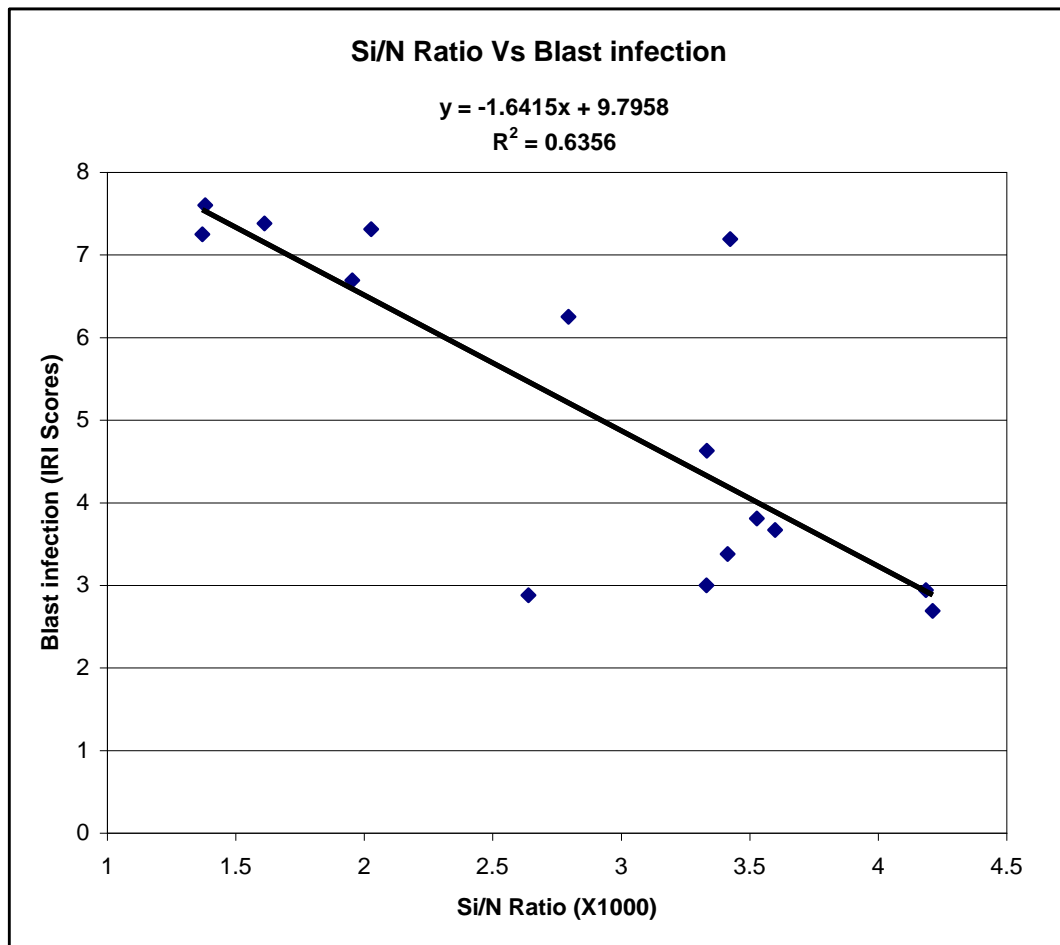


Figure 6. Response of rice blast to silicon/nitrogen ratio

Discussion

The trend indicated that as the level of nitrogen increased the rice blast incidence also increased with highest occurring at 120KgN/ha. The massive growth contributed by nitrogen may have provided the pathogen with readily available substrate. As reported by Marschner, (1995) an increase in low molecular weight of organic nitrogen compound as a substrate contributes to the high pathogen infection. Although the application was done in two splits the infection at this rate was high.

These results disagreed with what was found by Helms, (1990) and Kurschner *et al* (1992) who found that split application of nitrogen reduced the rice blast severity.

Soil in Mwea being vertisols could have had little leaching and probably the first split had not been exhausted by the time the second split was applied. Since the rice plants were grown in the pot there was no likely-hood of leaching of nitrogen hence retaining high amount of nitrogen in the soil readily available to the plants. Probably the result would have the same as those of Helms, (1990) and Kurschner *et al* (1992) if the trial was conducted in the field.

High infection of rice blast was also realized in the pots that had no nitrogen 0KgN/ha (N0) and in 40KgN (N1) which was a low application rate. This may have been contributed by the weakness of the plants due to limited supply of nitrogen. These results agreed with the findings of Talbot *et al.* (1997) who found that low nitrogen leads to increased disease due to lack of sufficient plant defense. Similarly Datnoff *et al.* (1990) and Datnoff *et al.* (2001) found that low silicon level led to increased susceptibility of rice to rice blast disease. Therefore much as high nitrogen level increased disease incidence, nitrogen deficiency is also detrimental and there is an optimal level of nitrogen required by the rice plant to enables it resist *Pyricularia oryzae*. These observations indicate that the disease incidence would occur if nitrogen is limiting or if applied in excess.

The plants that had higher silicon (1000 and 1500KgSi/ha) were moderately resistant to the pathogen and therefore silicon played a role in disease management probably through its deposition in the cell wall creating mechanical barrier to pathogen penetration.

From the results the leaves of rice plants, grown in the presence of silicon showed an erect growth. This improved distribution of light within the canopy creating unsuitable environment for the pathogens. The other likely scenario could be the strengthening of the leaves cell wall due to deposited silicon. As stated by Epstein (1999) silicon is associated with lignin-carbohydrate complexes in the cell wall of rice epidermal cells resulting in strengthening of the leaves making it difficult for the pathogen to penetrate.

Although the hypothesis was not established with the prevailing data, there is a possibility that silicon triggered the rice plant cell defense mechanism such that by the time the appressoria penetrated the plants were physiologically defensive. Since all plants produces phytoalexins against infection, it is unclear on how silicon contributes to plant disease resistance.

The interaction of silicon and nitrogen (Figure 6) showed that as silicon nitrogen ratio increased the disease pressure decreased indicating that there is a relationship between rice blast disease and the silicon levels. These result supported the findings of Epstein (2004) who associated silicon with lignifications of the plant cell wall.

4.5 CONCLUSION

The study showed that nutrient management is an important factor to the health of rice plants. Rice blast disease can be managed through proper nitrogen and silicon fertilization. Nitrogen applied at 120Kg/ha led to high rice blast infection and where no (0kgN/ha) silicon was applied. It can be concluded that when nitrogen is deficient or applied below 80kg/ha the plant resistance to rice blast was low indicating that

there is a threshold amount of fertilizer required for optimal rice growth and disease resistance.

Silicon was found to manage the disease at 1000 and 1500kgSi/ha and since the two levels were not significantly different it would be reasonable to adopt the former level of application. The interaction of silicon and nitrogen at 80KgN/ha, 1000kgSi/ha (N2S2) was found to manage rice blast. Since the trial was conducted under screen house condition further work is recommended verifying these results under field conditions.

CHAPTER FIVE

5.0 EFFECTIVENESS OF RICE BY-PRODUCTS AND COMMERCIAL SILICATE ON RICE BLAST MANAGEMENT

5.1 INTRODUCTION

Rice blast caused by *Pyricularia oryzae* is the most important fungal disease of rice (Couch and Kohn, 2002). It is reported to cause losses of more than 70-80 percent in infected fields (Awodero, 1990). Rice blast was reported for the first time at Mwea Irrigation Scheme (Central Province) in 2006. This scheme produces more than 50% of Kenyan rice therefore rice blast disease attacks is a threat to Kenyans food security unless affordable measures are taken to control it.

One of the approaches that will enable high productivity of rice in Mwea irrigation would be the use of plant nutrition targeting the use organic material as a source of silicon application. Silicon (Si) is not considered an essential element for plant but is responsible for the rigidity and lignification of the tissues resulting to disease and pest resistance (Ishiguru, 2001; Meyer and Keeping, 2001). The authors have showed elsewhere that silicon in form of calcium silicates is effective in the management of rice blast.

Si is usually deposited in various organs of rice that include the husks, leaf, leaf sheath, culm and roots (Zhu *et al.* 2004). Studies done by (Savant *et al.* 1997 and Epstein, 1999) have shown that rice plant residues could be a source of Si for rice nutrition and consequently improve yield and ameliorate biotic as well as abiotic stress of rice plant. For instance rice straw are applied as an alternative material to

inorganic silicate fertilizers in many countries such as South Korea, Taiwan, USA, Cuba and China (Lian, 1989). An annual production of rice straw is about 573 million tons worldwide (Shashirekha *et al.* 2005). Rice husk which is another by product of rice production accounts for 23% of total paddy weight. The disposal of this husk causes significant problems for the rice mill owners which end up being disposed and burned to ashes along the Mwea Nairobi high way (Muriithi *et al.* 2010). Other different ways have been adopted to utilize rice husk, in Thailand, for instance it is used as biomass fuel to help reduce global climate change and reserve fossil fuel resource (Shashirekha *et al.* 2005). However, rice straw and rice husk have been identified as rich in cellulose and silicon which was the important element in this study (Datta and Chakravarty, 2001; Obodai *et al.* 2003). The main objective of the study was to establish appropriate technologies for the management of rice blast in Mwea irrigation scheme of Kenya using cheap and available sources of silicon.

5.2 MATERIALS AND METHODS

Results

The study was carried out at Mwea Irrigation Agricultural Development (MIAD) in a green house in Kirinyaga district. The soils in this area are vertisols (Sombroek *et al.* 1982) which are characterized by imperfectly drained, very deep, dark grey to black, firm to very firm, cracking clay. Soil was analyzed for total and available nitrogen using Kjeldahl digestion method as in Okalebo *et al.* (2002). Phosphorus was analysed through Olsen method due to its suitability for a wide range of pH values as described by Okalebo *et al.* (2002). Silicon concentration was determined by the calorimetric molybdenum blue method (Imaizumi, and Yoshida., 1958). Sources of

Si were calcium silicate (CaSiO_3), and rice by-products (rice straw that was raw, straw ash and husk ash). There were four treatments (control, CaSiO_3 at 5000Kg/ha, (0.7tons husk ash), equivalent to 2ton/ha husk before burning and straw ash at 0.6tons/ha (equivalent to 2ton/ha straw before burning). The ashes from these materials were first analyzed for potassium, phosphorous, and silicon.

5.2.1 Phosphorus in husk and straw ashes.

One gram of ground plant tissue sample was weighed into a crucible 3mls of magnesium nitrate solution (50% percent $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) for each gram of plant material and sufficient water to wet the sample. The mixture was placed into a muffle furnace. Temperatures were slowly raised to 550°C and ashed for two hours until no black ash remained. The sample were removed from the furnace and allowed to cool as described by Gupta, (1999). The ash was dissolved using 5ml of 2N hydrochloric acid. 5 ml of the sample was pippered into 50mls volumetric flask and 4 drops of 0.5% penitrophenol indicator solution. Drop by drop of 6M NH_4OH solution was added to change the colour to yellow and brought to colourless by addition of 1N HNO_3 5mls of ammonium molybdate/ammonium vanadate was added and mixed well and then topped to 50mls volumetric flask. Contents were made to settle for 30min for colour development. Once the colour was developed the samples were run through 400nm and reading recorded.

5.2 2 Determination of potassium in rice plant

A 0.3 gram of oven died plant tissues was weighed and put in a dry and clean digestion tube. 4.4ml digestion mixture (selenium powder, lithium sulphate, hydrogen peroxide and sulphuric acid) was added into each tube and also the reagent

blanks. The solution was digested for three hours at 360⁰C. The content were allowed to cool and transferred to a 50ml volumetric flask and filled to the mark with distilled water. The standards were prepared through a stock potassium solution of 1000ppm. A 1.907 g potassium chloride was dissolved in distilled and made to a one litre wit distilled water. A 10ml of the stock solution was diluted to 100ml while the standard series of 0, 1,2,3,4 and 5ml of 100ppm potassium was pipetted into 100ml flask and filled to the mark with distilled water. These solutions had concentrations of 1, 10,20,30,40 and 50ppm respectively. A 2ml of the digest sample solution was pipetted into 50ml volumetric flask and filled to 50 mark with distilled water and mixed well. The working standard series, soil extract and the blank solution were aspirated into a flame photometer at a wave length of 766.5nm. The amount of potassium present in the solution was read from the calibrated curve prepared plotting absorbance readings against potassium concentration in the standards series. Corrections of reagent blank were made by subtracting the blank value from the sample concentration value.

Plant tissue sampling and analysis

At the fifth week of sampling some plants were cut at base, dried, ground and analyzed for nitrogen and silicon. The analysis for nitrogen was by Kjeldahl nitrogen method as described by Okalebo *et al.* (2002). Briefly, 0.3g of oven dried plant samples were digested in 2.5 ml of digestion mixture (Selenium powder, salicylic acid and sulphuric acid) at 110⁰C for one hour and cooled. Three successive 1ml potions of hydrogen peroxide (H₂O₂) were added. The digest was allowed to cool

and transferred to a 50mls volumetric flask and filled to the 50mls mark. 5 ml of the digest was then transferred into a distillation apparatus. 10mls of 40% sodium hydroxide added into it and the steam distilled immediately into a 10ml boric acid indicator solution containing 4 drops of mixed indicator. Distillation continued for two minutes until the indicator turned to green then removed for titration. Titration was done with a standardised 0.01N HCL through an automatic burette until the colour of the indicator changed to pink. Nitrogen was calculated and expressed as a percentage.

5.2 3 Silicon in rice straw and husk ashes

One gram of ground plant tissue sample was weighed into a crucible. Sample was fused into a muffle furnace and temperatures were slowly raised to 550⁰C and ashed for five hours until no black ash remained as described by Gupta, (1999). The sample were removed from the furnace and allowed to cool and weighed. 3mls of hydrochloric acid was added and heated four half an hour in a steam bath. The mixture was cooled and transferred into 100mls volumetric flasks and mixed immediately into a polythene bottle. 2mls of the aliquot samples were pipetted into 50mls and neutralized by adding a few drops of sodium hydroxide using phenothalein as indicator. The solution was neutralized by adding 1% hydrochloric acid. 1.25ml molybdate reagents was added, mixed and left for 10 minutes, 1.25ml of tartaric was added and left for 5minutes. 1ml of reducing solution was added and the flask allowed to settle for 15 min to allow colour development. The sample and the standard were read using spectrophotometer (model UV-mini 1240) at 810nm.

Calibration curve for the standard was constructed and the silicon in the sample aliquots obtained from the drawn curve in ppm.

The Standard was prepared by using 0.41g calcium silicate and 2gms of sodium carbonate that was dissolved in 1000mls distilled water. The mixture was boiled until it became clear. Standard of 0, 10,15,20,25 were prepared and filled to 100mls volumetric flask,

The treatments were arranged in completely randomized design (CRD) replicated four times in a greenhouse at ambient temperature. Calcium silicate was thoroughly mixed with the soil before potting. Straw and husk ashes were obtained by burning an equivalent of 2 tons straw or husk/ha to produce the respective weights (0.7 and 0.6 tons/ha). The rice straw was cut in small pieces before mixing with whole soil. Except for the control the rest of the treatments were mixed with 6 kg of soil per pot. Due to the effect of calcium in control of disease, calcium element was added in all the treatment that had no calcium silicate at 1.24gm/6kg soil. Calcium silicate was applied at 3.6gm/6kg soil. Basal fertilizers (30 kg K₂O/ha and 58 kg P₂O₅/ha) were thoroughly mixed with soil in all pots including the control. Nitrogen (N) was applied as a top dress (80 kg N/ha) in two splits. Straw, husk ash, straw ash and potting soil were initially analyzed for Si, N, K, and P. (Table 10).

Pot mixtures were well-irrigated 10 days before transplanting. At vegetative stage (2weeks after transplanting) the plants were inoculated with conidial suspension of *Pyricularia oryzae* (4×10^5 conidia/ ml) prepared as described by Pinnschmidt *et al.* (1992) and applied as a fine mist using of a hand sprayer. To maintain high relative

humidity, plants were flooded with water throughout the vegetative stage. From the six plants per pot, four plants of approximately equal size were randomly selected, tagged and used for all the measurements/assessments. Disease symptoms were recorded one week after inoculation and continued to late heading (75days after transplanting). The experiment was repeated in the second season (May-August 2009, Table 12).

5.2 4 Data collected and analysis

Data recorded included plant height, number of tillers both productive and non-productive, panicles size, disease score on 0-9 scale where 0 is no infection while 9 is completely attacked, plant biomass, grain yield adjusted to 13 % moisture content. Yield components

Data analysis was done using Statistical Analysis System (SAS) statistical package version 8.1. The analysis conducted were analysis of variance (ANOVA) and means separations using LSD.

5.2 5 Disease infection assessment

From the six plants, four plants were randomly selected and tagged and were used for all the measurements. Neck node and sheath symptoms were recorded one week after inoculation continuing weekly until early to late heading. Disease was rated at a score of 0-9 according to IRRI standards.

5.3 RESULTS AND DISCUSSION

Results

Results in table 10 showed the results of phosphorous, potassium and silicon obtained after the analysis of rice plant tissues in different forms.

Table 10 Levels of phosphorous, potassium and silicon from husk and straw ashes and raw straw of rice plants.

Treatments	P(ppm)	K(mg/kg)	Si (g/kg)
Husk Ash	3100.12	621.01	63.29
Straw Ash	3567.27	584.06	50.22
Straw	325	147.18	48.69

Results for the first season (October 2008-March 2009) are shown in Table 11 showed that chemical silicon (CaSiO_3) sources were significantly effective in increasing rice biomass (1.ton/ha) followed by rice by-products (1.2ton/ha). It also increased the total number of tillers (54 tillers) compared to husk ash (47 tillers). Straw ash (41 tillers) and raw straw were not significantly different in the number of tillers from control. Chemical sources of silicon (CaSiO_3) significantly increased the number of productive tillers (31 tillers) and also increased panicle filled grain (61.4). Straw and husk ashes were not significantly different from one another in the number filled grains in a panicle but however, there was a difference between straw (29.9) and control (21.7). Chemical sources (14.3g) of silicon and husk ash (14.2g) increased 1000-seed weight. Similarly this increased weight of a 1000seed resulted to increased yield from both chemical (2.52ton) and husk ash (2.42ton). Rice straw (12.8g) and straw ash (12g) and control (11.9g) did not significantly influence the weight of a 1000seed but straw ash attained a higher mean than the rest. Application

of CaSiO_3 resulted in significantly taller rice plants compared with silicon sources from rice by-products. The results in the second season followed similar trend as season one although the yield in the first season were higher.

Table 11. Effect of different sources of silicon on yield and yield components in season 1 (Oct 2008-March 2009)

Treatment	Total biomass (t/ha)	Total number of tillers	Number of productive tillers	Panicle size Length (cm)	Panicle filled grain (number)	1000–seed weight (g)	Plant height (cm)	Disease scores	Yield tons/ha
CaSiO ₃ (1500kg/ha)	1.2a	54a	32a	23.0a	61.4a	14.3a	100a	1.5d	2.52a
Husk-ash (0.7tons/ha)	1.09ab	47b	29ab	21.8a	44.5b	14.2a	93bc	3.5c	2.42a
Straw-ash (0.6tons/ha)	0.97b	41c	25bc	22.1a	39.9b	13.0b	88c	5.5b	1.93b
Straw only (2 tons/ha)	0.95b	40c	25bc	21.8a	29.1c	12.0b	80d	7.0a	1.70b
Control	0.79c	37c	23c	21.9a	21.7c	11.9b	79d	8.0a	1.5b
CV (%)	4.5	8.4	13.1	5.3	14.7	7.1	5.2	13.1	5.6
Means (p<0.05)	0.98	44	22.8	22.4	20.5	13.0	88	5.9	2.0

Means of the same the along the column are not significantly different

Table 12. Effect of different sources of silicon on yield and yield components in the second season (April-August 2009)

Treatment	Total biomass (tons/ha)	Number of total tillers	Number of productive tillers	Panicle size (cm)	Panicle filled grain (Number)	1000-seed weight (g)	Plant height (cm)	Rice blast infection scores	Grain yield tons/ha
Treatment	1.18a	46a	29a	22.4a	57.6a	14.3a	107a	1.5d	2.35a
CaSiO ₃ (1500kg/ha)	1.09ab	42a	29ab	20.1a	42.7b	13.3a	100ab	3.5c	2.23a
Husk-ash (0.7tons/ha)	1.06b	35b	24b	19.9a	35.6bc	12.1b	99ab	5.5b	1.63b
Straw-ash (0.6tons/ha)	0.84c	33bc	17c	19.7a	28.4cd	12.0b	97bc	7.0a	1.06c
Straw only (2 tons/ha)	0.74c	28c	16	19.6a	24.3d	11.3b	88c	8.0a	1.06c
CV (%)	7.2	10.9	8.3	11.04	14.0	5.7	6.3	18.2	5.6
Mean (p<0.05)	0.98	37	22	19.9	37.7	12.6	98.3	5.1	1.7

Means of the same the along the column are not significantly different

The results in figure 7 showed that calcium silicate was the most effective source of silicon in management of rice blast. Husk ash was also effective in controlling rice blast while rice straw and control got a higher disease infection.

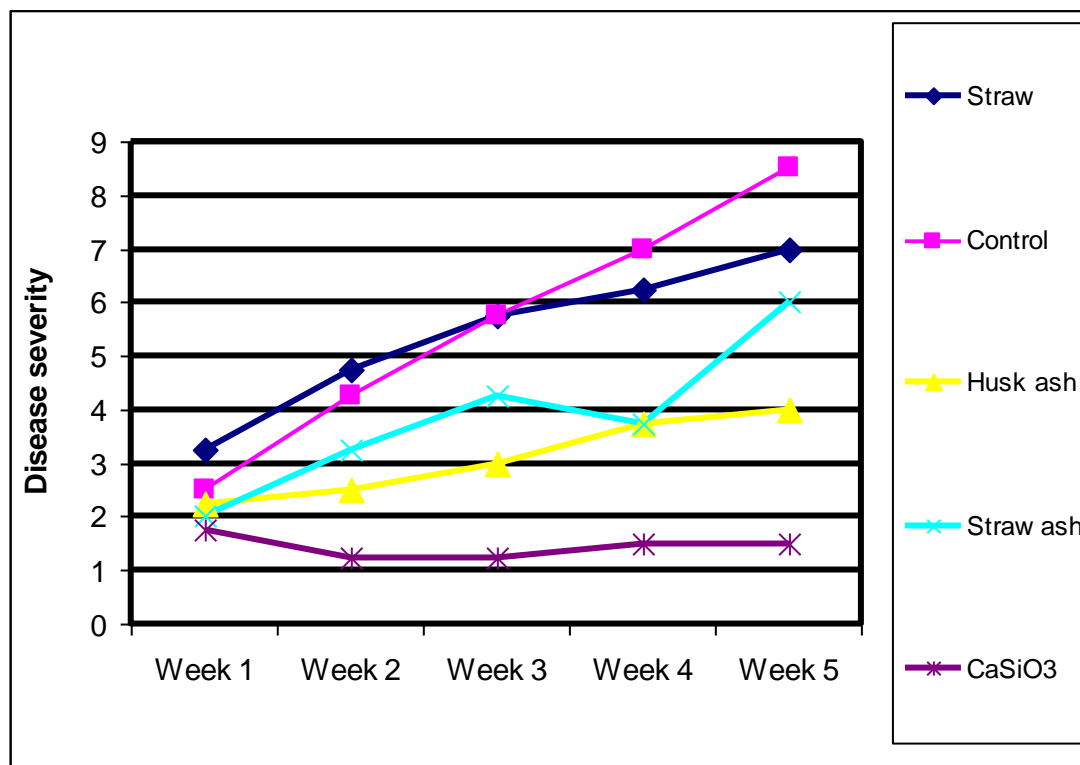


Figure 7. Rice blast disease severities with five treatments (Rice straw, husk ash, straw ash and calcium silicate

Discussion

Results in Table 11 showed that calcium silicate (CaSiO₃) effectively increased rice biomass. There is an indication that silicon had a positive effect on the increase of the tillers as well as biomass. Apart from the commercial sources of silicon the rice husk ash and straw ash (rice by products) would be a cheap and affordable for soil amendment. The results support the findings reported by Savant *et al.* (1997) who

reported an increase in dry matter in rice with additional silicon. Calcium silicate also increased the total number of tillers (54 tillers) compared to husk ash (47 tillers), Straw ash (41 tillers) and raw straw as well as number of productive tillers (31 tillers) and panicle filled grain (61.4). These increase brought about by the calcium silicate and ash husk may have indicated that there was no difference when silicate source was either from the chemical source or from the crop by-product. The husk ash could release the silicon as easily as the chemical silicate. The results agree with the findings of Ma *et al.* (1989) and Takahashi (1995) who demonstrated that silicon increased fertility of the spikelet which is an attribute of yield. Similarly Savant *et al.* (1997) also realized the benefit of silicon in the increased number of tillers. Weight of a 1000 seed and the total yield increased as a result of chemical sources (14.3g) of silicon and husk ash (14.2g). It may be attributed to the availability of the silicon from the two sources which the crop utilized leading to increased seed weight. On contrary rice straw (12.8g) and straw ash (12g) and control (11.9g) did not significantly influence the weight of a 1000seed but straw ash attained a higher mean than the rest. This was in line with what was reported by Muriithi *et al.* (2010). CaSiO_3 significantly resulted to taller rice plants compared with silicon sources from rice by-products which were attributed to the form in which the plants absorbed them.

Rice blast disease was effectively managed by calcium silicate as well as husk. Rice straw however showed higher disease incidence. This implied that silicon may have played a role in disease management probably through its deposition in the cell wall (Muriithi *et al.* 2010). The lignifications of the cell wall may have formed an effective barrier against fungal growth. According to (Deren *et al.* 1994) rice plants

that is grown entirely without silicon showed that disease was negatively correlated to with silicon content as compared to those supplied with silicon

The effectiveness of rice straw by-products in controlling blast was low. This could have been due to little silicon from the straw and probably it had not decomposed enough to release silicon to manage the disease. Chemical sources of silicon and husk ash were most effective in increasing grain yield compared to straw by-products and therefore application of commercial silicates and the bio-plants (husk ash) materials were able to manage the disease considerably and no yield reduction realized. On over all there was expectation that the performance of the rice straw would be poor compared to the control but on contrary it did perform well. The likely scenario was that when the materials were initially flooded and drained the decomposition was hastened resulting to release of the nutrients to the growing plants. According to Gopal *et al.* (2009) use of plants part in control of rice blast was a common practice where farmers possessed rich traditional ecological knowledge where they burned the straw and husks. This does not only manage the disease but adds nutrient to the soils.

5.4 CONCLUSION

Previous report shows that silicon provides many benefits to crops though not regarded as an essential nutrient. This element contributes to boost rice blast management and rice yield as well as other growth parameters such as number of productive tiller, panicle grain fill and weight of seeds. Integration of silicon with rice

fertilizers may contribute to the reduction of rice blast. Soil application of this nutrient may result in increased host resistance to the disease.

There is a need to develop cheaper and efficient sources of silicon that may be integrated with other nutrient management practices. Rice breeders could be encouraged to breed rice varieties with high silicon deposition which would offer inherent resistance to rice blast. Farmers should be encouraged to incorporate rice husks in the soil to contribute to soil and plant health.

CHAPTER SIX

6.0 EFFICACY OF SELECTED FUNGICIDES IN MANAGEMENT OF RICE BLAST DISEASES

6.1. INTRODUCTION

Rice blast disease reduces the quality and quantity of the rice crop. It attacks the crop at all stages of the crop and symptoms appear on the leaves, nodes as well as the panicle (Seebold *et al.*, 2004). The effects of rice blast are more severe in case of neck node blast leading to higher yield loss. The disease has been observed by literally every country where rice is grown and it is the most important disease of rice in the world (Couch and Kohn, 2002). For its management fungicide application has been in use in many countries to reduce the incidence and the severity of rice blast. Many of the fungicides developed to control the disease are often systemic fungicide although a prolonged use of similar mode of action may result to resistance of the pathogen (Kim *et al.*, 2008). Field trials conducted by Dubey, (1995) showed that use of Topsin M+Indofil M-45 proved to be effective on management of rice blast. Carbendazim, Pyroquilon thiophanate methyl and chlorbenthiazole as well as tricyclazole also proved to effectively control rice blast (Gouramanis, 1995). In Kenya there are no registered chemicals for rice but in an attempt to contain the disease, farmers have used various fungicides in the market. The chemicals are broad-spectrum and possess a systemic activity in them. They included Azoxystrobin, Thiophanatemethyl, Hexaconazole and Carbendazim among others. As found out by (Ou, 1985), there are some cases where five fungicide applications may be made in areas where conditions are favorable for severe epidemics. Moletti *et al.* (1998)

conducted a field trial and found that application of pyroquilon granules or wettable powder at two kilogram per hectare effectively controlled rice blast. Similarly Tirmali and Patil (2000) who worked on a susceptible rice cultivar found fungicides like Opus 15.5 SC and Ocatve 50 WP effectively eliminating rice blast particularly at when sprayed at tillering, booting and heading stages

Other work done on chemical control of rice blast has been reported in India indicating several spray schedules as reported by Kumbhar, (2005).

6.2. MATERIALS AND METHODS

Infected plants were obtained from MIAD and in the farmers' fields. The samples were taken to National Agricultural laboratories (NARL). The plant tissues were washed thoroughly using tap water. The process of sterilization identification and isolation was done as previously described. *Pyricularia oryzae* was challenged with four fungicides under three different concentrations 0.05% 0.075% and 0.10% (1, 1.5 and 2.0). These included Azoxystrobin (Ortiva 250 cs), Thiophanatemethyl 50% w/v (Topsin), Hexaconazole (Cotaf 5EC) and Carbendazim (Pearl 500G). The cultured plates were arranged in complete randomised design (CRD) and replicated four times.

6.2.1. Growth of *Pyricularia oryzae* in Agar medium

The test was based on the ability of *Pyricularia oryzae* to grow on a culture medium containing the test chemical and then measuring its mycelial growth. Poison food technique was employed by cultivating *Pyricularia oryzae* in a growth medium. The fungicides Azoxystrobin (Ortiva 250 cs), Thiophanatemethyl (Topsin), Hexaconazole (Cotaf) and Carbendazim (pearl) which are systemic and have both curative and

protective effect were individually incorporated and mixed well with autoclaved malt-extract agar at 50°C and poured into 90mm Petri dishes culture plates in standard quantities of a 100mls. With the use of cork borer (5-mm-diameter) a mycelial plug from the previously cultured fungi was seeded on the centre of the amended malt extract agar (MEA) and incubated for 24 hours. The sensitivity of the *Pyricularia Oryzae* isolate to fungicides was determined using 90mm Petri dishes containing MEA amended with the test fungicides. Growth rates were evaluated basing on daily records of mycelium growth by measuring the diameter (mm day⁻¹) across the Petri dish which excluded the diameter (0.5mm) of the inoculation plug for 21days.

The measurement on the Mycelial growth of the pathogen was expressed on percentage growth inhibition and expressed as either sensitive (<19%), intermediate (10-60%) and resistant (over 60%) respectively on fungicide amended relative to growth on the control as described by Tanaka, (1965)

Table 13. Fungicides used in Efficacy evaluation trial in control of rice blast

Manufacturer	Trade name	Common name	Formulation	Mode of action
Osho chemicals industries LTD	Cotaf 5EC	Hexaconazole	Soluble concentrate (SC)	Systemic broad-spectrum protective and curative
Syngenta Crop protection	Ortiva 250SC	Azoxystrobin 50G/L	Soluble concentrate (SC)	Systemic broad-spectrum protective and curative
Osho chemicals industries LTD	Pearl 500G	Carbendazim 500G	Soluble concentrate(SC)	Systemic broad-spectrum protective and curative
Farmchem	TopsinM	Thiophanate Methyl 50% w/v	Granules(G)	Systemic broad-spectrum protective and curative

Data analysis

Data on the percentage inhibition growth of the mycelia was analyzed using SAS statistical package version 8.1. The analysis conducted were (ANOVA) and means separations using LSD.

6.3 RESULTS AND DISCUSSIONS

Results

The results in experiment one (Table 14) and two (Table 15) showed that there was significant ($P < 0.05$) difference among the concentrations of the hexaconazole on the daily measurements. *Pyricularia oryzae* isolate was sensitive to hexaconazole at higher concentration (18.75mls/lit). At this rate there was no mycelial growth observed. A lower (6.25mls/lit) and medium (12.5mls/lit) concentrations were intermediate although the mean of mycelial growth was higher in a lower (6.25mls/lit) concentration than the medium rate (12.5mls/lit).

Table 14 Mycelial growth on hexaconazole amended agar at three concentrations (Experiment one)

Isolate	Fungicide	Concentration mls	Mycelial growth mm16 th day)	Host sensitivity
<i>Pyricularia Oryzae</i>	Hexaconazole	6.25	43.000b	Intermediate (10-60%)
		12.5	20.750c	Intermediate (10-60%)
		18.75	0.000d	Sensitive (<19%)
	Control		77.67a	Insensitive (Over 60%)

NB: Means followed by the same letter within the column are not significantly different (P>0.05)

Table 15. Mycelial growth on hexaconazole amended agar at three concentrations (Experiment two)

Isolate	Fungicide	Concentration mls	Mycelial growth (16 th day)	Host sensitivity
<i>Pyricularia Oryzae</i>	Hexaconazole	6.25	21.000b	Intermediate (10-60%)
		12.5	20.550c	Intermediate (10-60%)
		18.75	0.000d	Sensitive (<19%)
	Control		71.00a	Insensitive (Over 60%)

NB: Means followed by the same letter within the column are not significantly different (P>0.05)

The results indicated that there was significance ($P < 0.05$) difference among the three concentrations of the Azoxystrobin (Table 16 and 17) from measurement taken. *Pyricularia oryzae* isolate was insensitive to the untreated culture. In all the three concentrations (1, 2 and 3 mls/lit) the fungus was intermediary affected. The trend was similar when the experiment was repeated the second time. The response of the *Pyricularia oryzae* to azoxystrobin was intermediate (10-60%) in all the three rates (1, 2 and 3mls/lit)

Table 16. Mycelial growth on azoxystrobin amended agar at three concentrations (Experiment one).

Isolate	Fungicide	Concentration mls	Mycelial growth mm16 th day	Host sensitivity
<i>Pyricularia Oryzae</i>	Azoxystrobin	1	38.250b	Intermediate (10-60%)
		2	34.550c	Intermediate (10-60%)
		3	31.000d	Intermediate (10-60%)
	Control		79.00a	Insensitive (Over 60%)

NB: Means followed by the same letter within a column are not significantly different ($P > 0.05$)

Table 17. Mycelial growth on azoxystrobin amended agar at three concentrations (Experiment two).

Isolate	Fungicide	Concentration mls	Mycelial growth (16 th day)	Host sensitivity
<i>Pyricularia Oryzae</i>	Azoxystrobin	1	27.00b	Intermediate (10-60%)
		2	24.00c	Intermediate (10-60%)
		3	24.00c	Intermediate (10-60%)
	Control		70.00a	Insensitive (Over 60%)

NB: Means followed by the same letter within a column are not significantly different (P>0.05)

There was significant (P<0.05) difference in mycelial growth of the thiophanate methyl amended media at the three different rates (1, 2 and 3mls/lit). The response of the fungus to the lower rates (1mls/lit) was intermediate while it became sensitive to middle and the higher rates (2 and 3mls/lit) respectively (Table 18 and 19).

Table 18. Mycelial growth on thiophanate methyl amended agar at three concentration (Experiment one)

Isolate	Fungicide	Concentration mls	Mycelial growth (16 th day)	Host sensitivity
<i>Pyricularia Oryzae</i>	Thiophanate Methyl	1	30.500b	Intermediate (10-60%)
		2	0.000c	Sensitive (<19%)
		3	0.000c	Sensitive (<19%)
	Control	78.33a	Insensitive (over 60%)	

Means of the same letter within a column are not significantly different (P>0.05)

Table 19. Mycelial growth on thiophanate amended agar at three concentrations (Experiment two)

Isolate	Fungicide	Concentration mls	Mycelial growth mm(16 th day)	Host sensitivity
<i>Pyricularia Oryzae</i>	Thiophanate Methyl	1	20.750b	Intermediate (10-60%)
		2	0.000c	Sensitive (<19%)
		3	0.000c	Sensitive (<19%)
	Control	78.33a	Insensitive (over 60%)	

Means of the same letter within a column are not significantly different (P>0.05)

There was significance (P<0.05) difference among the three concentrations of carbendazim amended agar (Table 20). The *Pyricularia oryzae* was sensitive (<19%) to carbendazim at higher and medium (2 and 3mls/lit) concentrations.

The fungus was intermediate (10-60%) at low concentration compared to (over 60%) in control plates. Similar response was found in the second experiment (Table 21).

Table 20. Mycelial growth on carbendazim amended agar at three concentrations (Experiment one)

Isolate	Fungicide	Concentration	Mycelial growth (16th day)	Host sensitivity
<i>Pyricularia Oryzae</i>	Carbendazim	1	30.750a	Intermediate (10-60%)
		2	0.000a	Sensitive (<19%)
		3	0.000a	Sensitive (<19%)
	Control		78.00a	Insensitive (Over 60%)

Means of the same letter within a column are not significantly different (P>0.05)

Table 21. Mycelial growth on carbendazim amended agar at three concentrations Experiment two

Isolate	Fungicide	Concentration	Mycelia growth (16th day)	Host sensitivity
<i>Pyricularia Oryzae</i>	Carbendazim	1	25.750b	Intermediate (10-60%)
		2	7.000c	Sensitive (<19%)
		3	0.000c	Sensitive (<19%)
	Control		71.00a	Insensitive (Over 60%)

Means of the same letter within the column are not significantly different

Discussion

The results indicated that the *Pyricularia oryzae* was sensitive to hexaconazole at a higher concentration (18.75mls/lit) indicating that at this concentration the fungus may not have been able to reproduce resulting to no mycelial growth as compared to the un-amended media or medium and lower concentrations. At low concentration (6.25mls/lit) and medium (12.5mls/lit) application showed some mycelia growth indicating that the fungus was not sensitive and therefore the fungicide may not have been effective enough at these rates to control the rice blast. *Pyricularia oryzae* was insensitive in the agar that was not amended with the fungicide and this showed that at favourable environment the fungus expressed its potential growth. Work done by Ali and Shafi (2005) showed hexaconazole to effectively control rice blast when used as a seed dresser.

The results on Azoxystrobin showed that *P. oryzae* isolate was not sensitive to the untreated culture and in all the three concentrations (1, 2 and 3mls/lit) it was tested. This growth of mycelia showed the fungicides could not fully control the rice blast at the laboratory experiment. Instead of re-proving the fungicide at the in-vitro test it is however necessary to test the fungicide under field conditions in order to make a comprehensive conclusion for its efficiency. The same fungicide was used in the United States and it controlled the disease effectively when applied at panicle and heading stage Gerd, (2007). Since the rice blast attack the crop at all stages of plant growth, the chemical could be used with other chemicals fungicides that would be used in early stages of crop growth.

Pyricularia oryzae was sensitive to both Thiophanate Methyl and Carbendazim at medium and higher level (1, 2 and 3mls/lit) and no mycelial growth was observed. These fungicides being systemic, broad-spectrum, protective and curative, there is a possibility that they may have had a direct inhibitory activity against *Pyricularia oryzae* acting on primary metabolism as was also found by Woloshuk *et al.* (1980). These may include protein and phospholipids biosynthesis in the membrane. The results were supported by Ram *et al.*, (1991) who found that Thiophanate methyl gave positive response in controlling the disease. Similar work done by Gouramanis, (1995) found that carbendazim and thiophanate methyl gave positive results in the control of rice blast disease Dubey, (1995) conducted similar experiment and found that among the eight fungicides tested thiophanate methyl proved to be the most effective fungicides in rice blast.

6.4 CONCLUSION

The *Pyricularia oryzae* was found to be either being sensitive or intermediately or insensitive among the various fungicides tested. Carbendazim and Thiophanate Methyl 50% w/v were found to effectively control rice blast at (2 and 3ml/lit) concentration. Hexacanazole was also effective but required a higher concentration rate of (18.75mls/lit) than the carbendazim and Thiophanate. Azoxystrobin 50G/L was not effective in the management of rice blast in the two experiments carried at in-vitro. However, from the literature the fungicide is said to be effective at the heading stage (Gerd, 2007) and therefore would require to be combined with other fungicides like carbendazim that protect the crop at earlier growth before heading stage. A field trial would be recommended for the four fungicides since the condition in the

laboratory is different from those of the field and the response may be different once the fungicides are exposed to a outdoor/field environment.

CHAPTER SEVEN

7.0 GENERAL CONCLUSION AND RECOMMEDATION

7.1. CONCLUSION

From this work there is evidence that plant nutrition can influence disease incidence and severity. When nitrogen level increased from 80KgN/ha to 120KgN/ha disease pressure increased. At very low nitrogen application, the disease became severe. Silicon was found to manage rice blast but application rate was important in that 1000KgSi/ha was as effective as 1500KgSi/ha levels. An increased rate of silicon decreased the disease pressure but a lower rate of 500KgSi/ha did not manage the disease. It can be concluded that silicon and nitrogen interact to influence the yield and rice blast infection

The organic husk ash at 2 tons/ha was able to suppress the disease. Its performance was found to be as effective as the rate of 120kgN and 1000KgSi/ha. It may be used as an alternative source of silicate since it produced similar results as those produced by the commercial silicate.

The interaction between silicon and nitrogen at 80KgN/ha and 1000kgSi/ha (N2S2) was found to be effective in management of rice blast in Mwea. Planting resistant cultivars is the primary method of managing rice blast, in many areas of the world. However, fungicides have been used particularly where conditions are favorable for severe epidemics. The study revealed that Carbendazim500G, Thiophanate Methyl 50% w/v at 1mls/lit and 2mls/lit concentration were effective in controlling the pathogen while Hexaconazole controlled it at 6.25mls/lit. Azoxystrobin 50G/L was

however found to be in-effective in controlling the pathogen in-vitro as it allowed the growth of mycelia.

A rate of (80kgN/ha and 1000 KgSi/ha) gave the highest yield but not significantly different from N3S2, (120KgN and 1000 KgSi/ha) N3N3 (120KgN and 1500KgSi/ha), N2S3 (80KgN and 1500 KgSi/ha). This is an indication that the two elements are important in the rice growth and development. (80kgN/ha and 1000 Kg Si/ha) could be adopted for optimal yield rather than applying a higher level which will add more expenses.

7.2 RECOMMENDATION

- Routine analysis for silicon and nitrogen should be carried out for proper fertilizer management since the level of silicon is low and no external silicon is added
- Emphasis on proper use of fertilizer because over application of nitrogen causes rice blast disease infection
- Application of 80kgN/ha and 1000 Kg Si/ha should be adopted since it was found to be effective in disease management as well as the highest yield.
- Rice husk should be burned and ash applied in the field as a source of silicon to be used in management of rice blast since it is cheaper than commercial silicate and is readily available in the scheme.

- Carbendazim, Thiophanate Methyl 50% w/v at concentration level two (2mls/lit) should be used for rice blast management.
- Fungicide trial should be conducted in the field for further investigation to validate the in-vitro test
- There are business opportunities of importing the basic slag to add on rice as a fertilizer.
- Economic study to compare chemical and organic silicon source should be done
- Further studies on silicon for different varieties require to be studied.

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APPENDICES

Appendix I ANOVA table. Effect of silicon and nitrogen on rice yield season one

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	15	17.43688647	1.16245910	59.93	<.0001
Error	48	0.93103310	0.01939652		
Corrected Total	63	18.36791957			
	R-Square	Coeff Var	Root MSE	Yield t/ha	Mean
	0.949312	8.507881	0.139271	1.636969	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Nitrogen	3	7.59478929	2.53159643	130.52	<.0001
Silicon	3	7.81476230	2.60492077	134.30	<.0001
Nitrogen*silicon	9	2.02733489	0.22525943	11.61	<.0001

Df=degrees of freedom

Pr=Alpha level significance at 0.05

Appendix II. ANOVA table Rice blast infection rate over time

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	16	1023.485294	63.967831	285.23	<.0001
Error	255	57.187500	0.224265		
Corrected Total	271	1080.672794			

R-Square	Coeff Var	Root MSE	Date5 Mean		
0.947082	9.116061	0.473566	5.194853		
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Nitrogen	3	28.6250000	9.5416667	42.55	<.0001
Silicon	3	949.7812500	316.5937500	1411.70	<.0001
Nitrogen*Silicon	9	23.2812500	2.5868056	11.53	<.0001

Appendix III ANOVA table: The effect of available nitrogen on disease severity on diseased and healthy plants

		Sum of			
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	1	2.02279506	2.02279506	13.29	0.0026
Error	14	2.13125388	0.15223242		
Corrected Total	15	4.15404894			

R-Square	Coeff Var	Root MSE	Nitrogen Mean
0.486945	20.93255	0.390170	1.863938

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	1	2.02279506	2.02279506	13.29	0.0026

Appendix IV. ANOVA table: Effect of nitrogen silicon interaction and the leaf angle on rice plant.

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	16	320.9411765	20.0588235	42.62	<.0001
Error	51	24.0000000	0.4705882		
Corrected Total	67	344.9411765			
R-Square Coeff Var Root MSE LeafAngle Mean					
0.930423 19.76594 0.685994 3.470588					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Nitrogen	3	89.0000000	29.6666667	63.04	<.0001
silicon	3	200.5000000	66.8333333	142.02	<.0001
Nitrogen*silicon	9	30.5000000	3.3888889	7.20	<.0001

Appendix V. ANOVA table: Effect of fungicides on *Pyricularia oryzae* mycelial growth

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	11	25972.61019	2361.14638	1073.94	<.0001
Error	36	79.14908	2.19859		
Corrected Total	47	26051.75926			

R-Square	Coeff Var	Root MSE	D16 Mean
0.996962	2.040178	1.482763	72.67811

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	11	25972.61019	2361.14638	1073.94	<.0001

Appendix VI. ANOVA table: Silicon levels in plant tissues
Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	0.00075702	0.00037851	2601.88	<.0001
Error	21	0.00000305	0.00000015		

Corrected Total	23	0.00076008			
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R-Square	Coeff Var	Root MSE	Silicon ppm Mean
0.995981	1.549936	0.000381	0.024608

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	2	0.00075702	0.00037851	2601.88	<.0001

Appendix VII ANOVA: Nitrogen levels in infected and non-infected field

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.01470609	0.00490203	22.57	<.0001
Error	28	0.00608013	0.00021715		
Corrected Total	31	0.02078622			
	R-Square	Coeff Var	Root MSE	N Mean	
	0.707492	22.04532	0.014736	0.066844	

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	1	0.00664128	0.00664128	30.58	<.0001
Depthcm	1	0.00538203	0.00538203	24.79	<.0001
Treat*Depthcm	1	0.00268278	0.00268278	12.35	0.0015

Appendix VIII Media preparation

The media was prepared by dissolving 40gm of corn meal agar (CMA) powder in 1 litre of distilled water and mixed on hot plate/stirrer and mixed homogeneously before autoclaving /sterilizing for 15 min at 121⁰c (1.5psi) (Pascals per sq. inch).