EFFECT OF NITROGEN FERTILIZERS ON DEVELOPMENT OF RICE YELLOW MOTTLE VIRUS (RYMV) AND YIELD OF RICE (*Oryza sativa L.)*

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ABSTRACT

Rice Yellow Mottle Virus (RYMV) is the most important pest constraint of rice in Tanzania and other sub Saharan Africa countries. Fertilizer management is recommended to minimize incidence and development of the disease in affected rice ecosystems. Field research was carried out in the 2014 cropping season at Sokoine University of Agriculture to determine the effect of nitrogen fertilizers on the development of RYMV and grain yield of rice. Split-split plot in a randomized complete block design (RCBD) with three replications was adopted. Three RYMV inoculated rice cultivars namely Mwangaza (R), Salama (MR) and Supa (S) were fertilized with four different types of nitrogen fertilizers (DAP, NPK, SA and Urea) each at two levels (100kgN/ha and 120kgN/ha). Urea was applied in split and single dose. Significant differences for area under disease progress curve (AUDPCs) ($P<0.05$) were detected among the varieties, nitrogen fertilizers and their interactions. The variety Supa had the highest mean AUDPC (100.6) followed by Salama (51.7) and Mwangaza (30.8). Single dose Urea application had highest disease levels (62.9 AUDPC) followed by NPK (60.1), DAP (59.8), SA (60.0) and split urea (59.9) ($P<0.05$). Nitrogen fertilizer at higher levels did not show significant effect on the development of RYMV ($p\leq0.05$). The effect of nitrogen fertilizers on disease development depended on genotype. Yield was significantly affected by variety, type and amount of N fertilizer and their interactions ($P<0.05$). The variety Mwangaza had the highest grain yield (3.9ton/ha) followed by Salama (3.0ton/ha) and Supa (2.1ton/ha). Yield was highest with NPK (3.6ton/ha), DAP (3.5ton/ha), SA (3.5ton/ha) and split Urea (3.6ton/ha) and lowest was with Urea single dose (2.8ton/ha). Treatment with 120kgN/ha gave higher grain yield than 100kgN/ha. Urea decreased RYMV significantly but should always be applied in splits in order to fully utilize its potential for reducing RYMV and maximizing yield of rice.

DECLARATION

I, Martha Losindilo, do declare to the Senate of Sokoine University of Agriculture that, this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Martha Losindilo Date
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The above declaration is confirmed by

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Prof. A. J. P. Tarimo Date
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DEDICATION

I dedicate this work to my beloved brother Mr. Imani Losindilo, my aunt Ms. Fatuma Kimario, my friends Mr. Abihudi Zakaria, Mr. Edwin Mhede and Mr. Ezekiel Oluoch.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATE

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AUDPC	Area under Disease Progress Curve
DAP	Di-ammonium Phosphate
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
IITA	International Institute of Tropical Agriculture
INGER	International Network for the Genetic Evaluation of Rice
IRRI	International Rice Research Institute
PVY	Potato Virus Y
RYMV	Rice Yellow Mottle Virus
SA	Sulphate of Ammonia
TAMV	Tomato Aucuba Mosaic Virus
TMV	Tobacco Mosaic Virus
WARDA	West Africa Rice Development Association

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Rice (*Oryza spp*) is the second most important food and commercial crop in Tanzania after maize; it is among the major sources of employment, income and food security for Tanzania farming households. Tanzania is the second largest producer of rice in Southern Africa after Madagascar with production level of 818 000 tones (FAOSTAT, 2010). The cultivated area is 681 000 ha; this represents 18 % of Tanzania's cultivated land. Rice is grown in three agro- ecosystems namely, rainfed lowland (74%), rainfed upland (20%) and irrigated lowland (6%) (RLDC, 2009). The average yield in most farmers' fields is very low, 1-1.5 t per ha. Farmers grow a number of traditional varieties, some have long maturity period and yield is affected by irregular rainfall patterns, low soil fertility and occurrence of pests, which contribute to yield decline.

Rice Yellow Mottle Virus (RYMV) is the most important pest constraint of rice in Sub-Saharan Africa. The virus is endemic to the continent and causes a devastating disease on the crop. Bakker (1974) first reported the virus in 1966 in Kenya. Since then the virus has spread to most rice growing countries in the sub-continent, particularly in West Africa where the virus induces severe crop damage and yield losses (Kouassi *et al*., 2005).

In Tanzania, RYMV was not reported until mid 1980s when the disease was first observed on an introduced cultivar ITA 173 grown in farmers' fields at Mkindo rice irrigation project in Morogoro (Kanyeka *et al*., 1996). The virus has since then spread fast and is now found in all the major rice growing regions and in all rice ecosystems

(Traore *et al*., 2009). The most severely affected rice growing regions are Mbeya (Kyela Basin and Usangu plains in Mbarali district), Morogoro (Kilombero Valley), Kilimanjaro (Ndungu Plains) where the virus induces severe yield losses in farmers' fields (Kanyeka, 2006).

1.2 Research Problem and Justification

RYMV has gained substantial economic importance in Tanzania's rice cropping systems. It is particularly a devastating pest in most farmers' fields in Kilombero and other rice growing areas. The existence of different strains of RMYV with contrasting ecological and geographical distribution within a region and with different pathogenecity on susceptible cultivars has been reported (N'Guessan *et al*., 2001; Banwo, 2002).

Nitrogen fertilization has been shown to have impact on various plant diseases including those caused by viruses (Dordas, 2008). Singh, (1970) reported that the growth of *C. Amaranticolor*, a Tobaccco Mosaic Virus (TMV) increased with increase in nitrogen supply up to 630 ppm and further increase above (1050 ppm) retarded the growth. The number of local lesions produced was highest in plants grown at 1 050 ppm and lowest at 0 ppm nitrogen levels. The results indicated that the number of lesions produced is directly proportional to the supply of nitrogen, irrespective of host growth. In addition, Singh and Singh (1977) investigated the effect of several concentrations of nitrogen (0, 21, 70, 210, 420 and 630 ppm) on the growth and multiplication of cucumber mosaic virus in cucumber (*Cucumis sativus L. Barshati*) grown in sand culture and reported that the effect of nitrogen nutrition on the concentration of the virus appeared to be directly related to vegetative growth of the host. 420 ppm level yielding the best growth also showed the highest virus concentration and total nitrogen percentage at 30TH day of infection.

However, it is not just the amount of nitrogen that is important in influencing disease development; its form is also important, either nitrate, nitrite or ammonium. One disease (foliar, wilts, or root rots) may be made worse by application of ammonium nitrogen, or application of nitrate nitrogen (Henn, 2004). The form and amount of N are also important in plant diseases development hence proper selection of fertilizers could help to minimize the disease and the presence of nitrification inhibitors responsible for transformation of one form of nitrogen to another is important too (Celar, 2003; Harrison and Shew, 2001).

In a greenhouse study to determine how growth, grain yield, and yield components of oat *(Avena sativa* L.) and wheat *(Triticum aestivum* L.) plants given nutrient solutions containing different ratios of NO_3-N to NH_4-N would react to barley yellow dwarf virus (BYDV) infection conducted by Riedell (2000) showed that in the NH_4 nutrient solution treatments, BYDV infection significantly reduced individual kernel weight in oat and primary tiller height in wheat. These same measures were not significantly affected by BYDV infection in the NO_3 or NH_4NO_3 nutrient solution treatments. Nutrient solution treatments had no significant effect on grain yield, but BYDV infection reduced grain yield by 45% in oat and 46% in wheat. In conclusion, nutrient solution N form interacted with BYDV infection to alter disease tolerance in oat (kernel weight) and wheat (primary tiller height), but these alterations had no effect in ameliorating grain yield loss caused by BYDV disease.

There is little knowledge on the effect of the type and amount of nitrogen fertilizers on RYMV disease development. This study was conducted to determine the effect of type and amount of nitrogen fertilizers on the development of RYMV disease with the aim of providing insight on what types and amount of nitrogen fertilizers that can be used in the management of RYMV disease in vulnerable areas.

1.3 Research Objectives

1.3.1 Overall objective

To improve rice productivity through identifying types and amount of nitrogen fertilizers to be used in areas vulnerable to RYMV.

1.3.2 Specific objectives

1. To determine disease progress on rice cultivars differing in RYMV susceptibility under different types and amounts of nitrogen fertilizers.
2. To determine if rice cultivars with varying levels of resistance to RYMV respond differently to types and amounts of nitrogen fertilizers with regard to RYMV development.
3. To determine yield and yield components of rice cultivars as influenced by the effect of types and amounts of nitrogen fertilizer in RYMV infected plots.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rice Production in Tanzania

Tanzania is the second largest producer of rice in Eastern, Central, and Southern Africa after Madagascar. In 2008, the country produced about 899 000 t of rice from an area of 355 000 ha (FAOSTAT, 2010). About 74% of the rice in Tanzania is grown under rain fed lowland conditions by smallholder farmers, whereas upland and irrigated rice comprise about 20% and 6% of the area, respectively (RLDC, 2009). The average yield is very low, 1−1.5 t ha^{-1}, as farmers grow a number of traditional varieties that are tall and prone to lodging. Moreover, these varieties have long maturity period and are not suitable for areas with marginal rainfall pattern (FAOSTAT, 2010). The occurrence of rice blast and rice yellow mottle virus (RYMV) also contributes to the yield decline.

2.2 Rice Yellow Mottle Virus (RYMV)

2.2.1 History and geographical distribution of rice yellow mottle virus (RYMV)

RYMV is a member of the genus *Sobemovirus*. RYMV was identified for the first time on the rice cultivar “Sindano” by Bakker, in Otonglo near Lake Victoria in Kenya in 1966 (Bakker, 1974). It is the main viral disease of rice in Africa (Sere *et al*., 2008). So far, RYMV is reported in 23 countries in Africa South of the Sahara and in all rice ecosystems. In Tanzania, RYMV was not reported until mid 1980s when the disease was first observed on an introduced cultivar ITA 173 grown in farmers’ fields at Mkindo rice irrigation project in Morogoro (Kanyeka *et al*., 1996). Kanyeka *et al*. (2007) identified three types of RYMV strains in Tanzania. The topology of the phylogenetic tree allowed the discrimination of three major strains with strong bootstrap support of bifurcating branches. The strains were exclusively found in East Africa region and were designated as S4, S5, and S6. No major variant was identified as there were no intermediates between the main clusters.

Assessment of the distribution of the 3 strains revealed that strain S4 occurs predominantly in Kyela district. It was also found in the 3 districts of Morogoro region (Mvomero, Kilombero and Ulanga). By contrast, strain S5 is restricted to a few sites only in Kilombero district. Strain S6 is widely spread in East Africa and occurs predominantly in all the 3 districts in Morogoro region and in Same district, Kilimanjaro region (Kanyeka *et al*., 2007).

The isolates of strain S4 in Morogoro region were very closely related with percentage identity between 99.2 and 100. There was one exception of an isolate, which was more closely related to isolates from Kyela than to the rest of Morogoro. It is differed from other Morogoro isolates by percentage between 3.9 and 4.6. S4 isolates in Morogoro were more closely related to the Lake Malawi strain with percentage identity between 95.4 and 6.8 compared with those of Lake Victoria strain (93.2 to 94.3%) (Kanyeka *et al*., 2007).

2.2.2 Infection and symptoms of RYMV

The beetles of the family *Chrysomelideae* naturally transmit the virus. It is also sap transmissible under artificial conditions (Bakker, 1974). Several workers have given details of the characteristic symptoms of the disease on susceptible rice cultivars (Bakker, 1974). These include leaf yellowing and mottling, stunting of infected plants with narrowing of emerging leaves and incomplete panicle emergence with subsequent sterile spikelets. The initial symptom is yellowing of leaves which begin with an alternation of yellow and green spots, giving a mottled aspect to the leaf. Two weeks after infection, these spots develop and become parallel to the leaf veins (Plate 1). For some varieties, leaves become orange when plants get matured. The standard evaluation system (SES) on a scale of 1–9 is used for visual diagnostic of symptoms (Bakker, 1974). Other symptoms such as the decrease of the spikelet number, the partial or total sterility of rice panicles, the significant reduction of the yield, and the death of the infected plant are also observed (Abo *et al*., 2000).

Figure 1: Leaves and panicles of rice infected with RYMV

When the infection occurs in the early stages of plant development, susceptible cultivars normally die (Luzi-Kihupi *et al.*, 2000). Field yield losses of RYMV-infected rice cultivars ranging from 10-95% have been reported in many countries in West Africa (Mogga *et al*., 2012). In Tanzania, yield losses ranging from 20-100% have been observed in farmers' fields in the endemic areas (Luzi-Kihupi *et al.,* 2000).

2.2.3 Ecology and Epidemiology of RYMV

Geographical adaptation of RYMV strains as reported by many authors is probably related to climatic factors which either directly impact on the virus, or do so through alternative hosts, the occurrence of which depends on such factors. While investigating the factors which influence the development of the disease symptoms in Nairobi, Kenya, and in Wageningen, the Netherlands, Bakker (1974) observed that high temperatures (30 °C and above) would reduce the period for the expression of symptoms, and that low relative humidity would trigger the necrosis of infected rice leaves. Mogga *et al.* (2012) reported a variation of the incidence and severity of RYMV depending on climatic zones and the local environment in which rice is grown (type of rice cultivation). For example, with irrigated rice and in the Sahel, the disease incidence and severity are higher than in the tropical rain forest where they vary from low to moderate regardless of the type of rice cultivation (Mogga *et al.,* 2012).

The two types of RYMV's transmission now clearly admitted are: insect-borne transmission and transmission by artificial mechanical inoculation. Bakker (1974) reported in East Africa several leaf beetle species with the potential to transmit the virus, namely *Sesselia pussilla* (Galerucinae), *Chaetocnema pulla Chapius* (Halticinea), *C. dicladispa* (Chrysispa) *Kraatz* (Hispinae), *D. bayoni Gest* (Hispinae), and *Trichispa sericea Guer*in (Hispinae). Hispa (Dicladispa) *gestroi Chapius* (Hispinae) was also reported by Woin *et al* (2007) as a vector of the virus. However, the role of these vectors in rice yellow mottle disease epidemics is not well established. In East Africa, Bakker (1974) reported that the first diseased rice plants were consistently found at the borders of rice plots along irrigation bunds, which suggests transmission of the virus by insect vectors from external sources.

It was also formally established that RYMV's infection is not seedborne (Konaté *et al*., 2001) even though the virus was detected in all the rice seed parts (lemma, caryopsis, and embryo) (Konaté *et al*., 2001). It is generally admitted that viruses found in the embryo are seedborne (Konaté *et al*., 2001). As for RYMV, Konaté *et al*. (2001) have shown that absence of transmission was due to the inactivation of the virus during the maturation stage of the rice seed. Such inactivation phenomenon contradicts the results obtained by Uke *et al.* (2014) stating that empty spikelets and above ground contaminants of hulled rice seed were infectious RYMV sources.

Several other RYMV modes of transmission have been suspected and more or less verified. Thus, despite the abundant rice nematode fauna, none of the nematode species tested had been able to transmit RYMV (Abo *et al.*, 2004).

Abiotic transmission of RYMV was strongly suspected but is yet to be demonstrated. According to Woin *et al.* (2007), transplanting rice into a soil containing cow dung and poorly decomposed crop residues could be responsible for the mechanical transmission of RYMV. Similar types of transmission could be observed when rice is transplanted into a soil on which infected rice regrowths and roots have been manipulated during plowing operations (Uke *et al.*, 2014). RYMV could also be transmitted mechanically by contact of the gustation liquid and irrigation water with rice plants (Bakker, 1974). The role of man in RYMV mechanical transmission is highly suspected, mainly during cropping operations such as transplanting, fertilizer application, irrigation, harvesting, etc. Other assumptions include contact between infected agricultural implements and rice plants, between contaminated hands and rice plants, and between healthy and diseased plants under the effect of wind, man, or animals (Uke *et al.*, 2014). Infection of rice farms from nurseries infected through contact between diseased and healthy plantlets or between contaminated hands and rice plants was suspected as far back as 1974 by Bakker (1974). RYMV is always more severe in farms with transplanted rice than in farms with direct sowing, Traoré and Traoré (2000).

In East Africa, Bakker (1974) showed that the following Poacea species could be experimentally infected by RYMV and, therefore, are likely to host the virus: *Dinebra retroflexa (Vahl) Panz., Diplachne caudata K. Schum., Eragrostis aethiopica Chiov., Eragrostis ciliaris* (L.) R. Br., *Eragrostis namaquensis Nees var. namaquensis, Eragrostis tenella* (L.) *Reom. Et Schult., Oryza barthii A. Chev*., and *Oryza punctata Steud*. However, rice is the only host found with natural infection. In West Africa, Konaté *et al*., (2001) showed that *Echinochloa colona*, *Ischaemum rugosum, Panicum repens* L., *Oryza longistaminata A. Chev. Et Roehr* and *O. barthii A. Chev*. were naturally infected by RYMV. In Central Africa, Traoré (2000) reported natural infection on *Oryza barthii, O. longistaminata, Panicum subalbidum Kunth, Acroceras zizanioides, Sacciolepis africana, Eragrostis sp*, *and Setaria longiseta* in Cameroon and Chad. With regrowths, rice seems to offer the best source of preservation to the virus.

Bakker (1974) and Allarangaye *et al.* (2006) showed that RYMV could not be transmitted by the rice seed. However, Uke *et al.* (2014) could detect the virus in the lemma but not in the embryo. Konaté *et al*. (2001) subsequently proved that RYMV was present not only in the lemma but also in the aryopsis and the embryo and still was not seedborne. Investigating virus infectivity during seed maturation, they were able to find almost 100% loss of RYMV-infectious potential at maturity. The cause of the inactivation of the virus during the maturation phase of the seed is being investigated. The results now seem final that rice seed is not a survival source for RYMV (Konaté *et al*., 2001).

Several media have been suspected to host RYMV; irrigation water (Bakker 1974), crop residues (Uke *et al*., 2014), and animal waste (Woin *et al.,* 2007). Increasing use of cow dung as organic manure in rice cultivation prompted our team to look for RYMV in such an environment. It has been established that when a cow is fed with RYMV-infected rice straw, the infectious virus can easily be detected in the fresh dung using ELISA test. However, the virus is fast degraded and loses its infectious potential when the cow dung is left to dry in the conditions of the pen; it cannot be detected after 2–3 weeks (Abo *et al*., 2002). Consequently, cow dung is not a favorable environment for RYMV preservation. Sarra and Peters (2003) reported that RYMV would remain infectious in rice straw 10 months after harvest, and roughly 6 months after harvest in rice roots sampled in the soil during the dry season.

The site of RYMV was observed for the first time in leaf tissues of infected rice plants by Bakker (1974). He showed that the virus was present in the cells of the epidermis, mesophyll, and stomata. On the other hand, no virus particle could be observed in the chloroplasts and the cell nucleus. Ioannidou *et al*. (2000) reported a strong RYMV build-up in the xylem cells. RYMV migration was thought to take place mainly from this tissue via the plasmodesmata for short-distance (cell to cell) migration and sap vessels for long-distance migration.

2.3 Effects of Nitrogen Fertilizers on Plant Viral Diseases

Nutritional factors that favor growth of host plants also favor virus multiplication. This holds true particularly for Nitrogen (Spann and Schumann, 2010). Visible symptoms are dependent upon the competition for N between the virus and the host cells (Spann and Schumann, 2010). This competition varies with different diseases and can be influenced by environmental factors, such as temperature (Spann and Schumann, 2010).

Singh, (1970) reported that the growth of *C. Amaranticolor*, a Tobaccco Mosaic Virus (TMV) increased with increase in nitrogen supply up to 630 ppm and further increase above (1050 ppm) retarded the growth. The number of local lesions produced was highest in plants grown at 1050 ppm and lowest at 0 ppm nitrogen levels. The results indicated that the number of lesions produced was directly proportional to the supply of nitrogen, irrespective of host plant growth. In addition, Singh and Singh (1977) investigated on the effect of several concentrations of nitrogen (0, 21, 70, 210, 420 and 630 ppm) on the growth and multiplication of cucumber mosaic virus in cucumber (*Cucumis sativus L. Barshati*) grown in sand culture and reported that the effect of nitrogen nutrition on the concentration of the virus appeared to be directly related to vegetative growth of the host. 420 ppm level yielding the best growth also showed the highest virus concentration and total nitrogen percentage at 30th day of infection.

The form of nutrient solution nitrogen (either NH_4-N or NO_3-N or mixtures of the two) provided to plants influences the severity of many crop diseases. Riedell (2000) reported that in a greenhouse study conducted to determine how growth, grain yield, and yield components of oat *(Avena sativa* L.) and wheat *(Triticum aestivum* L.) plants given nutrient solutions containing different ratios of NO_3-N to NH_4-N would react to barley yellow dwarf virus (BYDV) infection, Oat and wheat plants given NH_4 had fewer tillers than plants given the other nutrient solution treatments. BYDV-infected oat and wheat plants were shorter than uninfected plants. All pots then received NO_3 nutrient solution until plant maturity, after which days to anthesis, primary tiller height, grain yield and yield components were measured. In the NH_4 nutrient solution treatments, BYDV infection significantly reduced individual kernel weight in oat and primary tiller height in wheat. These same measures were not significantly affected by BYDV infection in the NO_3 or NH_4NO_3 nutrient solution treatments. There was no other significant nutrient solution by BYDV infection interactions for any other dependent variable measured. Nutrient solution treatments had no significant effect on grain yield, but BYDV infection reduced grain yield by 45% in oat and 46% in wheat. In conclusion, nutrient solution N form interacted with BYDV infection to alter disease tolerance in oat (kernel weight) and wheat (primary tiller height), but these alterations had no effect in ameliorating grain yield loss caused by BYDV disease (Riedell, 2000).

The response of viral pathogen replication to nutrient addition has been shown to vary according to the host species tested (Rua *et al*., 2013). Lacroix *et al*. (2014) found that environmental nutrient supply rates modulated viral infection success differently for each virus species. Barley yellow dwarf virus-PAV (BYDV-PAV) was more effective than cereal yellow dwarf virus-RPV (CYDV-RPV) at infecting hosts at all N supply rates. These rates constituted two concentrations of N and P addition at concentrations equivalent to 0.2 and 10% of a half-strength Hoagland's nutrient solution, while concentrations of other macro- and micronutrients remained constant. Lacroix *et al*. (2014) also reported that environmental nutrient supply rate can alter virus prevalence and the interaction strength among viruses within a host.

The report further showed that there was evidence of antagonistic interactions among viruses and the presence of a competitive hierarchy based on level of nutrients: Barley yellow Dwarf Virus (BYDV-PAV) suppressed Cereal Yellow Dwarf Virus (CYDV-RPV) under conditions of low nutrients. It was also shown that N addition weakened the strength of the antagonistic interaction. Although these results did not point to a simple, clear tradeoff in use of N and P by these virus species, they demonstrated that even relatively closely related viral species can differ significantly in their response to altered N and P supplies to a host. Thus, these empirical results suggested that responses of viral species to the environmental N and P supply rates and ratios of their hosts is a viable framework for understanding the mechanisms of maintenance of virus diversity within a host, as in free-living organisms (Chesson, 2000). Differential responses of species to nutrient supply rates and ratios can provide a mechanism that mediates coexistence of competitor species and co-infection of a host (Miller *et al*., 2005).

Macromolecules such as proteins and nucleic acids are N-rich, and increasing N supply rate could increase the production rate of both virus particles (nucleic acids and capsid proteins) and host factors (e.g. nucleotides and proteins) that are required to complete the virus multiplication cycle (Haile *et al*., 2012). Thus, N addition could reduce N limitation and the strength of inter-specific virus competition for nutrients and host factors that is known to occur between viruses in animal hosts (Ge *et al*., 2012) and which is also likely to occur in plant hosts. N addition could thus facilitate the successful establishment of multiple virus infection.

VanGessel (1993) reported that neither moisture nor nitrogen stress had an additional impact on the severity of Maize Dwarf Mosaic Virus (MDMV) symptoms in corn seedlings. MDMV titer was similar for all plants receiving adequate nitrogen, regardless of moisture level. Corn with adequate moisture but nitrogen-stressed had the highest MDMV-A titer (VanGessel, 1993). Similarly, Olson *et al*. (1990) found that moisture stress did not influence MDMV titer in sweet corn grown with sufficient nitrogen.

In a research conducted by Ahmad *et al*. (2008), it was reported that the highly Cotton leaf curly Virus (CLCuV) infested genotypes showed better response to low plant-spacing and high nitrogen-fertilizer. From this study, it was concluded that the optimum yield of seed cotton can be achieved by increasing the plant population (decreasing plant-to-plant spacing) and high nitrogen-fertilizer. It was also concluded that the genotypes which are less susceptible to CLCuV showed negative response to high nitrogen-fertilizer and low spacing. The genotypes showed maximum seed-cotton yield under plant spacing of p x p=30cm and nitrogen fertilizer rate of 8.6 bags of urea/ha.

2.4 Effects of Nitrogen Fertilizers on Other Plant Diseases

Nitrogen is the most important nutrient for plant growth but has also been shown to influence diseases in many crops (Bhaduri *et al*., 2014). It is not only the amount but also the form of nitrogen which can reduce one disease and favor another. There are several reports of the effect of N on disease development that are inconsistent and contradict each other, and the real causes of this inconsistency are poorly understood (Hoffland *et al.*, 2000). These differences may be due to the form of N nutrition of the host (Celar, 2003; Harrison and Shew, 2001), the type of pathogen: obligate versus facultative parasites (Newmann *et al*., 2004; Bhaduri *et al*., 2014) or the developmental stage of N application (Snoeijers *et al.,* 2000). Also, there are no systematic and thorough studies about the effect of N supply on disease resistance, on bio-control agents' activity, and especially on the interaction among nutrient, pathogen, and bio-control organisms (Tziros *et al*., 2006).

A wide range of plant hosts and their pathogens are influenced by N, but the form of N available to the host plant or the pathogen may greatly influence the disease reaction, independently of the rate of N available, for example disease susceptibility in strawberry was affected by both N-concentration and N-source. *B. cinerea* lesions were largest in ammonium nitrate > ammonium sulphate > calcium nitrate. This data suggest that calcium nitrate may be a suitable source of nitrogen, helping growers to reduce disease risk (Walter *et al*., 2008). In wheat crop when compared with the nil N treatment, ammonium nitrogen fertilizers, either as ammonium sulphate or ammonium chloride drilled with the seed, lowered the severity of take-all of wheat (Thomason *et al*., 2001). Availability or failure to act, on both growth and organogenesis nitrogen is the main growth factor element in the plants. An increased nitrogen supply has a strong impact on leaf area and foliage density. Ammonia may have fungicidal effects on *V. dahliae* and some *Fusarium* (Tenuta and Lazarovits, 2002). The use of organic nitrogen base may also improve the competition between certain fungi and micro-organisms antagonistic to pathogens in favor of the latter, and thus limit disease outbreaks (Chau and Heong, 2005). In cases where ammonium has an unfavorable effect on the development of a disease, the use of nitrification inhibitors may be a way to limit the risk of infection while continuing to provide the host plant with sufficient nitrogen (Huber and Thompson, 2007).

Several authors have suggested that further development of the canopy could induce a favorable microclimate in infectious process (Champeil *et al*., 2004; Lecompte *et al*., 2010; Abro, 2014). This hypothesis is supported by the fact that a target size of leaves or fruit may limit the deleterious effect of nitrogen on an outbreak of *B. cinerea* (Lecompte *et al*., 2010). The proposed contradictions about the effect of nitrogen on the development of certain diseases can be overcome by considering the nitrogen supply in the form of ammonia or nitrate (Huber and Thompson, 2007). The germination and penetration into the host can be promoted or inhibited by the presence of leaf or root exudates from the host. For example, root exudates promote the germination of *Fusarium solani f. chlamidospores sp. phaseoli*, and the quantity and chemical composition of these exudates may be influenced by the type of nitrogen nutrition (Datnoff *et al*., 2006).

The effect of different nitrogen levels on disease severity of genotypes of wheat varying in quantitative resistance to yellow rust caused by *Puccinia striiformis*, in both years the disease severity increased strongly with increased N levels. The infection types also increased with increased N levels (Lecompte *et al*., 2010). In the field excessive amounts of N fertilizer had a marked tendency to increase the size of the late blight lesions on the potato leaves. In the greenhouse excessive use of N fertilizer did not increase the late blight lesions on the potato leaves. By increasing the amended N, there was a linear increase of 34.8% for AUIPC (Area under incidence progress curve) and 34.3% for

AUSPC (Area under severity progress curve) of Phoma spot of coffee. The dry matter of coffee seedlings increased linearly with the increase in the amended N. By increasing the amended N, a corresponding increase in shoot N content was observed (De Lima *et al*., 2010).

Long *et al*. (2000) reported that while nitrogen was essential for productivity, the severity of rice blast also increased with the rate of application. Several studies have shown that excessive nitrogen increases the susceptibility of the rice plant to rice blast (Kapoor and Sood, 2000). Kurschner *et al*. (1992) observed that split applications of nitrogen reduced excessive vegetative growth early in the season and reduced the severity of blast. Other workers have also noted that nitrogen fertilization influenced the size of leaf blast lesions (Muriithi *et al*., 2012).

Myint *et al. (*2007) reported that disease severity of bacterial blight of rice could be increased by the application of nitrogen fertilizer. Use of higher level of nitrogen fertilizer can cause higher disease severity of bacterial blight of rice. To maintain a tolerable disease severity and to minimize yield losses, the use of urea above 125.5 kg/ ha should be avoided (Myint *et al.,* 2007).

In a research conducted by Hoepting, (2010), an important observation in a small-plot on-farm field trial in onions grown on plastic the following were observed. The plots located at the bottom of the slope had 83% bacterial bulb decay at harvest. The amount of decay decreased progressively in each replicate moving up the slope to 58% to 17% to zero in the replicate at the top of the slope. This trial was located on a diversified farm that had been heavily manured. Perhaps, heavy rainfall had caused leaching of nitrogen from the top to the bottom of the slope, and thus, increased nitrogen at the bottom of the slope which may have contributed to the higher levels of bacterial bulb decay. The incidence of bacterial bulb decay at harvest was also assessed in a study designed to evaluate the effect of nitrogen on onion Thrips. In this study, onions grown with only 2.0 lb/A of applied nitrogen had 0.7% bacterial rot at harvest. Onions grown with the Cornell recommended rate of 125 lb/A of nitrogen had 10.8% bulb decay, which was 15 times more than the rot that occurred at the 2.0 lb/A rate. Compared to the recommended rate, onions grown with reduced rates of applied nitrogen, 62 and 94 lb/A, had significantly less than half (4.9%) and one third (7.3%), respectively, of bacterial decay without any significant differences in yield (Hoepting, 2010).

2.5 Management of RYMV

2.5.1 Host resistance

Host plant resistance is the most effective option (Banwo *et al*., 2004) because it is sustainable with minimal deleterious effects on the environment. Investigations have revealed the existence of three types of resistance to RYMV: partial natural, high natural and resistance obtained through genetic transformation (Sorho *et al*., 2005). The mechanism involved in partial resistance is retardation of virus movement, thereby lowering virus accumulation and symptom expression (Ioannidou *et al*., 2003). This type of resistance has been found in cv. *Azucena* and a few other cultivars of *Oryza sativa sub sp. japonica* (Ndjiondjop *et al*., 2001). High resistance is conferred by a single recessive gene *Rymv* 1 (Albar *et al*., 2003) which has been identified in the rice cv. *Gigante*. On the other hand, genetic transformation employs transgenic lines obtained by introducing the viral polymerase gene into the susceptible *O. indica cv. Bouake* 189 (Pinto *et al*., 2000).

In Tanzania, mutagenesis or induced mutation has been employed in the breeding program to reduce plant height and the maturation period of popular indigenous cultivars while maintaining the parents' good quality traits (Luzi-Kihupi and Zakayo, 2001). The lines/varieties that showed resistance were subsequently screened for resistance to RYMV under natural conditions in farmers' fields. Several genotypes showed resistance, including mutants that were derived from irradiating the popular local cultivar Supa and introduced varieties from the Africa Rice Center (WARDA) and the International Rice Research Institute (IRRI). One mutant, SSD 35, derived from irradiating Supa by 170 gray gamma rays, and one introduced line, H232-44-1- 1-1, were recommended for release (Luzi-Kihupi *et al.,* 2009).

2.5.2 Vector control and integrated management

Like so many problems that affect growing crops, there is no single method of approaching RYMV that is going to eliminate this insidious disease. Instead, there is always a need to combine elements to provide adequate relief. Along with the use of resistant varieties, the virus can also be managed through cultural practices such as early or late planting in order to avoid the peak vector population, and roguing of diseased plants. Insecticide application to control insects that carry the virus has also been recommended (Woin *et al*., 2007). Nwilene (2000) reported that Neem oil was not only more effective in controlling all three groups of vectors (beetles, grasshoppers and leaf-sucking bugs) compared to pawpaw extract, but it was also even better than commercial pesticide, Decis.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The experiment to investigate the effects of nitrogen fertilizers on the development of RYMV and yield of rice was conducted in 2014 cropping season at Sokoine University of Agriculture. Experiments were conducted at the Horticulture Unit of the Department of Crop Science and Production while laboratory analyses were conducted at the African Seed Health Centre (ASHC).

Table 1: Weather conditions in Morogoro during the experimental period

	Mean Temperature (°C)	Rainfall (mm)	Solar Radiation ($MJm^{-2}d^{-1}$)
February 2014	22.2	69.4	17.6
March 2014	21.8	182.7	16.8
April 2014	21.3	231.0	14.6
May 2014	19.8	113.0	13.9
June 2014	17.8	24.0	14.0

Source: Meteorological Station of Morogoro, Tanzania (2014).

The Horticulture unit is located at latitude $6^0$50'S and longitude $37^0$39'E and at an altitude of 526m asl. The location has a sub-humid tropical type of climate and a bimodal rainfall pattern. The short rains usually last from November to January, with a peak in December. The long and heavy rains start from March to May with a peak in April. The temperatures are lowest in June and July, and the highest in November to February. The mean monthly temperature ranges between 25 and 28^0C, with daily minimum of 26^0C and mean maximum of 30^0C (Meteorological Station of Morogoro, Tanzania (2014).

3.2 Soil Analysis

Physical and Chemical analyses of the soil of the study area were performed in the Department of Soil Science, Sokoine University of Agriculture, using standard methods (Landon, 1991). Measurement of soil pH was carried out using glass electrode pH meter in 1:2.5 mixtures (v/v) of soil and water. Soil texture was determined by Bouyoucos hydrometer method (Day, 1965). Phosphorus was extracted by Bray and Kurtz-1 method (Bray and Kurtz, 1945) and determined spectrophotometrically (Watanabe and Olsen, 1965). The cation exchange capacity (CEC) and exchangeable bases were extracted by saturating soil with neutral 1M NH4OAc (Thomas, 1982) and the adsorbed NH4+ displaced with K+ using 1M KCl and then determined by Kjeldahl distillation method for the estimation of CEC of soil. The bases Ca++, Mg++, Na+, and K+, displaced by NH4+ were measured by atomic absorption spectrophotometer. Organic carbon was determined by wet oxidation method of Walkley and Black (Nelson and Sommers, 1982) and converted to organic matter. Total N was determined with the Kjeldahl method (Bremner and Mulvaney, 1982).

3.3 Seed Source for Rice Varieties

Three rice cultivars were used in this study. A susceptible locally grown variety, Supa collected from Sokoine University of Agriculture seed storage facility; a resistant variety developed from SUA, SSD 35 (Mwangaza) and a moderately resistant variety, Salama M 30 collected from SUA seed storage facility.

3.4 Experimental Design

The experimental design was a split-split plot in randomized complete block (RCBD) arrangement with 3 replications. The cultivars served as factor A, types of nitrogen fertilizers as factor B and amount of nitrogen fertilizer as factor C. The ANOVA model for this design is given by:

$$yijkl = \mu+\rho l+\alpha i+(wp)il \quad +\beta j+(\alpha\beta)ij+(sp)ijl+ \quad \gamma k+(\alpha\gamma)ik+(\beta\gamma)jk+(\alpha\beta\gamma)ijk+(ssp)ijkl,$$

(Main-plot portion) (Split-plot portion) (Split-split-plot portion)

(i=1,...,a j=1,...,b k=1,...,c l=1,...,r)

Where:-

$(wp)il \sim N(0,\sigma 2wp),(sp)ijl\sim N(0,\sigma 2sp),(ssp)ijkl\sim N(0,\sigma 2ssp)$, and all random effects are independent.

μ is the grand mean, α_i is the additive main effect of level i from the first factor (i-th row in the contigency table), $^{\beta}j$ is the additive main effect of level j from the second factor (j-th column in the contigency table) and γij is the non-additive interaction effect of treatment (i, j) from both factors (cell at row i and column j in the contigency table).

3.5 Land Preparation, Planting and Fertilizer Application

The experimental plots were planted on 28 July 2014. The entire land was ploughed and harrowed by a tractor before layout. The size of each treatment sub-sub plot was 5 m^2. After layout each plot was raised up by hand hoe to create sunken pads. Two seeds of each cultivar were directly planted in holes at a spacing of 20 x 20 cm from row to row and plant to plant. Two seedlings were left per hill. The excessive seedlings per hill were thinned.

Four Nitrogen fertilizers namely SA (21% N, 24% S), NPK (20.10.12), DAP (18% N, 46% P,) and Urea (46% N) were applied to each rice cultivar. All fertilizers were applied at fourth leaf stage (initial tillering) by top dressing. Control plots received no fertilizer treatment. Each fertilizer type was applied in two different amounts, one as a recommended/optimum amount (100kg N/ha) and the other at a higher amount of 120kg

N/ha. The equivalent amounts applied in each plot varied with the type of fertilizer since each fertilizer has a different percentage of Nitrogen. For NPK the equivalent of 100kgN/ha was 250g per plot and for 120kgN/ha was 300g per plot. For DAP it was 277.8g and 333.3g, for Urea it was 108.7g and 130.4g, and for SA it was 238.1g and 285.7g respectively based on soil analysis of the study area.

All fertilizers were applied as a single dose except for additional plots in which each amount of Urea (120 kg N/ha and 100 kg N/ha) was applied in two equal splits to check for the effect of ammonia volatilization which is prominent when Urea is applied under flooded conditions. Additional Potassium and/or Phosphorus were added to plots that were not treated with NPK in order to ensure a balanced supply of these elements in the whole experimental area. Amount of 108.9g and 130.4g of P and 416.7g and 500g of K were added in those plots that received SA or Urea at 100 and 120kgN/ha, respectively. In addition, 416.7g and 500g of K were added to plots that received DAP at 100 and 120kgN/ha, respectively. Also, 138.9g and 166.7g of P were added to plots that received 100 and 120kgN/ha, respectively. All additions of P and K were done during planting by incorporating the fertilizers in the soil.

3.6 Crop Management Practices

Plots received identical cultural treatments in terms of ploughing, cultivation, seed rate, sowing method, and weed control. Chemical herbicide (Roundup) was applied at the rate of 120mls per 20 liters of water to control weeds during the course of crop growth along with hand weeding.

3.7 Virus Isolation and Inoculation Method

RYMV did not occur naturally at the experimental site. Artificial inoculation of the virus isolate was done to ensure high level of disease. Isolates of S6 RYMV strain were collected from rice fields in Kilombero valley, Morogoro. The isolates were first propagated in the susceptible rice variety, *Kihogo* by mechanical inoculation of 21 days old plants in the screen house. Four weeks after inoculation, leaves from each RYMV isolate with typical symptoms of RYMV disease were harvested and ground with 0.01M phosphate buffer pH 7.0 at the ratio of 1:10 (w/v). This method was used to standardize the inoculum of the isolates. The resulting homogenate was filtered through cheesecloth. Carborundum powder (600 mesh) was added to the inoculum to aid the penetration of the virus into leaf tissues. Plants were inoculated four weeks after planting (i.e. one week after fertilizer application) by rubbing the youngest emerging leaves with prepared inoculum.

3.8 Data Collection

3.8.1 Disease assessment

Disease was assessed from all leaves in 10 randomly selected plants in each plot. Each leaf was scored on a weekly basis for four weeks starting from two weeks after inoculation according to a standard evaluation system of 1-9 described by Sorho *et al.* (2005):

1= no visible symptoms,

3= green leaves with sparse dots of streaks and < 5% of height reduction,

5= green leaves or pale green leaf with mottling and 6-25% height reduction; flowering slightly delayed,

7= leaves pale yellow to yellow, with 26-75% height reduction; flowering delayed, 9= leaves turn yellow or orange, >75% plant height reduction; no flowering or some plants dead.

The average disease scores were used to calculate disease severity percent and then AUDPCs for analysis. The AUDPCs were calculated as described by (Madden *et al.,* 2007) as follows:-

$A(t_k)$, the AUDPC at $t = t_k$, is the total accumulated disease until $t = t_k$, given by.

$$A_k = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$$

Whereas: Ak= AUDPC at t= tk

Yi– disease severity at the ith observation

Xi– time (weeks) at the ith observation

n– Total number of observations

3.8.2 Assessment of yield and yield components

Plant height, grain yield, and yield components (number of panicles per square meter, number of grains per panicle, and grain weight per plant (measured from 1000 grains dried to 13% moisture content) were determined for each cultivar in treatment. Mean values of grain yield (ton/ha), plant height, number of panicles per m^2, number of grains per panicle and grain weight per plant were compared for fertilized and non-fertilized of inoculated plants of each cultivar as well as among the different types of fertilizers.

3.9 Data Analysis

The data on AUDPCs, plant height, yield and yield components, were subjected to analysis of variance (ANOVA) using GenStat software (GenStat, 2010) 15th edition, where significant treatments were detected from the ANOVA. Treatment means were separated using Fisher’s Protected as well as Duncan’s Multiple Range Test (DMRT) at the 5% level.

CHAPTER FOUR

4.0 RESULTS

4.1 Soil Analysis of the Experimental Area

The soil analysis of the experimental area revealed that the soil had 1.2% organic carbon, 0.09 mg/kg available nitrogen, 48.48 mg/kg available phosphorus, 0.72 mg/kg available potassium and zn1.84 mg/kg, and 36.25 mg/kg sulphate sulphur with 7.5 pH (Table 1).

Table 2: Some physical and chemical properties of the soil in the Experimental site

Characteristics	Value	Rating
Physical characteristics		
Soil depth, cm	0-25	
Clay (%)	24.1	
Silt (%)	6.9	
Sand (%)	69	
Texture		Sandy clay
Chemical characteristics		
pH, (in H_2O)	7.5	High
Org. carbon, %	1.3	Low
Total N, %	0.1	Low
C/N ratio	14.2	medium
Sulphate Sulphur, mg/kg	36.3	
Avail. P, mg/kg	48.5	Very low
CEC, cmol/kg	7.7	Low
Ca2+, cmol/kg	4.3	Medium
Mg2+, cmol/kg	2.1	Medium
K+, cmol/kg	0.7	High
Na+, cmol/kg	0.4	
Acidity (H+), cmol/kg	0.2	
Zn, mg/kg	1.8	Normal

4.2 Effect of Variety on RYMV Development

The main effect of variety on RYMV development was statistically significant ($p \leq 0.05$) (Table 3). RYMV development varied among varieties in all assessment dates. Across fertilizer treatments, Supa variety had the highest mean AUDPC (100.6) followed by Salama (51.7) and Mwangaza (30.8). Untreated plots had higher disease level for Supa

(108.8) and Salama (59.2) varieties compared with fertilized plots. The level of disease was the same (30.8) for Mwangaza variety in control and fertilizer treatments.

Table 3: Main Effect of Variety on RYMV Development

	Mean AUDPCs				
Variety	35DAP	42DAP	49DAP	56DAP	Mean
Salama	13.7^b	28.6^b	59.4^b	105.2^b	51.7^b
Supa	24.2^c	52.7^c	125.7^c	199.9^c	100.6^c
Mwangaza	11.6^a	24.1^a	37.2^a	50.4^a	30.8^a
Mean	16.5	35.1	74.1	118.5	61.1
$LSD_{(0.05)}$	0.8	0.9	1.0	0.7	0.9
CV%	3.1	1.5	0.9	0.3	1.5

Means in columns followed by the same letter (s) are not significantly different at P< 0.05 according to DMRT; LSD= Least Significant Difference; CV= Coefficient of Variation; DAP= Days after planting.

4.3 Effects of Type and Amount of Nitrogen Fertilizers on RYMV Development

Results showed that the type of Nitrogen fertilizers had significant influence on the development of RYMV (Table 4). Generally, untreated checks had the highest mean AUDPC (65.9). Among fertilizers treated plots, lowest mean AUDPC (59.8) was recorded in plots treated with DAP fertilizer which were not significantly different from NPK (60.1), SA (60.0) and urea split (59.9) treatments. Highest mean AUDPC (62.9) was recorded in plots treated with single dose urea application.

Disease development between the two amounts of nitrogen fertilizers (100 and 120kgN/ha) did not differ significantly as plots receiving 120kgN/ha and those receiving 100kgN/ha resulted into the same mean AUDPC (60.5).

Table 4: Main Effect of Type and Amount of Nitrogen Fertilizers on RYMV Development

		Mean AUDPCs				
Fertilizer	Rate	35DAP	42DAP	49DAP	56DAP	Mean
Control	0kg/ha	19.2^{d}	39.0d	79.7d	125.8c	65.9c
Urea single dose	100kg/ha	17.5^{c}	36.7^{c}	76.3^{c}	121.3^{b}	62.9^{b}
	120kg/ha	17.5^{c}	36.7^{c}	76.3^{c}	121.3^{b}	62.9^{b}
NPK	100kg/ha	16.0^{ab}	34.4^{b}	73.0^{ab}	116.9^{a}	60.1^{a}
	120kg/ha	16.0^{ab}	34.4^{b}	73.0^{ab}	116.9^{a}	60.1^{a}
DAP	100kg/ha	15.6^{a}	33.8^{a}	73.2^{a}	116.7^{a}	59.8^{a}
	120kg/ha	15.6^{a}	33.8^{a}	73.2^{a}	116.7^{a}	59.8^{a}
SA	100kg/ha	16.1^{b}	34.5^{b}	72.6^{a}	116.8^{a}	60.0^{a}
	120kg/ha	16.1^{b}	34.5^{b}	72.6^{a}	116.8^{a}	60.0^{a}
Urea split	100kg/ha	15.9^{ab}	34.2^{ab}	72.7^{b}	117.1^{a}	59.9^{a}
	120kg/ha	15.9^{ab}	34.2^{ab}	72.7^{b}	117.1^{a}	59.9^{a}
Mean		16.5	35.1	74.1	118.5	61.1
LSD$_{(0.05)}$		0.8	0.9	1.0	0.7	0.9
CV%		3.1	1.5	0.9	0.3	1.5

Means in columns followed by the same letter (s) are not significantly different at P< 0.05 according to DMRT; LSD= Least Significant Difference; CV= Coefficient of Variation; DAP= Days after planting.

4.4 Effect of Variety x Nitrogen Fertilizers Interaction on RYMV Disease Development

All three interactions between variety, nitrogen fertilizer type and nitrogen amount had significant effect on the development of RYMV disease ($p \leq 0.05$) (Table 5). Variety x nitrogen fertilizers interactions resulted into lower disease intensity compared to untreated checks. Mean AUDPCs significantly varied among varieties for all types of fertilizers (single dose urea, NPK, DAP, SA, and split urea). The application of any type of nitrogen fertilizer resulted into lowest mean AUDPCs for Mwangaza variety compared to other varieties. Highest mean AUDPCs were observed for Supa variety under the same treatments.

Table 5: Main Effect of Variety x Fertilizers Interaction on RYMV Development

			Mean AUDPCs				
Variety	Fertilizer	Rate	35DAP	42DAP	49DAP	56DAP	Mean
Salama	control		16.5^{d}	34^{e}	68.8^{e}	117.4^{d}	59.2^{d}
	Urea 1	100kg/ha	14.5^{c}	30.0^{d}	62.2^{d}	108.7^{c}	53.9^{c}
		120kg/ha	14.5^{c}	30.0^{d}	62.2^{d}	108.7^{c}	53.9^{c}
	NPK	100kg/ha	13.5^{b}	28.0^{c}	57.5^{bc}	103.0^{b}	50.5^{b}
		120kg/ha	13.5^{b}	28.0^{c}	57.5^{bc}	103.0^{b}	50.5^{b}
	DAP	100kg/ha	12.8^{b}	27.5^{bc}	58.8^{b}	102.7^{b}	50.4^{b}
		120kg/ha	12.8^{b}	27.5^{bc}	58.8^{b}	102.7^{b}	50.4^{b}
	SA	100kg/ha	13.3^{b}	27.6^{bc}	56.9^{bc}	102.8^{b}	50.2^{b}
		120kg/ha	13.3^{b}	27.6^{bc}	56.9^{bc}	102.8^{b}	50.2^{b}
	Urea split	100kg/ha	12.8^{b}	27.1^{b}	56.6^{c}	102.6^{b}	49.8^{b}
		120kg/ha	12.8^{b}	27.1^{b}	56.6^{c}	102.6^{b}	49.8^{b}
Supa	control		29.5^{h}	59^{j}	134.5^{i}	212.0^{h}	108.8^{h}
	Urea 1	100kg/ha	26.5^{g}	56.0^{i}	129.5^{h}	205.0^{g}	104.3^{g}
		120kg/ha	26.5^{g}	56.0^{i}	129.5^{h}	205.0^{g}	104.3^{g}
	NPK	100kg/ha	22.9^{ef}	51.2^{gh}	124.2^{fg}	197.3^{ef}	98.9^{ef}
		120kg/ha	22.9^{ef}	51.2^{gh}	124.2^{fg}	197.3^{ef}	98.9^{ef}
	DAP	100kg/ha	22.5^{e}	50.0^{f}	123.5^{f}	197.0^{e}	98.3^{e}
		120kg/ha	22.5^{e}	50.0^{f}	123.5^{f}	197.0^{e}	98.3^{e}
	SA	100kg/ha	23.5^{f}	52.0^{h}	123.7^{fg}	197.4^{ef}	99.2^{ef}
		120kg/ha	23.5^{f}	52.0^{h}	123.7^{fg}	197.4^{ef}	99.2^{ef}
	Urea split	100kg/ha	23.0^{ef}	51.0^{g}	124.2^{g}	198.0^{f}	99.1^{f}
		120kg/ha	23.0^{ef}	51.0^{g}	124.2^{g}	198.0^{f}	99.1^{f}
Mwangaza	control		11.5^{a}	24^{a}	37.2^{a}	50.3^{a}	30.8a
	Urea 1	100kg/ha	11.5^{a}	24.0^{a}	37.2^{a}	50.3^{a}	30.8^{a}
		120kg/ha	11.5^{a}	24.0^{a}	37.2^{a}	50.3^{a}	30.8^{a}
	NPK	100kg/ha	11.5^{a}	24.0^{a}	37.2^{a}	50.3^{a}	30.8^{a}
		120kg/ha	11.5^{a}	24.0^{a}	37.2^{a}	50.3^{a}	30.8^{a}
	DAP	100kg/ha	11.5^{a}	24.0^{a}	37.2^{a}	50.3^{a}	30.8_{a}
		120kg/ha	11.5^{a}	24.0^{a}	37.2^{a}	50.3^{a}	30.8^{a}
	SA	100kg/ha	11.5^{a}	24.0^{a}	37.2^{a}	50.3^{a}	30.8^{a}
		120kg/ha	11.5^{a}	24.0^{a}	37.2^{a}	50.3^{a}	30.8^{a}
	Urea split	100kg/ha	11.8^{a}	24.3^{a}	37.5^{a}	50.7^{a}	30.1^{a}
		120kg/ha	11.8^{a}	24.3^{a}	37.5^{a}	50.7^{a}	30.1^{a}
Mean			16.5	35.1	74.1	118.5	61.1
LSD$_{(0.05)}$			0.8	0.9	1.03	0.7	0.9
CV%			3.1	1.5	0.9	0.3	1.5

Means in columns followed by the same letter (s) are not significantly different at P< 0.05 according to DMRT; LSD= Least Significant Difference; CV= Coefficient of Variation; DAP= Days after planting.

The interaction between variety and nitrogen fertilizer amount had significant effect on the development of RYMV (Table 5). The application of either 100kgN/ha or 120kgN/ha resulted into lowest mean AUDPCs for Mwangaza variety compared to other varieties.

Highest mean AUDPCs were observed in Supa variety under the same treatments. Mean AUDPCs were lowest in Mwangaza variety for any of the types of nitrogen fertilizers applied at both nitrogen amounts and highest in Supa variety under the same treatments.

4.5 Effects of Variety on Plant Height, Yield and Yield Components

Variety had significant effects on plant height, yield and yield components ($p \leq 0.05$) (Table 6). Plant height is not a yield component especially in grain crops, but it indicates the influence of various nutrients on plant metabolism. Nitrogen fertilizer treated plots resulted into higher plant height, yield and yield components compared to untreated checks for all varieties. Mwangaza variety had highest plant height, 110.5 cm compared to Salama (100.7 cm) and Supa (93.1 cm). Mwangaza variety had the highest grain yield (3.9ton/ha) while Supa had the lowest yield (2.1ton/ha). All yield components (number of panicles/m^2, number of grains/panicle and grain weight) were significantly affected by variety ($p \leq 0.05$). Across fertilizer treatments, Mwangaza had the highest number of panicles/m^2 (166.9) followed by Salama (139.9) and Supa (131.8). Mwangaza variety had the highest number of grains per panicle (81.9) followed by Salama (66.9) and Supa (53.1). Mwangaza variety had the highest grain weight (34.2g) compared to Salama (30.5g) and Supa (25.9g).

Table 6: Main Effect of Variety on Plant Height, Yield and Yield components

Variety	Means				
	Plant height (cm)	Yield (Ton/ha)	No. of panicles/m^2	No. of grains/panicle	Grain wt. (gm)
Salama	100.7^{b}	2.8^{b}	139.9^{b}	66.9^{b}	30.5^{b}
Supa	93.1^{a}	2.1^{a}	131.8^{a}	53.1^{a}	25.9^{a}
Mwangaza	110.1^{c}	3.0^{c}	166.9^{c}	81.9^{c}	34.2^{c}
Mean	101.3	3.1	146.2	67.3	30.2
LSD$_{(0.05)}$	3.1	0.2	2.58	1.9	1.3
CV%	1.9	3.5	1.1	1.7	2.6

Means in columns followed by the same letter (s) are not significantly different at $P < 0.05$ according to DMRT; LSD= Least Significant Difference; CV= Coefficient of Variation.

4.6 Effects of Type and Amount of Nitrogen Fertilizers on Plant Height, Grain yield and yield components

Type of nitrogen fertilizers had significant influence on plant height, grain yield and yield components of rice ($p \leq 0.05$) (Table 7). The amount of nitrogen fertilizer significantly affected only grain yield and number of grains/panicle. All fertilizer treated plots had significantly taller plants compared with the untreated check (70.9cm). Across varieties and nitrogen amounts, SA gave the tallest plants (108.8 cm), which did not differ significantly from NPK (107.5 cm), DAP (107.2cm), and split Urea (106.8cm). Treatments that received single dose of Urea had lowest plant height (91.7cm). Plant height was higher (105cm) in those plots treated with 120kgN/ha compared with those treated with 100kgN/ha (103.4cm) although not significantly different.

Generally, all fertilizers treated plots had significantly higher grain yield compared with the untreated check (1.2ton/ha). Among fertilizer treatments, highest grain yield (3.6ton/ha) was recorded with NPK and split applied urea which did not differ significantly from treatments that received DAP (3.5ton/ha) and SA (3.5ton/ha). However, the lowest grain yield was recorded in those treatments that received single dose urea (2.8 ton/ha). Plots treated with 120 kg N/ha had significantly higher grain yield (3.4ton/ha) compared with those treated with 100 kg N/ha (3.2ton/ha).

Table 7: Main Effect of Fertilizers on Plant Height, Yield and Yield Components

		Means				
Fertilizer	Rate	Plant height (cm)	Yield (Ton/ha)	No. of pani-cles/m2	No. of grains/panicle	Grain wt. (g)
Control	0kg/ha	70.9^{a}	1.2^{a}	113.7^{a}	52.0^{a}	20.3^{a}
Urea 1	100kg/ha	90.7^{b}	2.6^{b}	137.9^{b}	64.1^{b}	28.2^{b}
	120kg/ha	92.7^{c}	2.9^{c}	139.3^{b}	66.6^{c}	29.1^{c}
NPK	100kg/ha	106.6^{def}	3.5^{f}	153.4^{e}	70.8^{fg}	32.0^{ef}
	120kg/ha	108.3^{gh}	3.7^{g}	155.8^{f}	72.2^{h}	33.0^{g}
DAP	100kg/ha	106.2^{de}	3.4^{e}	151.2^{d}	69.1^{d}	31.8^{de}
	120kg/ha	108.1^{fgh}	3.6^{g}	152.9^{e}	71.2^{gh}	32.6^{fg}
SA	100kg/ha	107.7^{efg}	3.3^{d}	150.0^{c}	69.0^{c}	31.7^{c}
	120kg/ha	109.8^{h}	3.6^{e}	151.0^{de}	71.1^{ef}	32.5^{d}
Urea split	100kg/ha	105.8^{d}	3.5^{ef}	151.6^{d}	69.3^{de}	32.1^{ef}
	120kg/ha	107.7^{efg}	3.7^{g}	153.2^{e}	71.7^{gh}	32.9^{g}
Mean		101.3	3.1	146.2	67.3	30.2
LSD$_{(0.05)}$		3.1	0.2	2.6	1.9	1.3
CV%		1.9	3.5	1.1	1.7	2.6

Means in columns followed by the same letter (s) are not significantly different at $P< 0.05$ according to DMRT; LSD= Least Significant Difference; CV= Coefficient of Variation.

All fertilizers treated plots had significantly higher number of panicles/m^2 compared with untreated check (113.7). Among fertilizers treatments, plots treated with NPK had the highest number of panicles/m^2 (154.6) but did not differ significantly from those with DAP (152.1), urea splits (152.4) and SA (150). However, lowest number of panicles/m^2 was recorded with single dose urea (138.6). Number of panicles/m^2 recorded from 120kgN/ha (150.4) was not significantly different from those observed in plots that received 100kgN/ha (148.5).

Mean number of grains/panicle was significantly higher with nitrogen fertilizers treated plots compared with untreated check (52.0) for all types of fertilizers ($p \leq 0.05$). Among varieties and nitrogen amounts, the highest number of grains/panicle (71.5) was recorded with NPK, which did not differ significantly from DAP (70.2), urea in split application (70.5) and SA (70.1). However, the lowest number of grains/panicle was recorded with single dose urea (65.4). Plots treated with 120kgN/ha gave significantly higher number of grains/panicle (70.5) compared with those treated with 100kgN/ha (67.9).

Among the nitrogen fertilizers treatments, the highest grain weight per plant (32.5g) was recorded with NPK and urea split application which were not significantly different from DAP (32.2g) and SA (32.1g). However, lowest grain weight (28.7g) was recorded with Single dose urea. Grain weight was numerically higher (31.7g) in plots treated with 120kgN/ha but this was not significantly different from plots treated with 100kgN/ha (30.6g).

4.7 Effect of Variety x Nitrogen Fertilizers Interaction on Plant Height, Yield and Yield Components

All three interactions among variety, fertilizer type and nitrogen had significant effect on plant height, yield and yield components of rice ($p \leq 0.05$) (Table 8). Nitrogen fertilizer types resulted into higher plant height, yield and yield components compared to untreated checks for all varieties. The application of Urea single dose, NPK, DAP, or Urea split resulted into highest plant height for Mwangaza variety compared to other varieties and lowest for Supa variety. On the contrary, SA fertilizer treatment did not result into significant different plant height between Mwangaza (116 cm) and Salama (113.2 cm) varieties but shorter plants (97.9 cm) was recorded in variety Supa.

Table 8: Effect of Variety x Fertilizer Interaction on Plant Height, Yield and Yield Components

			Means				
Variety	Fertilizer	Rate	Plant height (cm)	Yield (Ton/ha)	No. of panicles/m^{2}	No. of grains/panicle	Grain wt. (gm)
Salama	control		67.0^{b}	1.3^{a}	100.3^{a}	49^{a}	19^{a}
	Urea 1	100	81.0^{c}	3.3^{b}	141.7^{c}	61.0^{b}	28.0^{b}
		120	83.0^{c}	3.5^{b}	143.0^{c}	64.7^{cd}	29.0^{bc}
	NPK	100	106.7^{l}	4.1^{g}	144.7^{k}	71.0^{ef}	33.7^{ef}
		120	108.7^{lm}	4.2^{h}	145.7^{k}	73.0^{g}	34.0^{gh}
	DAP	100	107.7^{lm}	3.9^{d}	144.0^{de}	70.0^{cd}	32.0^{d}
		120	109.7^{lm}	4.0^{ef}	144.7^{ef}	72.7^{ef}	33.0^{de}
	SA	100	111.7^{no}	3.4^{c}	142.0^{d}	64.0^{c}	30.0^{c}
		120	114.7^{pq}	3.7^{de}	144.0^{de}	67.0^{e}	31.0^{d}
	Urea split	100	108.0^{lm}	3.9^{d}	144.3^{de}	70.3^{d}	32.3^{de}
		120	110.0^{mn}	4.1^{fg}	145.0^{ef}	73.0^{fg}	33.3^{de}
Supa	control		51.7^{a}	0.6^{c}	94.3^{j}	38^{b}	18^{bc}
	Urea 1	100	90.0^{d}	1.3^{ij}	112.7^{l}	49.7^{h}	23.7^{jk}
		120	92.0^{de}	1.5^{j}	114.3^{l}	52.3^{i}	24.3^{lm}
	NPK	100	99.7^{hi}	2.4^{no}	140.5^{m}	57.7^{lm}	28.3^{lm}
		120	101.0^{jk}	2.7^{r}	143.7^{n}	59.7^{n}	30.0^{mn}
	DAP	100	99.0^{gh}	2.0^{qr}	138.7^{no}	53.7^{kl}	27.3^{no}
		120	100.7^{ij}	2.2^{s}	140.7^{p}	57.7^{mn}	27.7^{op}
	SA	100	96.3^{fg}	1.6^{mn}	132.7^{no}	51.8^{ij}	23.3^{lm}
		120	97.7^{gh}	1.9^{qr}	135.3^{op}	55.6^{lm}	26.7^{mn}
	Urea split	100	97.0^{fg}	2.1^{r}	139.0^{no}	54.0^{kl}	27.7^{op}
		120	98.7^{gh}	2.3^{s}	141.0^{pb}	58.0^{n}	28.0^{p}
Mwangaza	control		94.0^{ef}	1.7^{b}	146.3^{b}	69^{jk}	24^{a}
	Urea 1	100	101.0^{jk}	3.4^{i}	159.3^{fg}	80.7^{op}	33.0^{de}
		120	103.0^{k}	3.7^{j}	160.7^{fg}	82.3^{q}	34.0^{fg}
	NPK	100	113.3^{pq}	4.0^{op}	166.0^{ij}	83.7^{pq}	34.0^{l}
		120	115.3^{rs}	4.3^{pq}	170.0^{j}	84.0^{q}	35.0^{lm}
	DAP	100	112.0^{op}	4.3^{lm}	170.0^{gh}	83.7^{pq}	36.0^{ij}
		120	114.0^{pq}	4.7^{mn}	173.3^{ij}	83.3^{pq}	37.0^{jk}
	SA	100	115.0^{pq}	3.5^{j}	171.0^{fg}	82.0^{o}	34.0^{gh}
		120	117.0^{s}	3.9^{kl}	173.3^{gh}	84.0^{pq}	35.0^{hi}
	Urea split	100	112.3^{op}	4.4^{mn}	171.3^{hi}	83.7^{pq}	36.3^{jk}
		120	114.3^{pq}	4.7^{no}	173.7^{ij}	84.0^{q}	37.3^{kl}
Mean			101.3	3.1	146.2	67.3	30.2
LSD$_{(0.05)}$			3.1	0.2	2.6	1.9	1.3
CV%			1.9	3.5	1.1	1.7	2.6

Means in columns followed by the same letter (s) are not significantly different at $P < 0.05$ according to DMRT; LSD= Least Significant Difference; CV= Coefficient of Variation.

The application of urea as single dose, DAP and Urea in splits resulted into highest grain yield in variety Mwangaza and lowest grain yield in variety Supa. In plots treated with NPK or SA fertilizers, there was no significant difference in grain yield between Mwangaza and Salama but low grain yield was recorded in variety Supa. For all types of nitrogen fertilizers variety Mwangaza had the highest number of panicles/m^2 and grains/panicle compared to other varieties. Lowest number of panicles/m^2 and grains/panicle were recorded in variety Supa. The application of Urea in single dose, DAP, SA and Urea in split resulted into highest grain weight in variety Mwangaza compared with the other varieties. Lowest grain weight was recorded in variety Supa. However, NPK fertilizer did not result into significant difference in grain weight between Mwangaza and Salama though Supa had lower grain weight.

Nitrogen fertilizers applied at the rate of 100kgN/ha and 120kgN/ha both resulted into tallest plants, greatest grain yield and yield components in variety Mwangaza and lowest in variety Supa.

CHAPTER FIVE

5.0 DISCUSSION

Several studies have shown that fertilizers can reduce development of plant diseases hence providing less expensive means of managing crop diseases (Ahmad *et al.*, 2008; Lacrois *et al.*, 2014). Viral disease symptoms, the infective potential of viral particles and the susceptibility of the host to virus have shown to be influenced by the nutrition status of the host plant (Datnoff *et al.,* 2006). In the current study, the effect of nitrogen fertilizers on the development of Rice Yellow Mottle Virus (RYMV) and yield response of rice was investigated.

5.1 Effects of Variety on RYMV Development

The observed differences in RYMV disease development among varieties in this study were due to differences in inherent resistance traits of the genotypes. Mwangaza, a highly resistant variety to RYMV (Luzi-Kihupi and Zakayo, 2001), had the lowest disease severity levels followed by Salama a moderately resistant and Supa a highly susceptible variety. The results suggest that nitrogen fertilizers did not have impact on the inherent resistance of the genotypes. Similar results were reported by Rua *et al*. (2013). Reduction of disease levels observed in N fertilized Supa and Salama compared to untreated checks suggests that N fertilizers application reduced susceptibility of the two varieties to RYMV disease. Spann and Schumann (2010) observed that as a rule, plants with an optimal nutritional status have the highest resistance (tolerance) to insect pests and diseases. Susceptibility increases as nutrient concentrations deviate from this optimum. Direct influence of N nutrition on viral multiplication in plants has also been reported by Pennazio and Roggero (1997).

5.2 Effects of Type and Amount of Nitrogen Fertilizers on RYMV Development

Nitrogen fertilizers reduced RYMV disease on susceptible and moderately resistant varieties. The fact that Nitrogen is the most important nutrient for plant growth and disease development (Datnoff *et al.*, 2006) provides less expensive means of crop disease management. Adequate N nutrition is generally required to maintain a high level of disease resistance (Abro, 2014). Johnson, (2007) reported that potatoes deficient in either N or P were more susceptible to early blight (*Alternaria solani*). Nitrogen fertilizers have also been reported to reduce the incidence of "take-all” (*Gaeumannomyces graminis*) in barley and wheat (Kwak and Weller, 2013).

One of the mechanisms by which N influences plant viruses is through altering the physiology of host plant through its effect on the synthesis of various metabolites principally amino acids and proteins, which are directly linked to the synthesis of molecules involved in plant defense mechanisms: plant hormones mainly cytokinins and auxins (Sakakibara *et al.*, 2006) and Proline-rich proteins (PRP) and/or phytoalexins (Huber and Thompson 2007).

In this experiment, urea in splits, DAP, NPK and SA fertilizers application ensured optimum availability of N to plants hence reduced RYMV disease levels in susceptible and moderately resistant varieties. Kurschner *et al*. (1992) reported that split applications of nitrogen reduced excessive vegetative growth early in the season and reduced the severity of rice blast. Similar mechanisms could explain reduced RYMV following application of N fertilizers observed in this study.

On the other hand, urea applied in single dose had the highest disease intensity than the rest of fertilizers. This could probably be due to loss of nitrogen in the form of vapour (volatilization) which could have resulted into less than optimum nitrogen nutrients available to plants in a single dose treatment considering the fact that the study was conducted under lowland flooded conditions. Ammonia volatilization losses occur in flooded rice soils in moderately to slightly acid soils, although losses are higher in alkaline soils as reported by Choudhury and Kennedy (2005). VanGessel (1993) reported similar findings in which maize that were nitrogen-stressed had the highest Maize Dwarf Mosaic Virus (MDMV-A) titer compared to maize supplied with sufficient nitrogen. Graham and Webb (1991) reported that Take-all and Tan spot of wheat and Stalk rot of corn decreased in severity as N levels increased from deficiency to physiological sufficiency.

The form of nitrogen has also been shown to influence other diseases in plants. Johnson, (2007) reported that nitrate N can moderate the severity of black scurf (*Rhizoctonia solani*) in potatoes, root rots of beans and peas, and foot rot in wheat while ammonium N aggravates these diseases. However, soil-borne pathogens such as potato scab (*Streptomyces scabies*) and early dying of potatoes are aggravated by NO_3-N and moderated by NH_4-N (Engelhard, 1993).

The non-significant difference in disease development between the two amounts of Nitrogen (120kg/ha and 100kg/ha) is contrary to other studies on the effect of nitrogen amount on the development of viral and fungal diseases in which the increased amount of nitrogen resulted in increased disease symptoms (Singh, 1970; Singh and Singh, 1977). However, a possible explanation to this is that despite the rapid multiplication of the virus, visible symptoms of the infection do not necessarily correspond to an increase in mineral nutrient supply to the host plant (Spann and Schumann 2010). Symptoms of virus infections sometimes disappear when N supplies are large, even though the entire plant is infected (Spann and Schumann 2010). Visible symptoms are dependent upon the competition for N between the virus and the host cells (Spann and Schumann 2010). This competition varies with different diseases and can be influenced by environmental factors, such as temperature (Spann and Schumann 2010).

Marschner (1995) reported that in some situations, symptoms of viral diseases can be made to lessen or disappear with improved nutrition. However, this disappearance does not mean that the virus is not present. For example, sugar beets with beet mild yellowing virus (BMYV) exhibited symptoms similar to Mn deficiency. When beets with both Mn deficiency and BMYV infection were foliar fertilized with Mn, the visual symptoms of both problems lessened or even disappeared completely. However, working with this combined problem they found that the percent of infected plants decreased from 75% in the Mn deficient plants to 40% infection after the Mn deficiency was corrected. Despite this being a large decrease, there remained a significant infection in the field (Marschner, 1995).

5.3 Effect of Variety x Fertilizer Interactions on RYMV Disease Development

The results of this study have shown that the effect of the type and/amount of nitrogen fertilizer on the development of RYMV disease depended on the genotype suggesting strong interactions between the two. These findings corroborate reports by Ahmad *et al.* (2008) in which interaction between variety and nitrogen fertilizer was found to be significant for Cotton leaf Curly Virus (CLCuV) infestation. This implies that the influence of type and amount of nitrogen fertilizers on RYMV disease development depends on the susceptibility/resistance of the variety. The least susceptible variety had lowest disease level under the influence of any type and amount of nitrogen fertilizer and vice versa. It is apparent therefore that farmers who combine resistant varieties and better choice of N fertilizer have advantage of having low level of RYMV in the field.

5.4 Effects of Variety on Plant Height, Yield and Yield Components of Rice

Comparisons between untreated checks and fertilized treatments suggest that N fertilizers reduced level of disease in susceptible varieties which led to higher grain yield and yield components. However, the effect of inherent resistance/susceptibility to RYMV infection led to different level of disease and hence different plant height, grain yield, number of panicles/m^2, number of grains/panicle and grain weight among varieties. The genotype that was most resistant to RYMV disease also resulted into highest yield and yield components and vice versa. Similar results were reported by Juarez *et al*. (2000) in which two varieties with different levels of resistance to Potato Late Blight responded differently under the influence of Nitrogen fertilizations. Mwangaza is naturally tall, high yielding and resistant to RYMV hence its height and yield were not affected much by the disease while the height and yield of variety Supa were lower due to its high susceptibility to the RYMV disease even under the influence of N fertilizers. Infection by RYMV can result into stunting of infected plants (Abo *et al.*, 2004). Abo *et al*. (2002) reported that RYMV induces severe crop damage and yield losses to susceptible cultivars.

5.5 Effects of Type and Amount of Nitrogen Fertilizers on Plant Height, Yield and Yield Components of Rice

Nitrogen fertilizers reduced RYMV disease severity which in turn increased plant height, yield and yield components of susceptible rice varieties compared to untreated check. This observation is similar to the report by (Vennila *et al.*, 2007) in which nitrogen application increased the number of grains per panicle, 1000-grain weight and hence the grain yield of rice. Bahmanyar and Ranjbar (2007) also reported that nitrogen fertilizers which are able to provide suitable conditions for producing filled grains can play an important role in increasing grain yield of rice.

Urea applied in splits, DAP, NPK and SA fertilizers ensured enough availability of nitrogen nutrients for defense against RYMV attack and ultimately increased yield of rice compared to urea in single dose. The reduction in yield in plots treated with urea in single dose is attributed to high disease severity observed in this treatment. Tajani *et al*. (1997) reported that the highest yield of rice infected with blast was obtained by application of equal split of urea top dressed between rows at the four critical stages of rice growth (25% at 4 to 5 leaves stage; 25% ten days later; 25% twenty days after 4 to 5 leaves stage and, 25% at panicle initiation stage). This finding is also supported by (Kapoor and Sood, 2000).

The higher paddy yield at 120 kg N/ha application compared to 100 kg N/ha was due to higher number of grains per panicle and 1000 grain weight at this nitrogen rate since the treatment did not increase RYMV symptoms significantly to affect yield potential. Kaushal *et al*. (2010) also reported that the number of filled grain per panicle increased with increase in N amount. The more number of grains per panicle obtained in treatments at higher nitrogen rates was probably due to better nitrogen status of plant during panicle growth period. Studies of plant N content in relation to yield is a possible area for future research. The increase in grain weight at higher nitrogen rates could also be primarily due to increase in chlorophyll content of leaves which led to higher photosynthetic rate and ultimately plenty of photosynthates available during grain development (Reddy, 2000).

5.6 Effect of Variety x Nitrogen Fertilizer Interactions on Plant Height, Yield and Yield Components

The significant variety x fertilizer type x amount interactions in this study suggests that the effect of fertilizer type and/or amount on plant height, yield and yield components depended on the genotype ability to withstand RYMV infestation. Nitrogen fertilizer treatments greatly increased yield in the least susceptible variety (Salama) compared to the more susceptible one (Supa). Ahmad *et al*. (2008) reported that interaction between variety and nitrogen fertilizer was significant for plant height and seed-cotton yield under the influence of Cotton leaf Curly Virus (CLCuV). The significant difference in these variables leads to the conclusion that genotypes and nitrogen fertilizer changed the plant-height and yield.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results from this study have shown that the response of RYMV disease intensity and yield of rice to different types and amount of nitrogen fertilizers provide possible cultural management strategy in the control of RYMV and increase of rice yield for rice growers. The application of urea in two splits, and/or DAP, SA, NPK fertilizers significantly reduced the severity of RYMV disease by 9.1% compared with untreated check. On the other hand, urea applied in single dose reduced the severity of the disease by 4.5% suggesting that urea fertilizer is more superior compared to other N forms in suppressing RYMV. Yield and yield components responses were affected by variety, the type and amount of nitrogen fertilizer as well as their interactions. Treatments that had lowest level of disease intensity also resulted into highest grain yield and yield components. Urea in splits, NPK, SA and DAP fertilizers which had lowest disease level also increased yield by 200% compared to untreated check. Highest disease intensity observed with urea in single dose also resulted into lowest yield and yield components. Specifically, Urea single dose increased yield by 133.3%.

6.2 Recommendations

The results of this study have shown that urea is the best fertilizer for reducing the severity of RYMV and it is recommended that Urea should always be applied in splits rather than single doses in order to explore its full potential of reducing RYMV intensity and maximizing yield of rice. Farmers are advised to prioritize on the use of resistant varieties in RYMV vulnerable areas since the effectiveness of different nitrogen fertilizers as shown in this study depends on the genotype. The highly susceptible varieties are more likely going to be affected by RYMV more compared to the less susceptible ones regardless of which type and amount of nitrogen fertilizers are applied. In addition future research on the effect of nitrogen amount on RYMV disease that include higher amounts of nitrogen should be carried out in order to establish levels that could have high impact on the disease under various rice farming systems. Further research is needed to be carried out in more than one season to confirm the consistence of results. Finally, this work was related to only one isolate of RYMV. Because of the biological diversity of this virus isolates, it proves to be necessary to test a wide range of isolates in various localities.

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APPENDICES

Appendix 1: ANOVA for AUDPC Week 1

Variate: AUDPC_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	47.6566	23.8283	80.22	
REP.*Units* stratum					
VARIETY	2	2484.8687	1242.4343	4182.82	<.001
FERT_TYPE	10	41.1313	4.1131	13.85	<.001
VARIETY.FERT_TYPE	20	24.6869	1.2343	4.16	<.001
Residual	64	19.0101	0.2970		
Total	98	2617.3535			

Appendix 2: ANOVA for AUDPC Week 2

Variate: AUDPC_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	152.7475	76.3737	99.24	
REP.*Units* stratum					
VARIETY	2	14752.2626	7376.1313	9584.74	<.001
FERT_TYPE	10	192.2828	19.2283	24.99	<.001
VARIETY.FERT_TYPE	20	106.6263	5.3313	6.93	<.001
Residual	64	49.2525	0.7696		
Total	98	15253.1717			

Appendix 3: ANOVA for AUDPC Week 3

Variate: AUDPC_3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	251.152	125.576	53.99	
REP.*Units* stratum					
VARIETY	2	140944.788	70472.394	30300.83	<.001
FERT_TYPE	10	442.949	44.295	19.05	<.001
VARIETY.FERT_TYPE	20	238.990	11.949	5.14	<.001
Residual	64	148.848	2.326		
Total	98	142026.727			

Appendix 4: ANOVA for AUDPC Week 4

Variate: AUDPC_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	398.242	199.121	39.77	
REP.*Units* stratum					
VARIETY	2	381568.061	190784.030	38106.29	<.001
FERT_TYPE	10	786.404	78.640	15.71	<.001
VARIETY.FERT_TYPE	20	417.051	20.853	4.16	<.001
Residual	64	320.424	5.007		
Total	98	383490.182			

Appendix 5: ANOVA for Grain yield (kg/ha)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.06109	1.03054	21.61	
REP.*Units* stratum					
VARIETY	2	390.00395	195.00197	4089.69	<.001
FERT_TYPE	8	11.29040	1.41130	29.60	<.001
VARIETY.FERT_TYPE	16	2.46622	0.15414	3.23	<.001
Residual	52	2.47943	0.04768		
Total	80	408.30109			

Appendix 6: ANOVA for Plant Height (cm)

Variate: PLANT_ HEIGHT_ cm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	118.364	59.182	16.45	
REP.*Units* stratum					
VARIETY	2	4818.970	2409.485	669.58	<.001
FERT_TYPE	10	12898.242	1289.824	358.44	<.001
VARIETY.FERT_TYPE	20	2301.030	115.052	31.97	<.001
Residual	64	230.303	3.598		
Total	98	20366.909			

Appendix 7: ANOVA for No. of panicles/m^2

Variate: PANICLES_M2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	12.747	6.374	2.55	
REP.*Units* stratum					
VARIETY	2	22271.596	11135.798	4456.57	<.001
FERT_TYPE	10	13449.111	1344.911	538.24	<.001
VARIETY.FERT_TYPE	20	3099.737	154.987	62.03	<.001
Residual	64	159.919	2.499		
Total	98	38993.111			

Appendix 8: ANOVA for No. of grains/panicle

Variate: GRAINS_PANICLE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	17.152	8.576	6.68	
REP.*Units* stratum					
VARIETY	2	13653.152	6826.576	5316.27	<.001
FERT_TYPE	10	2947.859	294.786	229.57	<.001
VARIETY.FERT_TYPE	20	243.293	12.165	9.47	<.001
Residual	64	82.182	1.284		
Total	98	16943.636			

Appendix 9: ANOVA for Grain weight (gm)

Variate: GRAIN_ WT_ gm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	70.2424	35.1212	58.50	
REP.*Units* stratum					
VARIETY	2	1125.5152	562.7576	937.34	<.001
FERT_TYPE	10	1206.9495	120.6949	201.03	<.001
VARIETY.FERT_TYPE	20	77.5960	3.8798	6.46	<.001
Residual	64	38.4242	0.6004		
Total	98	2518.7273			

Appendix 10: Fertilizers Allocation

Fertilizer	**Rate (Kg/ha)**	**Equivalent Amount per plot**
Urea	100kg/ha	108.7g
	120kg/ha	130.4g
Diammonium Phosphate (DAP)	100kg/ha	277.8g
	120kg/ha	333.3g
NPK	100kg/ha	250g
	120kg/ha	300g
Sulphate of Ammonium (SA)	100kg/ha	238.1g
	120kg/ha	285.7g
Urea Split Application	100kg/ha	54.4g x 2
	120kg/ha	65.2g x 2

Appendix 11: Field Layout