

**SCREENING FARMERS' SAVED RICE SEED FOR DISCOLOURATION AND
INCIDENCE OF SEED-BORNE PATHOGENS**



BY

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REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP
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MOROGORO, TANZANIA.**

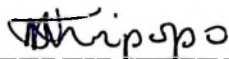
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ABSTRACT

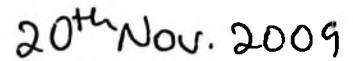
A study was conducted to screen for seed-borne pathogens causing discolouration in farmers' saved rice seeds produced in Shinyanga, Mbeya and Morogoro regions in Tanzania. A total of 37 seed samples were tested for the presence of seed-borne pathogens. The blotter method was used to detect fungal pathogens which were identified based on growth habit and morphological characteristics of the fruiting bodies and spores/conidia. The slide cassette holder method was used to detect bacterial pathogens. The isolated bacteria were characterized based on physiological and biochemical tests, hypersensitive reaction and pathogenicity tests. The data were analyzed using MSTAT-C Computer package and mean separation test using Duncan's Multiple Range Test at $P < 0.05$. The identified seed-borne fungal pathogens associated with seed discolouration were *Fusarium moniliforme*, *Phoma* sp., *Bipolaris oryzae* and *Alternaria padwickii*. All farmers' saved rice seed samples tested were infected. The highest seed infection was caused by *Fusarium moniliforme* (16.75%), (26.0%), (13.5%), *Bipolaris oryzae* (2.0%), (0.5%), (17.25%), *Phoma* sp. (5.0%), (2.25%), (2.25%) for samples from Shinyanga, Mbeya and Morogoro, respectively. The lowest seed infection was caused by *Alternaria padwickii* (0.25%), (0.75%) for samples from Shinyanga and Mbeya, respectively. Seed-borne bacterial pathogens causing grain discolouration were identified. The highest seed infection with *Acidovorax avenae* subsp. *avenae* was from Shinyanga region, *Pseudomonas syringae* subsp. *syringae* from Morogoro region and *Pseudomonas fuscovaginae* infection from samples collected in Morogoro and Mbeya regions. Rice seed from Shinyanga region had high seed infection followed by Morogoro. The results stress the need to encourage farmers to use certified seed which are clean and healthy for planting rather than using their owned saved seeds.

DECLARATION

I, Nuru Grace E. Mwipopo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is the result of my own original work and has not been submitted for a degree award in any other University.

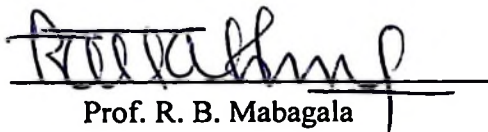


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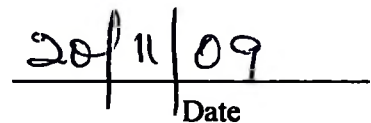


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I deeply thank my beloved husband James Mgale and our children, Yohana, Sarah, Joel, Jeremiah and Sechelela for their patience, endurance and perseverance during the entire period of my studies, may God bless you all.

DEDICATION

This work is dedicated to my beloved parents, Mr. Eliezer Mwipopo and the late Christina Mwipopo who took the trouble and responsibility of my existence in this world. The work is also dedicated to my beloved husband James Mgale and our children Yohana, Sarah, Joel, Jeremiah and Sechelela.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	- Analysis of Variance
AfSHC	- African Seed Health Centre
a. s. l.	- Above sea level
cfu	- Colony forming unit
cm	- Centimeter
°C	- Degree Celsius
CRD	- Completely Randomized Design
CZ	- Central Zone
DALDO	- District Agricultural and Livestock Development Officer
e.g.	- Example
FAO	- Food and Agriculture Organization
g	- Gramme(s)
ha	- Hectare
HR	- Hypersensitive reaction
i.e.	- That is
IRRI	- International Rice Research Institute
ISTA	- International Seed Testing Association
KB	- King's B medium
Kg	- Kilogramme(s)
KOH	- Potassium hydroxide
MAFSC	- Ministry of Agriculture, Food Security and Cooperatives
m	- Meter
m. c	- Moisture content

mls	-	Milliliters
mm	-	Millimeter(s)
NA	-	Nutrient Agar
N	-	Nitrogen
QDS	-	Quality Declared Seed
SNA	-	Sucrose Nutrient Agar
SUA	-	Sokoine University of Agriculture
TOSCI	-	Tanzania Official Seed Certification Institute
URT	-	United Republic of Tanzania
VEO	-	Village Extension Officer
TANSEED	-	Tanzania Seed Company

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Rice (*Oryza sativa* L.) is among the major leading food crops and second to wheat in terms of production in the world (FAO, 2004). Globally, rice is the staple food of more than half of the world's population. About 90% of the World's rice is produced and consumed in Asia (IRRI, 1993). In Africa, rice production is a strategic component of food security and a crucial element in the staple food economies of many countries (Kaungzan *et al.*, 1984). Rice is the second important food crop after maize which feeds 60% of population in Tanzania (FAO, 2001).

Tanzania is the major producer and consumer of rice in East Africa and is about 90% self-sufficient in rice production (FAO, 2001). The crop is grown in all regions of the country with varying levels of importance. The principal rice growing regions in the country are Shinyanga, Morogoro, Mwanza, Tabora, and Mbeya (FAO, 2003). Rice yield in Tanzania is low (1 - 1.5 tons ha⁻¹) because farmers use low yielding varieties and lack of irrigation facilities, poor soil, traditional methods of cultivation, poor weed control, insects and diseases (Ching'ang'a, 1984; MAFS, 1998).

Seed is the starting unit and may be the end point of any plant cycle (Maude, 1996). Seed provides the maximum limit of a crop yield of all other production inputs. Unlike fertilizer and pesticide, farmers can not produce without seed (Temu and Mtenga, 2001). Seed is a primordial input for production and it is embodiment of the past harvests and the promise of the future (Lowaars and Marreijk, 1996). In many developing countries including Tanzania, farmers use seed retained from the previous season for sowing in the next season

(Kibiby *et al.*, 2001). Such farmers' saved seed may be carriers of seed-borne pathogens (fungi, bacteria, viruses, insects and nematodes), which are transmitted when such seed are sown under suitable environmental condition for disease development (Neegaard, 1977; Hampton, 1982).

Some of seed-borne fungal pathogens in rice associated with discoloured grains include *Magnaphocete oryzae* (blast), *Bipolaris oryzae* (brown spot), *Alternaria padwickii* (stackburn), *Rhizoctonia solani* (sheath blight), *Fusarium graminearum* (scab), *Gibberella fujikuroi* complex (Bakanae), *Phoma* sp. (seedling blight and leaf spot), *Nigrospora oryzae* (black kernel). Most of storage moulds which are species of *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Rhizopus*, *Monilia*, *Streptomyces*, also cause seed discolouration (Agarwal *et al.*, 1989). Bacterial pathogens include, *Acidovorax avenae* subsp. *avenae* (brown stripe), *Pseudomonas fuscovaginae* (sheath rot), *Pseudomonas syringae* pv. *syringae* (bacterial sheath rot), *Burkholderia glumae* (grain rot), *Burkholderia plantarii* (seed blight), *Xanthomonas oryzae* pv. *oryzae* (bacterial leaf blight), *Xanthomonas oryzae* pv. *oryzicola* (bacteria leaf streak) and *Pantoea agglomerans* (palea browning) (Agarwal *et al.*, 1989; Zeigler and Alvarez, 1990; Ashura *et al.*, 1999).

Infection and discolouration of rice seed may take place before or after harvest. Rainfall and high humidity during seed production and lodging of rice plants, which places the panicle in a wet or humid environment increases grain discolouration. In storage, seed with moisture content above 13% allows invasion by microorganisms which cause seed discolouration (Agarwal *et al.*, 1989; Robert and Pamela, 1992). Discolouration of grain causes deterioration in grain quality (Zeigler *et al.*, 1987). Reduction in seed viability and such grains on planting usually exhibit pre-emergence or post-emergence death of

seedlings (Duraismamy and Mariappan, 1983b). Iizuka (1958) reported toxin production by storage moulds associated with discoloured grains.

1.2 Justification

The use of certified rice seed is very limited and only about 1% of rice seed is sold as certified seed in Tanzania (Mortensen and Mabagala, 2005). Farmers tend to continue using their own saved seed even when there are better alternatives due to inadequate access to seeds from the formal seed sector. The formal seed sector often provides seeds of limited range of cultivars and varieties which do not always fulfil the needs of the farmers to diversify their production. Certified seeds are of high price and not readily available (MAFSC, 2001). Seed discolouration reduces the market value of the seed, increases the possibility of spreading seed-borne pathogens in areas which were free from the disease as a result, cause economical losses to rice growers (Ashura *et al.*, 1999). Seed-borne pathogens carried with, in or on seed can be multiplied with the seed if the seed is sown in the next season (Maude, 1996; Ashura *et al.*, 1999).

By screening farmers' saved rice seeds collected from Shinyanga, Morogoro and Mbeya regions, seed-borne pathogens causing discolouration will be identified. The results of this study therefore, will be useful for rice seed producers and growers in Tanzania in managing microorganisms causing rice seed discolouration. Findings of the studies will also be used by extension workers to educate rice farmers on the risk of using farm-saved seed of low quality in rice production.

1.3 Overall Objective

To screen farmers' rice saved seed for discolouration and incidence of seed-borne pathogens in order to recommend ways to minimize seed-borne infection and manage diseases in rice production.

1.4 Specific Objectives

- i. To screen and determine the incidence of seed-borne pathogens causing discolouration in farmers' saved rice seed.
- ii. To determine the level of seed infection in rice seed samples collected from three selected regions with different agro-ecosystems.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Seed

Seed is the most important basic input and it is the critical factor in increasing agricultural productivity of any crop. It provides the maximum limit of crop yield of all other production inputs (Temu and Mtenga, 2001). Seed is the carrier of important characteristics for crop production (Louwaars and Marrewijk, 1996). The ability of seed to be viable from one crop season to another is essential to the survival of mankind (Douglas, 1975). Seeds are an essential component of world trade and are distributed nationally and globally, for food or to be used to grow crops for consumption (Maude, 1996).

2.2 Seed Quality

Seed quality is a concept that comprises of a series of characteristic attributes that determine its value for sowing (ISTA, 2005). The most relevant parameters are genetical (cultivar) quality, physical (analytical) quality, physiological quality (germination and vigour), moisture content (m.c) and freedom from seed-borne pathogens (seed health).

2.2.1 Genetical (cultivar) purity

Genetic seed quality refers to the plant's characteristics that result from genetic potential of the embryo. It includes genetic variations within a seed lot. The cultivars have to be maintained through all stages of multiplication and certified seeds which are to be distributed to farmers (ISTA, 2005).

2.2.2 Physical (analytical) purity

Analytical quality indicates how much of the material in a seed lot is intact seed of the species named on the label (ISTA, 2005). During the analysis, impurities (seed of other crop species, weed seeds and inert matter such as broken seeds, chaffs, pieces of leaf and soil particles) are separated from pure seed, weighed and expressed as the percentage by weight of the whole sample.

2.2.3 Physiological quality

2.2.3.1 Germination capacity

Germination of seed refers to emergence and development of the seedling to a stage where its essential structures indicates whether it is able to develop further into satisfactory plant under favourable conditions in the soil (ISTA, 2005). The germination capacity is expressed in percentage of normal seedlings. Seed lots with a higher germination capacity will always prove to establish more seedlings than those with a lower capacity, especially under sub-optimal conditions.

2.2.3.2 Seed vigour

Seed vigour is defined by ISTA (2005) as the sum total of those properties of the seed which determine the level of activity and performance of the seed lot during germination and seedling emergence, seed vigour is influenced by genetic constitution, environment and nutrition of the mother plant, stage of maturity at harvest, seed size, weight and specific gravity, mechanical integrity, deterioration, ageing and seed-borne pathogens present. Seeds, which perform well, are termed “high vigour seeds.”

2.2.4 Moisture content

The moisture content of a seed sample is the loss in weight when it is dried (ISTA, 2005). Seeds for planting should be stored under a correct moisture content to reduce effects which are associated with high moisture content such as increased respiration rate to both seeds and microorganisms.

2.2.5 Seed health

Seed health refers primarily to the presence or absence of disease-causing organisms, such as fungi, bacteria, viruses, nematodes and animal pests, such as eelworms and insects (ISTA, 1996).

2.3 Importance of Healthy Seed

Seed health refers to presence or absence of seed-borne pathogens like fungi, bacteria, viruses and nematodes. Healthy seeds are important in preventing the introduction of new pathogen into an area (ISTA, 2005). The main purpose of seed health testing is to reveal the level of pathogen contamination of a given seed lot (ISTA, 1996). Seed health can be useful in preventing the introduction of new pathogens into an area. It is a tool for seed-borne pathogens risk management and is a part of the entire seed-borne pathogen control programme (Shephard and Cockerell, 2000). It is related to food production because by using healthy seeds, effects which are caused by seed-borne pathogens are avoided and a crop growing from such seed will have normal vigour hence increased agriculture productivity and improved food safety (Louwaars and Marrewijk, 1996).

2.4 Seed Production Systems

Production of seed involves total physical, organizational and institutional components, their action and interactions that determine the seed supply and use in qualitative and

quantitative terms. There are two types of seed production (informal and formal) systems (Louwaars and Marrewijk, 1996).

2.4.1 Informal seed production

The informal seed production is made up of unregulated and uncontrolled seed operations. It is largely represented by on-farm seed selection and multiplication efforts by the farmers themselves, seed exchanges among farmers and use of planting materials saved from previous crop harvest and set aside for seed during the succeeding cropping cycle. Informal seed production is characterized by absence of interventions by external organizations, divorced from research and seed quality control, confined to seeds which the formal sector largely does not consider profitable to embark on and dependent on mostly traditional cultivars (MAFSC, 2002). The amount of seed supplied by informal sector has been reported to be 90% (Maingu T. Z., personal communication, 2007).

2.4.2 Formal seed production

The formal seed sector is the seed certification system that has all the essential sections involved in seed quality control in any country (MAFSC, 2001). The system ensures that only improved, high quality seed crop varieties are available for use by farmers. However, the amount of seed supplied is very little compared to the requirements of the farmers (MAFSC, 2001). The amount of seed supplied by the formal sector has been reported to be 10% (Maingu T. Z., personal communication, 2007).

2.5 Seed Production in Tanzania

Tanzania has all essential sections of the formal seed sector, a well established National research system, seed multiplication farms, public and private seed companies, seed quality control services and seed distribution and sales outlets (Kibiby *et al.*, 2001). The

distribution and purchases of certified seed has dropped drastically in Tanzania due to high costs of seed production, removal of subsidies as a result of liberalization and reduced production of certified seeds by seed companies relative to demand.

Production of breeder seed in Tanzania is done at research institutes and certified seed production is done by contract growers vested in Arusha, Morogoro and Iringa regions. The Tanzania Official Seed Certification Institute (TOSCI) is responsible for quality control standards to stipulate minimum rates of seed purity, disease contamination, germination and maximum allowable moisture; from the foundation seed farm stage up to the sale of certified seed to the farmers. The main seeds produced are hybrid and composite maize, sorghum, beans, wheat and sunflower.

Quality declared seed (QDS) is a semi-informal system which was developed to complement the seed certification system especially in developing countries (Ministry of Agriculture and Food Security, 2001). The system was developed to make the best use of resources already available in seed production organizations, where human and physical resources for quality control are limited.

Examples of private companies involved in seed production and distribution are Cargill Hybrid Seeds, Pioneer Hybrid International, Paunar and Rotian Seeds Company (Temu and Mtenga, 2001). Minimum standards for crop species concerned (Table 1) have been subjected to the standard measures (MAFSC, 2001). This grade aims at increasing the supply of good seeds from certified seed supply network and to improve food security in the farmers' households. However, due to the low percentage of seed under this system the outputs and improvement in seed production have not been optimal.

Table 1: Minimum and maximum standards for rice seed in Tanzania

Factor	Class		
	Basic	Certified 1	Certified 2
	(%)	(%)	(%)
Pure seed (min)	98.0	98.0	98.0
Other Seed (max)	0.01	0.01	0.01
Other Varieties (max)	0.0	0.0	0.0
Moisture Content (max)	13.0	13.0	13.0
Germination (min)	80	80	80
Blast - <i>Magnaphocte oryzae</i> (max)	1.0	0.2	0.2
Bacterial Leaf Blight - <i>X. oryzae</i> pv. <i>oryzae</i> (max)	1.0	0.2	0.2
Objectionable Weed Seed (max)	0.0	0.0	0.0
Restricted Noxious Weed Seed (max)	4 per kg	4 per kg	4 per kg

min = minimum, max = maximum

Source: Ministry of Agriculture, Food Security and Cooperatives 2001; URT, 2003

2.6 Importance of Seed-borne Pathogens

Seed-borne pathogens play a negative role to crop production (Jka, 1993). When infected seed is used, germination ability of the seed will be lowered, the resulting crop may be affected by seed-borne pathogens, the diseases may be introduced to areas that were free from diseases and the market value of the seed decrease (Maude, 1996). The current study will examine the discolouration and the incidence of seed-borne pathogens in farmers' saved rice seed.

2.7 Seed Discolouration

Discolouration of rice seeds is generally due to infection by certain microorganisms on the glumes, kernels or both. It may also occur due to certain factors other than microorganisms such as high moisture content in grain (>15%) and high relative humidity of >65% (Agarwal *et al.*, 1989; Robert and Pamela, 1992). Discolouration of rice grain causes deterioration in grain quality due to mycotoxin production (Zeigler *et al.*, 1987). Reduction in seed viability in planting, i.e. it usually exhibits pre-emergence or post-emergence death of seedling (Iizuka, 1958; Duraiswamy and Mariappan, 1983b). Discolouration and poorly filled grains causes economic losses to rice farmers (Agarwal *et al.*, 1989; Ashura *et al.*, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Field Survey and Collection of Rice Seed Samples

Surveys were conducted in Shinyanga Rural and Kahama districts in Shinyanga region (low rainfall, medium altitude zones), Kilosa and Kilombero districts in Morogoro region (medium rainfall, low altitude) and Mbeya Rural and Mbarali districts in Mbeya region (high rainfall, high altitude), to collect farmers' saved rice seeds. Farmers were interviewed individually using a check list questionnaire to obtain the information on farmer's saved rice seeds production, management and production constraints, such as diseases, insect pests, date of planting, harvesting and the storage facilities.

In each district, six rice seed samples were collected from six randomly selected farmers. In addition, one sample (certified seed from Msimba Seed Farm) was collected as a control. A total of thirty-seven seed samples were collected between October 2007 and November 2007. The weight of each rice seed sample collected was 1 kg. The seed samples were packed separately in bags and labelled: variety name, name of the farmer, area collected, date of planting, date of harvest, and area planted; name of collector and date collected. The collected rice seed samples were transported to the laboratory at Sokoine University of Agriculture and stored at 4 °C for further processing.

3.2 Seed Health Testing

Seed health testing was conducted at the African Seed Health Centre at Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Following the standard blotter method

(Mathur and Kongsdal, 2003) for the detection of fungi and the seedling symptom test developed for the detection of bacteria (Agarwal *et al.*, 1989; Mortensen, 2005).

3.3 Experimental Design

A completely randomized design (CRD) with three replications was used. Each of the regions was regarded as an experimental unit because of different climatic factors i.e. Mbeya (high rainfall, high altitude), Morogoro (medium rainfall, low altitude) and Shinyanga (low rainfall, medium altitude zones), which influence the occurrence of the seed-borne pathogens causing diseases. The treatments were the different seed varieties collected from each region.

3.4 Seed Samples for Health Testing

3.4.1 Submitted seed samples

A total of thirty-seven rice seed samples were used in this study (Appendix 1). All samples used were harvested between April and July 2007. The working samples and determination of seed quality standards were done following the procedures described by ISTA (2005). Accession numbers were given to each sample collected and deposited at the African Seed Health Centre (AfSHC) for testing.

3.4.2 Working samples

The working samples were obtained by the following procedures described in International Rules of ISTA (2005). The working sample was obtained by the Mechanical Divider Method where a conical divider apparatus was used.

3.4.3 Mechanical division

A large seeded divider the conical divider (Boerner type), with the following dimensions; 19 channels and 19 spaces, each 25.4 mm wide, the overall dimensions was 812.8 mm high and 368.3 in diameter was used. The apparatus divides a sample passed through it into two equal parts. The submitted samples were mixed by passing it through the divider, recombining the parts and passing the whole samples through a second time, and similarly, the third time. The samples were reduced by passing the seed through repeatedly and removing parts on each occasion, as described by ISTA (2005). This process of reduction continued until working samples were obtained using the weighing balance (Santorious BP 310S) used to determine the required amount of working sample (the sub-samples) of 40g as recommended by Mathur and Kongsdal (2003).

3.4.4 Sorting discoloured seeds

Discoloured seeds were obtained through sorting seeds by visual inspection. Seeds which deviated from normal colour, showed generally discrete brown to dark brown spots on glumes or diffused type of discolouration of different shades covering part of the seed or rarely whole seed (Agarwal *et al.*, 1989) were considered as discoloured seeds, hence were sorted for further analysis.

3.5 Purity, Moisture Content Determination and Germination

3.5.1 Purity analysis

The working sample (40g) of each rice seed sample was examined and separated into its component parts (i.e. pure seed, seeds of other crops and inert matter) using magnifying lamp, forceps and edge spatula. Each component part was weighed in grams and results expressed as percentage of the total working sample as recommended by ISTA (2005).

3.5.2 Moisture content determination

For determination of moisture content of rice seed, 40g of working sample were used by the high constant temperature oven method as described by ISTA (2005). Seeds were first fine ground using a mini grinding mill (J IKA A10). The containers and their covers were weighed before filling them with ground seeds. The containers with flour content and cover were then placed in an oven maintained at 130 °C and dried for 2 hours. After drying samples were removed from the oven and placed in the desiccator for 30 minutes for cooling, and weighed again to get the weight of dried samples. The moisture content of each ground rice seed samples was determined by using the formula: $MC = (M_2 - M_3) \times 100 / M_2 - M_1$; where M_1 was the weight in grams of the container with its cover, M_2 was the weight in grams of container, cover and the content before drying; M_3 was the weight in grams of the container, cover and the content after drying. The moisture content of each ground rice seed sample was determined by calculating the loss in weight after oven drying. The results were expressed as a percentage of the weight of the original sample (ISTA, 2005).

3.5.3 Seed germination

Four hundred seeds of each rice seed sample from the pure seed fraction were spaced uniformly and adequately apart (50 seeds x 8 replications) on moist substrate (between germination papers). Seeds were germinated between two layers of germination papers, rolled and wrapped in plastic bags, placed in an upright position in a plastic basket and incubated at 25 - 28 °C for 14 days. The resulting seedlings were evaluated as normal, abnormal, hard and dead seed following described procedures (Mathur *et al.*, 2003; ISTA (2005). The results were expressed in percentage germination.

3.6 Seed Health Testing

3.6.1 Detection and identification of seed-borne fungal pathogens

Detection of fungal pathogens in seed was done using the blotter method described by Mathur and Kongsdal (2003). Four hundred seeds of each rice seed sample were plated on well water-soaked three layer blotters (filter papers) in petri dishes (25 seeds/ dish) in four replication of 100 seeds each. The petri dishes with seeds were incubated at 25 °C - 28 °C for 7 days in alternating cycles of 12 hours darkness and 12 hours light. After seven days fungi that developed on each seed were examined under different magnifications with a stereomicroscope (WILD Heerbrugs Switzerland). The mycelia of the fungi were placed in a sterile drop of water and placed covered by a glass slip onto a sterile glass slide and observed with the compound microscope (Leica MSS). The examination was done under the magnification of x40 and x100 and the identification of fungi was based on growth habit and on the morphology of the fruiting bodies and spores/conidia observed under the compound microscope.

3.6.2 Detection and isolation of bacteria from rice seed samples

Three steps for detection of plant pathogenic bacteria from rice seed were used as described by Agarwal *et al.* (1989). Detection of bacteria from rice seedlings was done using the Slide Cassette Holder method as described by Shakya and Chung (1983). The detected bacteria were isolated and purified by a series of single colony transfers on King's B medium (KB) and Nutrient Agar (NA) for identification. Identification of pure bacterial isolates were based on colony morphology and pigment production on KB/NA, Gram reaction based on potassium hydroxide solubility test (Lelliot and Stead, 1987), oxidase reaction (Kovac, 1959; Hilderbrand and Schroth, 1968; Schaad *et al.*, 2001), arginine dihydrolase, levan formation (Lelliot and Stead, 1987), gelatin hydrolysis, potato soft rot,

nitrate reduction (Fahy and Persley, 1983; Schaad *et al.*, 2001), oxidative/fermentative metabolism of glucose procedures (Fahy and Parsley, 1983), hypersensitive reaction (HR) tests (Klement, 1983) and pathogenicity tests with rice host plants.

3.6.3 Slide Cassette Holder Method (Seedling symptom test)

Two pieces of filter papers with dimensions of 4.5 cm x 4.5 cm were placed in every second slot of the slide cassette holder. The cassette holders holding the filter papers were placed in a plastic tray containing urea (H_2NCONH_2) solution (230 ppm N) and allowed the filter papers to absorb the urea solution. Four hundred rice seed per sample replicated four times with 100 seed per replicate. Four rice seeds were placed between each pair of moistened filter paper. The trays with cassettes were incubated at 27 - 30 °C up to 14 days. Seed germinated were observed for presence of disease symptoms like bacterial stripe, bacterial sheath browning, yellowing of leaves and other disease symptoms. Seedlings with symptoms were counted and recorded.

Four to five seedlings showing typical bacterial brown stripe symptoms on the coleoptile, leaf blade and leaf sheaths; and other symptoms such as sheath browning, tip drying and yellowing of leaves were selected from each sample for isolation of pathogenic bacteria. Small areas of the diseased tissue including the health portions of the neighbouring tissue were placed on a flame sterilized glass slide in a sterile drop of water. A cover slip was put on a drop and the sample was examined for bacterial streaming under a compound microscope at x40 to x100 magnifications. Isolations were made from leaf tissues which were positive for bacterial streaming. Cover slips were removed, and the leaf tissues were teased apart in a few drops of sterile distilled water and left to stand for 10 - 15 minutes to allow the bacterial cells to move out of the macerated tissue. Using a sterile bacteriological

loop, bacterial suspensions were streaked a number of times onto nutrient agar (NA) plates in triplicate. Inoculated plates were incubated at 25 °C to 30 °C for 2 to 3 days. The bacterial colonies were purified by a series of single colony transfers on KB and NA for further characterisation and identification (Agarwal *et al.*, 1989).

3.6.4 Identification of bacteria isolates

Pure bacterial isolates from the seedlings were identified based on colony morphology on NA, pigment production on KB, Gram reaction based on potassium hydroxide solubility tests (Lelliot and Stead, 1987), oxidase reaction (Kovac, 1959; Hildebrand and Schroth, 1968; Schaad *et al.*, 2001), arginine hydrolase, gelatin hydrolysis, potato soft rot, nitrate reduction (Fahy and Persley, 1983; Schaad *et al.*, 2001) starch hydrolysis (Fahy and Persley, 1983), oxidative/fermentative metabolism of glucose (Fahy and Persley, 1983), hypersensitive reaction on tobacco plant (Klement, 1983), pathogenicity tests on rice seedlings (Shakya and Chung, 1983).

3.6.4.1 Gram reaction

Bacterial isolates were tested for gram reaction by using three percent potassium hydroxide (KOH) solubility test (Lelliot and Stead, 1987), on a glass slide. Loopful of 24 - 48 hour-old bacterial cultures were mixed, for about 10 seconds, with drops of three percent aqueous solution. A toothpick stick was used for picking bacteria from a colony and mixing thoroughly to create a bacterial suspension, and the toothpick was raised a few centimetres from the suspension on the glass slide. Presence of strands of viscid material indicated that the bacteria tested were Gram negative, Gram positive bacteria remained unchanged without the production of viscid material.

3.6.4.2 Kovac's Oxidase test

The Kovac's Oxidase test was used to determine the presence of cytochrome-c (oxidase enzymes of the respiratory chain) as described by Kovac (1959) and Hildebrand and Schroth (1968). Using a platinum loop wire a loopful of 24 - 48 hour-old bacterial cultures grown on KB or NA were streaked onto a Whatman filter paper No. 1 which was placed in a petri dish and previously made moist with 2 - 3 drops of freshly prepared 1 % aqueous solution of tetramethyl-p phenylenediamine dihydrochloride. A change in colour of the reagent to purple within 10 seconds of application of the culture was recorded as a positive reaction.

3.6.4.3 Gelatin hydrolysis

Tubes containing gelatin were stab- inoculated by 24 hour-old bacterial cultures grown on KB or NA medium. Isolated bacteria were grown in a nutrient medium solidified by gelatin, where proteolytic bacteria decompose gelatin with loss of gelling properties. Negative and positive reference strains, un-inoculated control were included. The inoculated test tubes were incubated at 28 °C for 7 - 14 days. Liquefaction of the medium was recorded every 2 - 3 days. Inoculated tubes were cooled at 5 °C for 30 minutes, incubated in a tilted position at room temperature for 30 minutes before recording the results. Hydrolyzed gelatin were recorded as a positive reaction and negative reaction was indicated when the test culture took as long as the control for liquefaction (Fahy and Persley, 1983; Lelliot and Stead, 1987).

3.6.4.4 Arginine dihydrolase

The test measures the ability of an organism to catabolize arginine. The arginine dihydrolase enzyme system couples arginine degradation to adenosine triphosphate (ATP)

generation, thus permitting the organism to grow under anaerobic condition. A procedure described by Lelliot and Stead (1987) was followed: a culture of fluorescent pseudomonad isolates grown for 24 hours on KB was stab inoculated into the medium. A control tube without bacterial inoculum organism was also included for comparison. All inoculated tubes were overlaid with 1 ml of sterile mineral oil to create anaerobic conditions. The inoculated tubes were incubated for 3 days at 28 °C. A change of colour to red (alkaline) was recorded as positive reaction, due to production of ammonia, and no change of colour indicating that arginine was not degraded, thus was recorded as negative.

3.6.4.5 Levan (poly- fructose) formation

Pseudomonas syringae produces white, domed, shining, mucoid, levan type colonies in 5 percent sucrose nutrient agar (SNA). Levan, formed by enzyme levan sucrase (Lelliot and Stead, 1987), was produced from most fluorescent pseudomonads, which utilize sucrose. A 24 hour-old pure culture of the bacterium was inoculated by streaking onto SNA medium. Positive (*Pseudomonas syringae* pv. *syringae*) and negative (*Acidovorax avenae* subsp. *avenae*) reference strains were included as controls. The inoculated plates were incubated for three days at 28 °C and the developed bacterial colonies were observed after three days.

3.6.4.6 Oxidative/Fermentative (O/F) metabolism of carbohydrates

Utilization of carbohydrate tests can be used to determine whether a bacterium has a respiratory (oxidative) or fermentative metabolism (creating anaerobic conditions by covering with sterile mineral oil). When glucose is oxidized in the presence of oxygen, it is an aerobic process, and bacteria that oxidize carbohydrates are aerobic, while fermentation is an anaerobic process where bacteria ferment carbohydrates. Facultative anaerobes are organisms that can grow and reproduce in either aerobic or anaerobic conditions. The

procedures described by Fahy and Parseley (1983) were used to test for oxidative/fermentative (O/F) metabolism of glucose. A 24 hour-old culture grown on NA medium was stab-inoculated in duplicate test tubes containing O/F semi-solid medium with 1% glucose. Control tubes in duplicates: un-inoculated and one inoculated with reference strain were included as controls. One of the test tubes was covered with sterile mineral oil to a depth of 2 cm and incubated at 28 °C and examined daily for up to 7 days. The oxidative metabolism of glucose with acid production causes a yellow colour change only at the top, 1 - 2 cm, of the unsealed tube. The yellow colour in both sealed and unsealed tubes indicated fermentation, while no colour change in both tubes indicated neither oxidation nor fermentation had occurred.

3.6.4.7 Nitrate reduction

Nitrate reduction test determines the ability of an organism to reduce nitrate to nitrite, which can be further reduced to free nitrogen gas. The procedure was described by Fahy and Parsley (1983). A loopful of 24 hour-old bacterial cultures grown on NA medium were inoculated in duplicate tubes containing molten nitrate semi-solid medium which was mixed by rotation between palms before setting of agar. An un-inoculated, a positive and a negative reference strains were included, then the test tubes were incubated at 28 °C for 7 days. Then three drops of Nitrate reagent A (starch iodide solution) and three drops of Nitrate reagent B (Hydrochloric acid solution) were added to an incubated nitrate agar tubes and results were recorded. A change in colour to blue-black within seconds was recorded as positive (the organism reduced nitrate to nitrite). While no blue-black colour seen was recorded as negative. If no colour develops, a speck of zinc powder was added for further observations. Presence of the blue-black colour indicated that nitrate was not

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reduced (the results were negative) and if no colour develops was considered positive (the nitrogen has been reduced beyond nitrite to gaseous).

3.6.4.8 Starch hydrolysis

Starch hydrolysis test shows the ability of an organism to hydrolyze starch by enzymatic activity. This was done following procedures described by Lelliot and Stead (1987). Starch medium agar was inoculated by streaking with a 24 hour-old pure cultures grown on NA medium. Also the reference strain: positive (*Xanthomonas oryzae* pv. *oryzicola*) and negative (*Xanthomonas oryzae* pv. *oryzae*) controls were included. The inoculated plates were incubated at 28 °C for 4 days, and then were flooded directly with Lugol's iodine and appearance of yellowish, clear zones around bacterial growth indicated a positive reaction. Staining blue-black that indicating lack of starch hydrolysis was recorded negative.

3.6.4.9 Potato soft rot

Potato soft rot test is useful and a determinative character in bacterial identification. The procedure described by Lelliot and Stead (1987) was used. The round potato tubers were washed and surface disinfected by dipping in 70 percent alcohol, flamed, then were sliced aseptically 1 cm thick, and placed on moistened sterile petri dishes. A groove was made in the sliced potato and smeared with bacterial growth of 24 hour-old agar cultures, a control of un-inoculated slice were included. The petri dishes with inoculated potato slices were incubated in darkness at 27 °C for 24 hours. The results were recoded by using an inoculating loop passing across the surface of the slice to determine whether the slice had rotted beyond the inoculation point. A black rotted tissue was recorded as positive for potato soft rot while slight rot only at the inoculating point was recorded as negative.

3.6.4.10 Hypersensitive reaction

Most bacterial plant pathogens can induce a hypersensitive response (HR) when injected into the tissue of non-susceptible host plant unlike non-pathogenic bacteria. The reaction can provide a useful presumptive diagnostic technique (Klement, 1983). The reaction is the result of an incompatible plant bacteria interaction thus leading to rapid collapse of the infiltrated areas and water soaked followed by dry, light brown necrosis at the infected site. A variety of plants may be used, but for many bacteria, tobacco is used in these tests as described by Lelliot and Stead (1987).

An aqueous suspension from 24 hour-old bacterial cultures grown on KB and NA media were prepared to a 10^8 cfu/ml. The mesophyll of the leaf blades of the tobacco plants were injected with the suspension by inserting the needle of a hypodermic syringe into the cavity, along the side of the lateral veins. Sufficient inoculum was injected into the leaves. A culture suspension of the reference strain was also injected as positive, negative and sterile distilled water as control. Injected areas were labelled and the inoculated plants were incubated at 27 °C. A rapid collapse and water soaking of inoculated tissue within 24 - 48 hours followed by a dry, light brown localized necrosis within 3 days indicated as positive hypersensitive reaction (isolates were pathogenic). Yellowing or browning without collapse or no visible reaction were considered negative, hence isolates were non-pathogenic.

3.6.4.11 Pathogenicity tests on host plants

The aim of pathogenicity test was to determine whether isolated bacteria were pathogenic to the host plant from which it was isolated. Pathogenicity tests were done as described by Lelliot and Stead (1987). A loopful of 24 - 48 hour-old bacterial cultures grown on NA and

KB media were suspended in sterile distilled water at the concentration of 10^8 cfu/ml and vortexed to form a uniform bacterial suspension.

Three-week-old rice seedlings of variety (Supamarti, 2 - 3 leave stage) were selected, labelled with isolate number, inoculation method and date. Zero point one millilitre bacterial suspensions were injected into the stems of the host plants, using a syringe attached with a fine hypodermic needle (23 G X 1 1/4 for rice). A known bacterial culture (*Acidovorax avenae* subsp. *avenae*, *Pseudomonas syringae* pv. *syringae*, *P. fuscovaginae*, *Burkholderia glumae*) as positive controls and distilled water (negative control) were included. The plants were incubated in polyethylene sheets arranged to prevent the sheet from touching the inoculated plants.

3.6.4.12 Data analysis

Each 400 seed tested per sample for each test represented four replications (100 seeds per replication). The analysis of variance (ANOVA) for the incidences of seed-borne fungal pathogens and the level of seed infection per seed sample were analyzed using Mstat-C computer package, and the mean separation tests were done using the Duncan's Multiple Range Test ($P=0.05$).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Purity, Germination Test and Moisture Content Determination

The purity test of farmers' saved rice seed samples tested showed that 39% of the rice seed samples (three accessions from Shinyanga, seven from Mbeya and four from Morogoro regions) were above the minimum recommended standards (98%) (Tables 2, 3 and 4) (MAFSC, 2001). While 61% had purity levels below the recommended standards (ten accessions from Shinyanga, four from Mbeya and eight from Morogoro regions), most of the farmers' saved rice seed samples collected from Shinyanga had low percentage of purity due to high content of inert matter in the seed samples collected. The method used during threshing encouraged incorporation of inert matter into the seed because it was done on the ground allowing contamination with seed and other impurities.

Germination tests conducted using the between paper method, indicated that the number of normal rice seedlings varied significantly ($P \leq 0.05$) between the rice seed samples tested (Table 2). The variety Mzuri Hajipaki Mafuta, Acc. No. 07/92 collected from Panagichiza in Shinyanga Rural had the highest number of normal rice seedlings (82%). Rice seed sample Acc. No. 07/87 (Supa India variety) and 07/91 (Kalamata variety) from Bukooba in Kahama district and Bumva in Kahama district had the least number of normal seedlings, 56% each. Rice seed sample Acc. No. 07/92 was the only rice seed sample which met the minimum germination standards for rice (80%) (MAFSC, 2001). Other rice seed samples tested were below the recommended germination standards, including the control (Acc. No. 07/122) from Msimba seed farm (certified seed) which had low germination (73%) (Table 2). A high number of seed-borne pathogens and storage moulds infecting the seeds

thus, may have affected the germination capacity of the seeds. A possible reason for the difference in germination percentage was due to factors such as high temperature and humidity in Shinyanga region which influenced the occurrence of the fungal pathogens or probably that the seeds were harvested from infected plants. The moisture content of farmers' saved rice seed collected from Shinyanga region ranged from 11.2 % to 12.7%, all of the 13 seed samples tested had moisture content within recommended standards for rice (max. 13%) (MAFSC, 2001) as shown in Table 2.

Table 2: Percentage germination, purity and moisture content of farmers' saved rice seed (*Oryza sativa* L.) collected from farmers in Shinyanga region in 2006/07

Sample No.	Germination (%)				Purity (%)			Moisture content (%)
	Normal seedlings	Abnormal seedlings	Dead seed	Hard seed	Pure seed	Inert matter	Other seed	
07/86	62 ^{b-d}	22	12 ^{a-d}	4	99.0	1.0	0.0	12.7
07/87	56 ^d	32	7 ^{de}	4 ^{cd}	94.1	5.9	0.0	11.5
07/88	61 ^{b-d}	22	12 ^{a-c}	5 ^{b-d}	97.1	2.9	0.0	12.5
07/89	58 ^{cd}	19	13 ^{ab}	9 ^{ab}	96.2	3.8	0.0	11.9
07/90	70 ^{a-d}	13	10 ^{b-d}	8 ^{a-c}	97.6	2.4	0.0	11.4
07/91	56 ^d	20	15 ^a	10 ^a	91.6	8.4	0.0	11.7
07/92	82 ^a	31	4 ^e	2 ^d	99.6	0.4	0.0	12.2
07/93	72 ^{a-c}	20	4 ^e	4 ^{cd}	94.5	5.5	0.0	11.5
07/94	58 ^{cd}	21	10 ^{b-d}	11 ^a	89.0	11.0	0.0	11.3
07/95	65 ^{b-d}	22	8 ^{b-c}	5 ^{b-d}	92.7	7.3	0.0	12.0
07/96	69 ^{a-d}	16	10 ^{b-d}	5 ^{b-d}	98.0	2.0	0.0	11.9
07/97	72 ^{a-c}	18	8 ^{c-e}	2 ^d	96.2	3.8	0.0	11.7
Control	73 ^{ab}	14	11 ^{b-d}	2 ^d	97.4	2.6	0.0	11.2
Mean	66	21	10	6	-	-	-	-
LSD	12	-	4	4	-	-	-	-
CV (%)	13	60	30	49	-	-	-	-

Means followed by the same letter(s) within a column are not significantly different at 5% level based on the Duncan's Multiple Range Test. Each value is a mean of 400 seed (4 replications).

Germination test results from farmers' saved rice seed samples tested from Mbeya region varied significantly at ($P \leq 0.05$) between the rice samples tested (Table 3). Variety Rubi Acc. No. 07/108 collected from Mwanavala in Mbarali district had the highest number of normal seedlings (76%), while rice samples Acc. No. 07/98 (variety, India Rangi ya Mkia) and 07/99 (variety, India Rangi ya Mkia) from Mwatenga in Mbeya Rural and Azimio in Mbeya Rural had the least number of normal rice seedlings both of 56%. In Mbeya region all the seed samples tested were below the minimum germination standard (80%). A high number of storage fungi, moulds and seed-borne pathogens infecting the seeds recorded to have reduced germination capacity (Plate 1); although Mbeya region had low temperature and low humidity, such conditions are known to favour the occurrence of certain fungal pathogens in rice seed. *Fusarium moniliforme* had high incidence in rice seeds in Mbeya (26%), resulting into low germination of seeds (Nsemwa and Wolffhechel, 1999).



Plate 1: Germination (A) and fungal infection (B) of rice seed sample Acc. No. 07/101 (variety Zambia) collected from Mbeya Rural in Mbeya region tested by the between naner method.

The moisture content of rice seed of samples collected from Mbeya region ranged from 11.2 to 12.8%. All the samples tested had the moisture content within the recommended standards for rice (max. 13%) (Table 3).

Table 3: Percentage germination, purity and moisture content of farmers' saved rice seed (*Oryza sativa* L.) collected from farmers in Mbeya region in 2006/07

Sample No.	Germination (%)				Purity (%)			Moisture content (%)
	Normal seedlings	Abnormal seedlings	Dead seed	Hard seed	Pure seed	Inert matter	Other seed	
07/98	56 ^d	23 ^a	16 ^a	6 ^{b-d}	98.6	1.4	0.0	12.3
07/99	56 ^d	19 ^{a-d}	15 ^{ab}	7 ^{ab}	98.1	1.9	0.0	12.8
07/100	72 ^{ad}	13 ^{d-e}	13 ^{a-c}	4 ^{b-e}	98.0	2.0	0.0	11.8
07/101	66 ^{a-d}	11 ^e	13 ^{a-c}	10 ^a	99.5	0.5	0.0	12.2
07/102	71 ^{ab}	23 ^a	3 ^e	3 ^{b-e}	98.0	2.0	0.0	11.4
07/103	60 ^{cd}	24 ^a	12 ^{a-c}	5 ^{b-e}	95.3	4.7	0.0	12.1
07/104	74 ^{ab}	14 ^{c-e}	10 ^{b-d}	3 ^{c-e}	95.6	4.4	0.0	11.4
07/105	63 ^{b-d}	22 ^{ab}	13 ^{a-c}	2 ^{de}	97.3	2.7	0.0	11.9
07/106	58 ^{cd}	26 ^a	9 ^{cd}	7 ^{a-c}	99.1	0.9	0.0	11.6
07/107	69 ^{a-c}	21 ^{a-c}	6 ^{de}	5 ^{b-e}	97.7	2.3	0.0	11.8
07/108	76 ^a	15 ^{b-e}	8 ^{c-e}	2 ^e	96.9	3.1	0.0	11.7
07/109	73 ^{ab}	14 ^{c-e}	8 ^{c-e}	5 ^{b-e}	98.4	1.6	0.0	11.7
Control	73 ^{ab}	14 ^{c-e}	11 ^{b-d}	2 ^d	97.4	2.6	0.0	11.2
Mean	67	18	10	5	-	-	-	-
LSD	10	7	5	3	-	-	-	-
CV (%)	11	26	35	53	-	-	-	-

Means followed by the same letter(s) within a column are not significantly different at 5% level based on the Duncan's Multiple Range Test. Each value is a mean of 400 seed (4 replications).

Results of seeds collected from Morogoro region, showed that there was no significant difference between the number of normal rice seedlings between samples tested ($P \leq 0.05$) (Table 4). The number of abnormal rice seedlings and dead seeds was significantly different between the seed samples tested ($P \leq 0.05$).

Table 4: Percentage germination, purity and moisture content of farmers' saved rice seed (*Oryza sativa* L.) collected from farmers in Morogoro region in 2006/07

Sample No.	Germination (%)				Purity (%)			Moisture content (%)
	Normal seedlings	Abnormal seedlings	Dead seed	Hard seed	Pure seed	Inert matter	Other seed	
07/110	81	9 ^{de}	8 ^{a-c}	3 ^{d-f}	98.3	1.7	0.0	12.7
07/111	73	14 ^{a-c}	9 ^{a-c}	4 ^{b-e}	98.2	1.8	0.0	11.6
07/112	72	16 ^{a-d}	8 ^{a-c}	4 ^{b-e}	99.5	0.5	0.0	12.0
07/113	63	19 ^a	12 ^a	6 ^{ab}	97.6	2.4	0.0	12.8
07/114	62	18 ^{ab}	11 ^{ab}	8 ^a	96.4	3.6	0.0	12.4
07/115	66	18 ^{ab}	11 ^{ab}	6 ^{a-c}	96.9	3.1	0.0	11.9
07/116	72	10 ^{c-e}	13 ^a	5 ^{b-d}	97.6	2.4	0.0	12.5
07/117	80	11 ^{b-d}	6 ^{a-c}	2 ^{ef}	95.9	4.1	0.0	11.5
07/118	76	10 ^{c-e}	11 ^{ab}	4 ^{c-f}	93.9	6.3	0.0	11.1
07/119	74	10 ^{c-e}	12 ^a	4 ^{c-f}	98.0	2.0	0.0	11.3
07/120	85	9 ^{de}	5 ^{bc}	2 ^{ef}	95.2	4.8	0.0	11.8
07/121	67	8 ^e	3 ^c	1 ^f	97.4	2.6	0.0	11.5
Control	73	14 ^{a-c}	11 ^{ab}	2 ^{ef}	97.4	2.6	0.0	11.2
Mean	73	13	9	4	-	-	-	-
LSD	20	7	6	2	-	-	-	-
CV (%)	19	37	46	39	-	-	-	-

Means followed by the same letter(s) within a column are not significantly different at 5% level based on the Duncan's Multiple Range Test. Each value is a mean of 400 seed (4 replications).

The highest number of abnormal rice seedlings and dead seeds were recorded in rice seed samples Acc. No. 07/113 and 07/116 collected from Mgudeni in Kilombero district and Kilangali in Kilosa district, respectively. The lowest number of abnormal rice seedlings and dead seeds were recorded in rice sample Acc. No. 07/121 from Ilonga in Kilosa district (Table 4). The moisture content of rice seed samples ranged from 11.1 to 12.8%, which was within the recommended standards for rice (max. 13%) (Table 4).

4.2 Seed-borne Fungal Pathogens Detected in Farmers' Saved Rice Seed Samples

Results from the blotter method (Fig. 1) from Shinyanga, Mbeya and Morogoro regions evaluated for seed-borne pathogens revealed that all of the 36 samples plus the control were infected by seed-borne fungal pathogens. The variation of seed infection between the regions was significantly different ($P \leq 0.05$) in Duncan's Multiple Range Test.

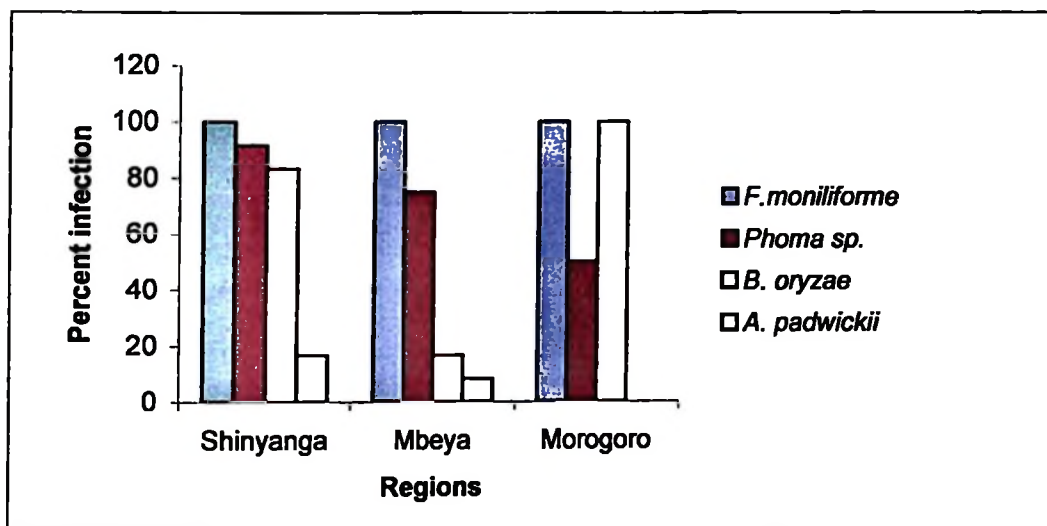


Figure 1: Percent infection of seed-borne fungi detected in farmers' saved rice seed from Shinyanga, Morogoro and Mbeya regions.

In general, the seed samples collected from the three regions under this study were infected by a number of seed-borne fungal pathogens associated with rice seed discolouration. The infection rate was higher in Shinyanga region followed by Morogoro region and Mbeya region. *Fusarium moniliforme* was detected in all seed samples tested, irrespective of the sampling area. Shinyanga region which had low rainfall, 600mm - 800mm per year, medium altitude 1100 - 1500m above sea level (a. s. l.), had high infection of seed-borne pathogens. Storage of rice was done using nylon bags and piled in stores where there was no ventilation which may cause pathogen multiplication in storage, explaining the high infection levels.

Morogoro region had medium rainfall, 600mm - 1200mm per year and low altitude 526 m a. s. l. The region is hot and humid and such conditions may be responsible for the observed levels of seed infection by seed-borne pathogens. Mbeya region has high rainfall 650mm - 2600mm per year, high altitude 1372 – 6500 m a. s. l., and the region is cool and humid, condition which may be favourable to some of seed-borne pathogens.

The infection of rice seed in Mbeya, Morogoro and Shinyanga regions particularly with *F. moniliforme*, may be favoured with such environmental conditions. Thus, the difference in agro-ecosystem in the three regions had an influence in occurrence of some of seed-borne fungal pathogens (Fig. 1). *Fusarium moniliforme* was detected in all rice seed samples collected from the three regions.

In Shinyanga region (Table 5, Acc. No. 07/86 – 07/97, Table 6 and Fig. 2), the infection ranging from 0.75 (variety Mzuri Hajipaki Mafuta) to as high as 16.75% (variety Rangi Mbili).

Table 5: Infection levels of various seed-borne fungi detected from farmers' saved rice seed collected from Shinyanga region

Acc. No.	Variety	Pathogen identified and percent infection (in bracket)
07/86	Mbegu Bora	<i>Alternaria padwickii</i> (0.25%), <i>Bipolaris oryzae</i> (0.25%), <i>Fusarium moniliforme</i> (7.75%), <i>Phoma</i> sp. (2.5%).
07/87	Supa India	<i>Bipolaris oryzae</i> (1.25%), <i>Fusarium moniliforme</i> (8.25%), <i>Phoma</i> sp. (2.75%).
07/88	Beneko	<i>Bipolaris oryzae</i> (2%), <i>Fusarium moniliforme</i> (0.75%), <i>Phoma</i> sp. (5%).
07/89	Kalamata	<i>Alternaria padwickii</i> (0.25%), <i>Fusarium moniliforme</i> (4%), <i>Phoma</i> sp. (0.75%).
07/90	Kalamata	<i>Fusarium moniliforme</i> (8.75%), <i>Phoma</i> sp. (0.25%).
07/91	Kalamata	<i>Bipolaris oryzae</i> (0.25%), <i>Fusarium moniliforme</i> (5.25%),
07/92	Mzuri Hajipaki Mafuta	<i>Bipolaris oryzae</i> (0.25%), <i>Fusarium moniliforme</i> (0.75%),
07/93	Kalamata	<i>Bipolaris oryzae</i> (1.25%), <i>Fusarium moniliforme</i> (1.25%), <i>Phoma</i> sp. (2.25%).
07/94	Mzuri Hajipaki Mafuta	<i>Bipolaris oryzae</i> (0.5%), <i>Fusarium moniliforme</i> (9.25%), <i>Phoma</i> sp. (3.75%).
07/95	Rangi Mbili	<i>Bipolaris oryzae</i> (1.5%), <i>Fusarium moniliforme</i> (16.75%), <i>Phoma</i> sp. (1.25%).
07/96	Supa India	<i>Bipolaris oryzae</i> (0.5%), <i>Fusarium moniliforme</i> (6%), <i>Phoma</i> sp. (1%).
07/97	Daniel	<i>Bipolaris oryzae</i> (0.5%), <i>Fusarium moniliforme</i> (14.5%), <i>Phoma</i> sp. (3.75%).
Control	Supa	<i>Bipolaris oryzae</i> (2%), <i>Fusarium moniliforme</i> (1.75%),

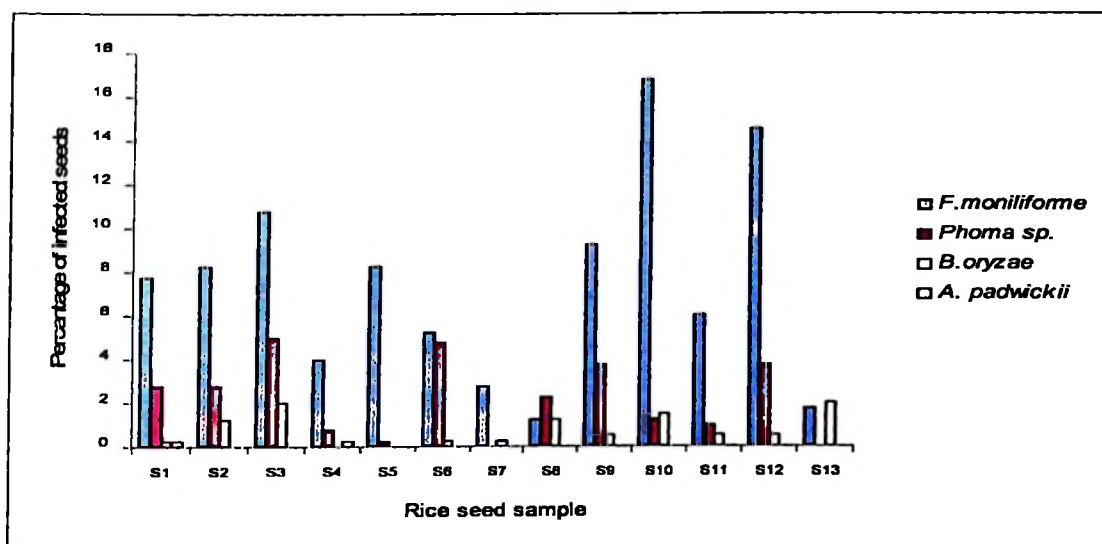


Figure 2: Percent frequency of seed-borne fungi detected on rice seed collected from Shinyanga region,

S1 = Mbegu Bora, S2 = Supa India, S3 = Beneko, S4 = Kalamata, S5 = Kalamata, S6 = Kalamata, S7 = Mzuri Hajipaki Mafuta, S8 = Kalamata, S9 = Mzuri Hajipaki Mafuta, S10 = Rangi Mbili, S11 = Supa India, S12 = Daniel, S13 = Supa.

Phoma sp. dominated in most of the seed samples ranging from 0.25 (variety Kalamata) to as high as 5.0% (variety Beneko) with low infection. *Bipolaris oryzae* was detected in 10 rice seed samples with low infection ranging from 0.25 - 2.0% (Tables 5 and 6). *Alternaria padwickii* was detected only in two rice seed samples with infection levels of 0.25% (Mbegu Bora and Kalamata) (Table 6).

Table 6: Range of infection of seed-borne fungal pathogens in farmers' saved rice seed (*Oryza sativa* L.) samples collected from Shinyanga region

Pathogen	Samples infection (Nos.)	Range of infection in percentage				
		0 - 1	2 - 3	4 - 10	11-16	17-30
<i>Fusarium moniliforme</i>	12	-	2	7	2	1
<i>Phoma</i> sp.	11	3	4	4	-	-
<i>Bipolaris oryzae</i>	10	6	4	-	-	-
<i>Alternaria padwickii</i>	2	2	-	-	-	-

In Mbeya region, *F. moniliforme* was detected in all rice seed samples (Table 7 Acc. No. 07/98 – 07/109, Table 8 and Fig. 3), Seed infection ranged from 0.5 (variety Rubi) to as high as 26.0% (variety Zambia). *Phoma* sp. was also detected, the infection was low ranging from 0.25 – 2.25% (Table 7).

Table 7: Infection levels of various seed-borne fungi detected from farmers' saved rice seed collected from Mbeya region

Acc. No.	Variety	Pathogen identified and percent infection (in bracket)
07/98	India Rangi ya Mkia	<i>Bipolaris oryzae</i> (0.5%), <i>Fusarium moniliforme</i> (4.25%),
07/99	India Rangi ya Mkia	<i>Fusarium moniliforme</i> (18%), <i>Phoma</i> sp. (0.25%).
07/100	India Rangi ya Mkia	<i>Fusarium moniliforme</i> (6%), <i>Phoma</i> sp. (2.5%).
07/101	Zambia	<i>Fusarium moniliforme</i> (26%), <i>Phoma</i> sp. (0.5%).
07/102	Mwenda Mbio	<i>Fusarium moniliforme</i> (8%).
07/103	India Rangi ya Mkia	<i>Fusarium moniliforme</i> (5.25%), <i>Phoma</i> sp. (0.5%).
07/104	Supamarti	<i>Fusarium moniliforme</i> (3.75%), <i>Phoma</i> sp. (0.5%).
07/105	India Rangi ya Mkia	<i>Fusarium moniliforme</i> (5.25%).
07/106	Zambia	<i>Alternaria padwickii</i> (0.75%), <i>Fusarium moniliforme</i> (12.25%), <i>Phoma</i> sp. (2.25%).
07/107	Cherehani	<i>Bipolaris oryzae</i> (0.25%), <i>Fusarium moniliforme</i> (3.75%), <i>Phoma</i> sp. (0.5%).
07/108	Rubi	<i>Fusarium moniliforme</i> (0.5%), <i>Phoma</i> sp. (0.5%).
07/109	Kilombero	<i>Fusarium moniliforme</i> (6.5%).
Control	Supa	<i>Bipolaris oryzae</i> (2%), <i>Fusarium moniliforme</i> (1.75%).

Bipolaris oryzae was detected only in two samples with infection levels of 0.25 (variety Cherehani) and 0.5% (variety India Rangi ya Mkia) respectively. *Alternaria padwickii* was detected only in one sample with infection levels of 0.75% (variety Zambia) (Tables 7 and 8).

Table 8: Range of infection of seed-borne fungal pathogens in farmers' saved rice seed (*Oryza sativa* L.) samples collected from Mbeya region

Pathogen	Samples infection (Nos.)	Range of infection in percentage				
		0 - 1	2 - 3	4 -10	11-16	17-30
<i>Fusarium moniliforme</i>	12	1	-	8	1	2
<i>Phoma</i> sp.	9	6	2	1	-	-
<i>Bipolaris oryzae</i>	2	2	-	-	-	-
<i>Alternaria padwickii</i>	1	1	-	-	-	-

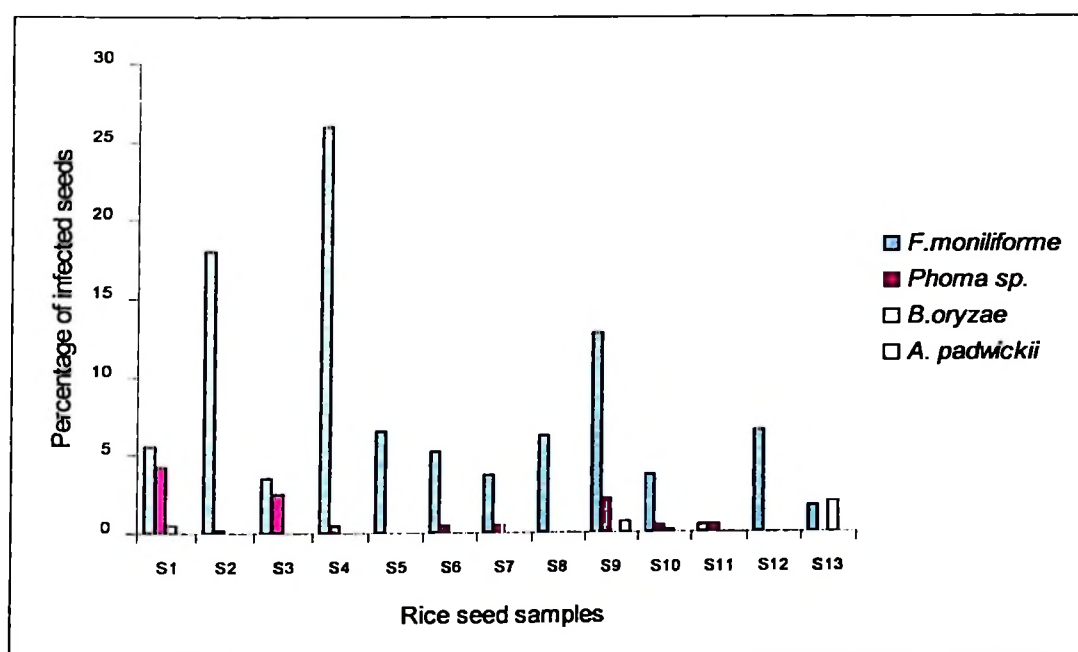


Figure 3: Percent frequency of seed-borne fungi detected on rice seed collected from Mbeya region.

S1 = India Rangi ya Mkia, S2 = India Rangi ya Mkia, S3 = India Rangi ya Mkia, S4 = Zambia, S5 = Mwenda Mbio, S6 = India Rangi ya Mkia, S7 = Supamarti, S8 = India Rangi ya Mkia, S9 = Zambia, S10 = Cherehani, S11 = Rubi, S12 = Kilombero, S13 = Supa

While in Morogoro region, *Fusarium moniliforme* was detected in all farmers' saved rice seeds collected (Table 9 Acc. No. 07/110 - 07/121, Table 10 and Fig. 4) with infection ranging from 1.5 (variety Kisegese) to 13.5% (variety India Supa). *Phoma* sp. was detected with low infection ranging from 0.25 (variety Masantula) to 2.25% (India Supa) (Table 9).

Table 9: Infection levels of various seed-borne fungi detected from farmers' saved rice seed collected from Morogoro region

Acc. No.	Variety	Pathogen identified and percent infection (in bracket)
07/110	Kalimawangu	<i>Bipolaris oryzae</i> (3%), <i>Fusarium moniliforme</i> (8.75%), <i>Phoma</i> sp. (1.75%).
07/111	Kangaga	<i>Bipolaris oryzae</i> (1%), <i>Fusarium moniliforme</i> (2.25%).
07/112	Kisegese	<i>Bipolaris oryzae</i> (0.5%), <i>Fusarium moniliforme</i> (1.5%).
07/113	India Supa	<i>Bipolaris oryzae</i> (17.25%), <i>Fusarium moniliforme</i> (13.5%), <i>Phoma</i> sp. (2.25%).
07/114	Saro	<i>Bipolaris oryzae</i> (3.25%), <i>Fusarium moniliforme</i> (3.25%), <i>Phoma</i> sp. (0.5%).
07/115	Saro	<i>Bipolaris oryzae</i> (3.25%), <i>Fusarium moniliforme</i> (7.25%), <i>Phoma</i> sp. (0.5%).
07/116	Afaa Kilombero	<i>Bipolaris oryzae</i> (2.75%), <i>Fusarium moniliforme</i> (4%), <i>Phoma</i> sp. (0.5%).
07/117	Jaribu	<i>Bipolaris oryzae</i> (2.75%), <i>Fusarium moniliforme</i> (4%), <i>Phoma</i> sp. (0.5%).
07/118	Masantula	<i>Bipolaris oryzae</i> (1.75%), <i>Fusarium moniliforme</i> (8%), <i>Phoma</i> sp. (0.25%).
07/119	Domo la Fisi	<i>Bipolaris oryzae</i> (4%), <i>Fusarium moniliforme</i> (7%).
07/120	Kisegese	<i>Bipolaris oryzae</i> (0.5%), <i>Fusarium moniliforme</i> (5%).
07/121	Rufiji	<i>Bipolaris oryzae</i> (1%), <i>Fusarium moniliforme</i> (4.5%).
Control	Supa	<i>Bipolaris oryzae</i> (2%), <i>Fusarium moniliforme</i> (1.75%).

Bipolaris oryzae was detected in all rice seed samples (Table 10 and Fig. 4) with infection ranging from 0.5 (variety Kisegeese) to 17.25% (variety India Supa) (Table 9). *Alternaria padwickii* was not detected in any sample from Morogoro region. The control sample collected from Msimba Seed Farm (certified seed), had 2% infection with *B. oryzae* and 1.75% infection with *F. moniliforme* (Table 9).

Table 10: Range of infection of seed-borne fungal pathogens in farmers' saved rice seed (*Oryza sativa* L.) samples collected from Morogoro region

Pathogen	Samples infection (Nos.)	Range of infection in percentage				
		0-1	2-3	4-10	11-16	17-30
<i>Fusarium moniliforme</i>	12	-	4	7	1	-
<i>Phoma</i> sp.	6	4	2	-	-	-
<i>Bipolaris oryzae</i>	12	5	4	2	-	-
<i>Alternaria padwickii</i>	0	-	-	-	-	-

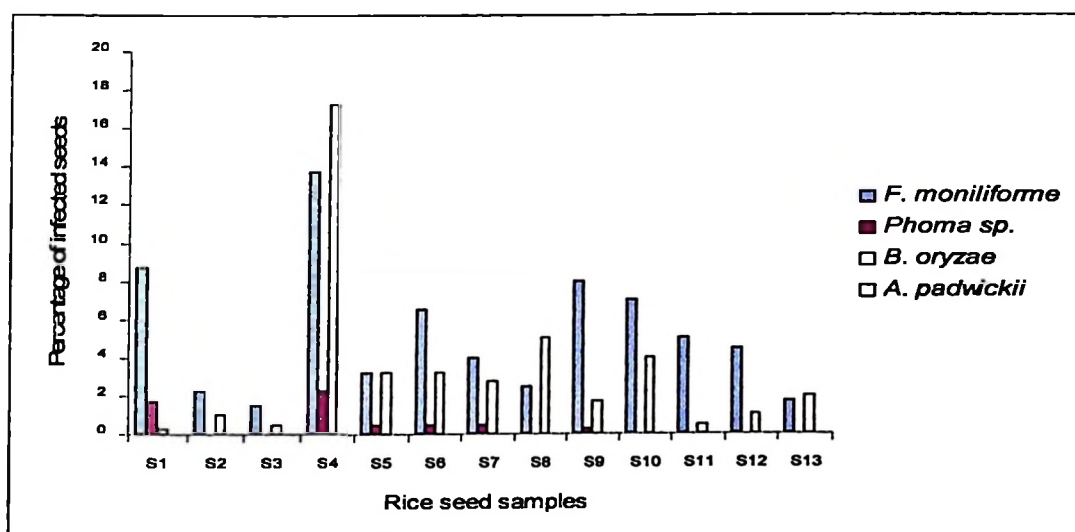


Figure 4: Percent frequency of seed-borne fungi detected on rice seed collected from Morogoro region.

S1 = Kalimawangu, S2 = Kangaga, S3 = Kisegeese, S4 = India Supa, S5 = Saro, S6 = Saro, S7 = Afaa Kilombero, S8 = Jaribu, S9 = Masantula, S10 = Domo la Fisi, S11 = Kisegeese, S12 = Rufiji, S13 = Supa

Fusarium moniliforme has been reported to be economically important in cereals such as maize and rice. The report by Owolade *et al.* (2001) indicated that *F. verticillioides* (syn. *F. moniliforme*) was associated with different types of maize (*Zea mays*) and rice seed discolouration, Bakanae disease (a complex of disease symptoms) and seed rot in rice (Mathur and Manandhara, 2003). The disease can result in yield losses of up to 50% (Webster and Gunnell, 1992). In addition, Nsemwa and Wolffhechel (1999) reported that, *F. moniliforme* as one of the important seed-borne pathogen in rice in Southern Tanzania has wide range of hosts. This fungal pathogen has been reported to produce mycotoxins in rice seeds (Edicar Wulff, personal communication 2009).

Bipolaris oryzae causes brown spot, seedling blight of *O. sativa* and seed discolouration. The fungus can survive in infected grain for a varying length of time depending on the storage conditions; it can survive in the infected seed for at least four years and lowers seed germination from 11 - 29% (Agarwal *et al.*, 1989; Mathur and Manandhar, 2003).

The highest incidence of *Phoma* sp. (causative agent of leaf spot) dominated most of the seed samples. Leaf spot and brown spot have been reported to adversely affect the yield and milling quality of the rice grain; and yield loss estimates range from 16 - 40 percent (Webster and Gunnell, 1992) Seed-borne fungal *Alternaria padwickii* had low incidence in all of samples under study. The fungus has been reported to be associated with seed discolouration and results in poor germination, rotting of seed, root and coleoptile, in the ultimate death of young seedlings (Agarwal *et al.*, 1989).

4.3 Isolation and identification of bacteria

A total of 52 Gram-negative bacterial isolates were obtained from rice seed samples collected from the three regions and two Gram-negative bacterial isolates from the control samples collected from Msimba Seed Farm. The results of percentage germination and seedlings with bacterial disease symptoms are shown in (Table 11). The germination percentage of most rice varieties were above 50%. The percentage of seedlings with bacterial disease symptoms varied from 26.3% for accession 07/108 to 81.5% for accession no. 07/122 (Table 11). The symptoms which were observed on the seedlings in the Cassette Holder Method included brown lesions on the coleoptile, leaf sheaths and stripes extended to the leaf blades. Leaf yellowing, stunted seedlings and brown lesions on the leaf tips (Plate 2).



Plate 2: Seedling symptoms test using the Slide Cassette Holder method showing rice seedlings with disease symptoms (A).

The bacterial pathogens isolated were mostly *A. a. subsp. avenae* (11 samples); *P. s. pv. syringae* (5 samples) and *P. fuscovaginae* (5 samples including the control) (Table 11). Pathogenic bacteria were not isolated from varieties with accession nos. Mbegu Bora (07/86), Supa India (07/87), Beneko (07/88), Mzuri Hajipakia Mafuta (07/92), India Rangi ya Mkia (07/98), India Rangi ya Mkia (07/100), Zambia (07/101), Mwenda Mbio (07/102), India Rangi ya Mkia (07/103), Supamarti (07/104), Cherehani (07/107), Rubi (07/109), Kisegese (07/112), Saro (07/114), Saro (07/115) and Rufiji (07/121).

Table 11: Percentage of seedlings showing symptoms of various bacterial pathogens isolated using the seedling symptom Slide Cassette-Holder Method

Acc. No.	Variety	Source	Germination (%)	Seedlings w/Symptoms (%)	Pathogen(s) Isolated
07/89	Kalamata	Kahama	50.8	46.3	<i>A. avenae</i> subsp. <i>avenae</i>
07/90	Kalamata	Kahama	61.3	58.8	<i>A. avenae</i> subsp. <i>avenae</i>
07/91	Kalamata	Kahama	55.0	50.0	<i>A. avenae</i> subsp. <i>avenae</i>
07/93	Malamata	Shinyanga (R)	73.8	68.0	<i>A. avenae</i> subsp. <i>avenae</i>
07/94	Mzuri Hajipaki Mafuta	Shinyanga (R)	70.0	65.3	<i>A. avenae</i> subsp. <i>avenae</i>
07/95	Rangi Mbili	Shinyanga (R)	67.5	65.0	<i>A. avenae</i> subsp. <i>avenae</i>
07/96	Supa India	Shinyanga (R)	72.5	68.8	<i>A. avenae</i> subsp. <i>avenae</i>
07/97	Daniel	Shinyanga (R)	70.0	64.0	<i>A. avenae</i> subsp. <i>avenae</i>
07/99	India Rangi ya Mkia	Mbeya (R)	92.0	31.3	<i>A. avenae</i> subsp. <i>avenae</i>
07/105	India Rangi ya Mkia	Mbarali	95.0	35.0	<i>P. fuscovaginae</i>
07/106	Zambia	Mbarali	91.3	30.0	<i>A. avenae</i> subsp. <i>avenae</i>
07/108	Rubi	Mbarali	91.5	26.3	<i>P. fuscovaginae</i>
07/110	Kalimawangu	Kilombero	88.0	48.3	<i>A. avenae</i> subsp. <i>avenae</i>
07/111	Kangaga	Kilombero	85.5	43.8	<i>P. fuscovaginae</i>
07/113	India Supa	Kilombero	80.3	37.5	<i>P. syringae</i> pv. <i>syringae</i>
07/116	Afaa Kilombero	Kilosa	87.3	40.0	<i>P. syringae</i> pv. <i>syringae</i>
07/117	Jaribu	Kilosa	80.0	42.5	<i>P. syringae</i> pv. <i>syringae</i>
07/118	Masantula	Kilosa	75.0	45.0	<i>P. syringae</i> pv. <i>syringae</i>
07/119	Domo la Fisi	Kilosa	81.0	36.5	<i>P. syringae</i> pv. <i>syringae</i>
07/120	Kisegese	Kilosa	82.5	40.0	<i>P. fuscovaginae</i>
07/122	Supa	Kilosa	86.5	81.5	<i>P. fuscovaginae</i>

Some of the bacterial isolates produced a brown diffusible pigment on KB medium and had variable hypersensitive reaction on tobacco, were not pathogenic on rice seedlings using the stem inoculation methods, thus were discarded. The characteristics of bacteria isolated from rice seed grown using the slide cassette holder method are summarized in Table 12.

Table 12: Biochemical characteristics of bacterial strains detected using seedling symptoms slide cassette-holder method

No. of samples	Pathogen	Biochemical tests											
		Pigme nt	GR	Oxidas e	Arg d	Po	O/ F	N R	G L	LF	HR/ T	Pathog	
11	<i>A.avenae</i> subsp. <i>avenae</i>	-	-	+	-	-	+/-	+	-	N	+	+	
5	<i>P. syringae</i> pv. <i>syringae</i>	+	-	-	-	-	+/-	-	+	+	+	+	
5	<i>P. fuscovaginae</i>	+	-	+	+	-	+/-	-	+	-	+	+	

GR= Gram reaction; Argd = arginine dihydrolase; Po = Potato soft rot; O/F = oxidative/fermentative metabolism of glucose; NR = nitrate reduction; GL = gelatin liquification; LF = levan formation from sucrose;

HR = hypersensitive reaction, T = tobacco;

Pathog = pathogenicity; ND = not done; + = positive reaction; - = negative reaction.

4.4 Hypersensitive reaction

Of the 54 Gram-negative bacterial isolates from rice seed subjected to hypersensitive reaction on tobacco plants, 21 isolates showed positive reaction and 33 isolates gave negative reaction. The pathogenic bacteria induced symptoms which were a rapid collapse and water soaking of inoculated tobacco areas within 24 hrs followed by a dry, light-brown localized necrosis within three days (Plate 3).

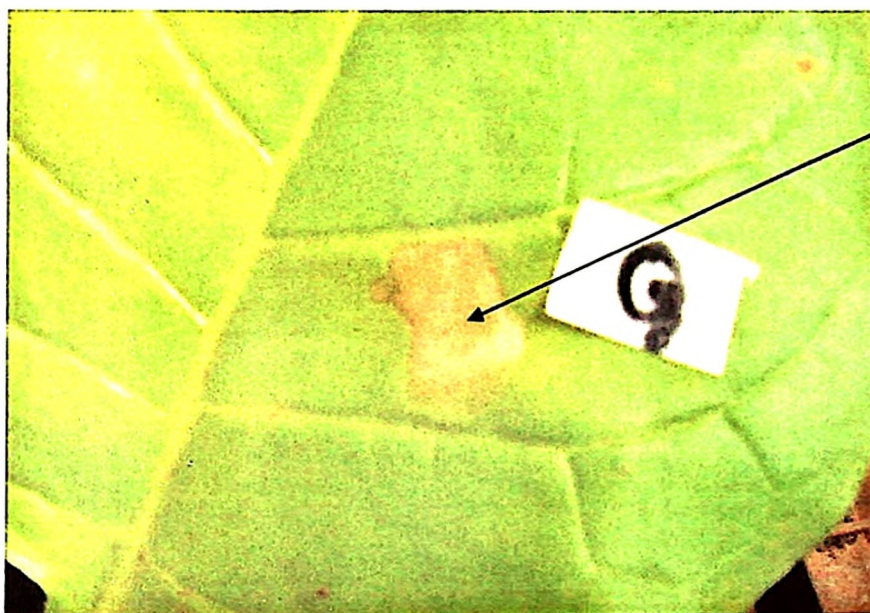


Plate 3: Hypersensitive reaction on tobacco leaf inoculated with *Acidovorax avenae* subsp. *avenae* (arrow).

4.5 Pathogenicity Tests

Of the 54 Gram-negative bacterial isolates subjected to pathogenicity test by the stem inoculation on rice seedlings, only 21 bacterial isolates produced plant disease symptoms similar to the reference bacterial strain. Known bacterial reference strains (*A. a.* subsp. *avenae* (Nepal), *P. s.* pv. *syringae*, *P. fuscovaginae*, *Burkholderia glumae* (NCCPB 2391) were included as positive controls.

Several bacterial isolates were associated with rice varieties, Kalamata Acc. Nos. (07/89), (07/90), (07/91), (07/93), Mzuri Hajipaki Mafuta (07/94), Rangi Mbili (07/95), Supa India (07/96), Daniel (07/97), India Rangi ya Mkia (07/99), Zambia (07/106) and Kalimawangu (07/110). Acc. No. 07/89 to 07/97 were collected from Shinyanga region (8 isolates), 07/99 and 07/106 from Mbeya region and 07/110 was collected from Morogoro region. Strains from these collections showed similar disease symptoms to those induced by the reference bacterial strain of *A. a. subsp. avenae* (Nepal), causing disease bacterial stripe symptoms, dark brown stripes on leaves and yellow discolouration of seedlings (Plate 4). Severe infection caused stunting and seedling death. Seeds showed moderate to severe discolouration of the glumes and endosperm. These results indicate that rice samples from Shinyanga region were mostly infected by *A. a. subsp. avenae* when compared to Morogoro (2 isolates) and Mbeya (1 isolate).

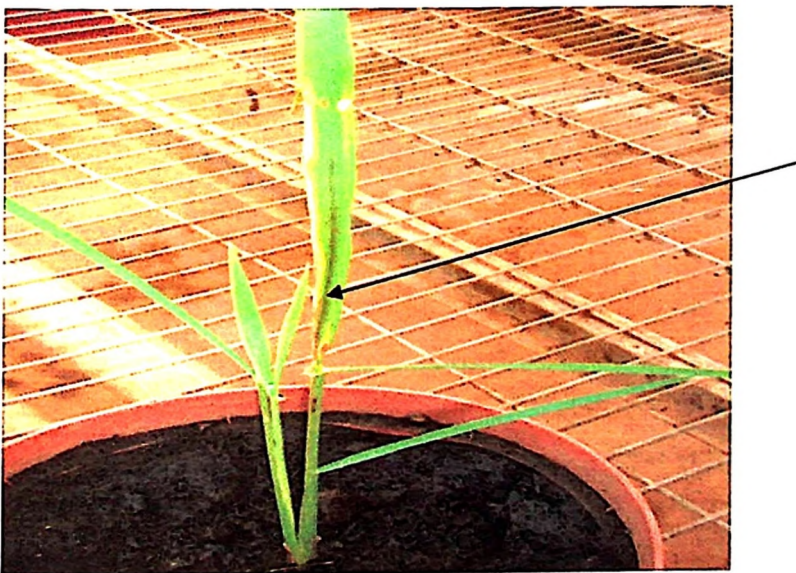


Plate 4: Symptoms of *Acidovorax avenae* subsp. *avenae* on rice seedling artificially inoculated (arrow) on variety Kalamata Acc. No. 07/93 from Shilabela in Shinyanga Rural district.

Detection of *A. a. subsp. avenae* in most of the rice seed samples tested indicates that this pathogen is widely distributed in Tanzania. Ashura *et al.* (1999) reported this pathogen as a common contaminant of rice seeds in Tanzania. The pathogen has been shown to have a wide distribution in other countries including Colombia, Uganda, Bukina Faso (CABI, 2005). Most of the farmers in Tanzania retain seed from the previous year for planting the next season. In addition, the bacterium can survive in rice seed up to 8 years at 5 °C (Shakya and Chung, 1985). This means that the disease can spread very fast. Effective control of brown stripe can be achieved by using pathogen-free seeds, hot air seed treatment and the use of resistant varieties (Zeigler *et al.*, 1987). Such an approach may prove to be very expensive for the resource poor farmers in Tanzania. The use of chemicals is also limited by the risk of environment pollution and the increase in production cost and limited availability, especially for small-holder farmers.

Five bacterial isolates were obtained from rice varieties India Supa Acc. No. (07/113), Afaa Kilombero (07/116), Jaribu (07/117), Masantula (07/118) and Domo la Fisi (07/119). All the bacterial isolates from Morogoro region induced similar disease symptoms as the reference bacterial strain *P. s. pv. syringae* causing sheath rot with brown or black lesions on sheaths, nodes and stems. Sheath discolouration lesions elongated, turned brown to reddish and the resulting necrosis lead to drying of the entire plants (Plate 5). Grain discoloration, seed infection and sterility occurred in extreme cases. Control of the seed-borne bacterial pathogens may be done using clean/pathogen-free seeds and resistant varieties (Zeigler *et al.*, 1987).



Plate 5: Symptoms caused by *Pseudomonas syringae* pv. *syringae* on rice seedling artificially inoculated (arrows) on variety India Supa Acc. No. 07/113 from Mgudeni in Kilombero district.

Bacterial isolates from varieties India Rangi ya Mkia Acc. No. 07/105, Rubi (07/108), Kangaga (07/111), Kisege (07/120) and Supa (07/122) control. Acc. Nos. 07/105 and 07/108 were collected from Mbeya region, 07/111, 07/120 and 07/122 were isolated from rice seed collected from Morogoro region. These isolates induced similar disease symptoms to those induced by *P. fuscovaginae* reference strain which caused bacterial sheath brown rot symptoms (Plate 6) brown water soaked necrotic areas on the leaf sheaths and death of seedlings. Bacteria were isolated from only one sample collected from Mbeya region and two samples from Morogoro region. Zeigler and Alvarez (1987) reported that the rate of pathogen transmission can be very high even when low levels of seed infection are found in samples.

Heat treatment at 65 °C for 6 days has been recommended for the eradication of the bacterium from the infected seed lots (Zeigler *et al.*, 1987). Such approach may prove to be very expensive for the resource poor farmers in Tanzania, but can be applied to reduce inoculum in basic seed.



Plate 6: Symptoms caused by *Pseudomonas fuscovaginae* on rice seedling artificially inoculated (arrow) on variety Kisegese Acc. No. 07/120 from Chazulu in Kilosa district.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The results of the current study reveal the presence of seed-borne pathogens on farmers' saved rice seed samples collected from Shinyanga, Mbeya and Morogoro regions. All rice seed samples tested were infected by seed-borne fungal pathogens. *Fusarium moniliforme*, *Phoma* sp., *Bipolaris oryzae* and *Alternaria padwickii* were commonly detected in farmers' saved rice seeds from the three regions and caused seed discolouration.

Of the seed-borne fungal pathogens identified in farmers' saved rice seeds, *F. moniliforme*, the causal agent of Bakanae disease and foot rot in rice caused the highest seed infection compared to other pathogens. It was detected in all rice seed samples tested followed by *B. oryzae* which was detected in all seed samples collected from Morogoro. Seed infection by *Phoma* sp. was the lowest while *A. padwickii* was only detected in three rice samples.

The study indicated that the control variety Supa (certified) from Msimba seed farm was also infected by *B. oryzae*. Therefore, the certified rice seeds from which the sample was collected when sold to farmers for planting were already contaminated with a fungal pathogen. Therefore, there is a need for ensuring that certified rice seed sold to farmers are healthy. Seed-borne bacteria also caused discolouration on farmers' saved rice seeds. These included *A. a. subsp. avenae*, *P. s. pv. syringae* and *P. fuscovaginae*. *Acidovorax avenae* subsp. *avenae* dominated most rice seed samples tested compared to other bacteria detected.

The results indicate that rice seed samples from Shinyanga region had higher seed infection by *A. a. subsp. avenae* (8 samples), than samples collected from Mbeya region (2

samples) and Morogoro region (1 sample). *Pseudomonas syringae* pv. *syringae* was detected in 5 seed samples collected from Morogoro region. *Pseudomonas fuscovaginae* was detected in 5 seed samples, (3 samples) collected from Morogoro and (2 samples) from Mbeya.

The germination capacity of most of the seed samples tested was found to be below the national minimum recommended standards. Thus, there is a need for farmers to use certified seeds produced from seed farms with acceptable levels of germination.

5.2 Recommendations

1. This study has shown that, most of the farmers' saved rice seeds from Shinyanga, Mbeya and Morogoro regions were infected by seed-borne fungal and bacterial pathogens and caused discolouration and low germination. Hence, there is a need for encouraging farmers to use certified healthy seed in order to reduce losses and the spread of diseases caused by seed-borne pathogens.
2. The availability of certified rice seed was limited to Morogoro region only and not available in Shinyanga and Mbeya regions. There is a need for these regions to encourage the government to make available certified rice seed to farmers.
3. The current study has also shown that rice seeds collected from Msimba seed farm (certified seed) and used as controls had low germination percentage below the national recommended germination level. It is very important that farmers are assured of clean and healthy rice seeds and therefore, Msimba seed farm should be more careful to ensure production of good quality seed with recommended germination levels and pathogen free.

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APPENDENCES

Appendix 1: Farmers' saved rice seed varieties, location where obtained, harvest date and method of cultivation used

Acc. No.	Variety	Location	Harvest date	Cultivation method
07/86	Mbegu Bora	Kahama	April 2007	Rainfed lowland
07/87	Supa India	Kahama	June 2007	Rainfed lowland
07/88	Beneko	Kahama	June 2007	Rainfed lowland
07/89	Kalamata	Kahama	April 2007	Rainfed lowland
07/90	Kalamata	Kahama	May 2007	Rainfed lowland
07/91	Kalamata	Kahama	June 2007	Rainfed lowland
07/92	Mzuri Hajipaki Mafuta	Shinyanga Rural	May 2007	Rainfed lowland
07/93	Kalamata	Shinyanga Rural	April 2007	Rainfed lowland
07/94	Mzuri Hajipaki Mafuta	Shinyanga Rural	April 2007	Rainfed lowland
07/95	Rangi Mbili	Shinyanga Rural	March 2007	Rainfed lowland
07/96	Supa India	Shinyanga Rural	April 2007	Rainfed lowland
07/97	Daniel	Shinyanga Rural	April 2007	Rainfed lowland
07/98	India Rangi ya Mkia	Mbeya Rural	June 2007	Rainfed lowland
07/99	India Rangi ya Mkia	Mbeya Rural	June 2007	Rainfed lowland
07/100	India Rangi ya Mkia	Mbeya Rural	June 2007	Rainfed lowland
07/101	Zambia	Mbeya Rural	June 2007	Rainfed lowland
07/102	Mwenda Mbio	Mbeya Rural	May 2007	Rainfed lowland
07/103	India Rangi ya Mkia	Mbeya Rural	April 2007	Rainfed lowland
07/104	Supamarti	Mbarali	June 2007	Rainfed lowland
07/105	India Rangi ya Mkia	Mbarali	May 2007	Rainfed lowland
07/106	Zambia	Mbarali	June 2007	Rainfed lowland
07/107	Cherehani	Mbarali	June 2007	Rainfed lowland

07/108	Rubi	Mbarali	May 2007	Rainfed lowland
07/109	Kilombero	Mbarali	June 2007	Rainfed lowland
07/110	Kalimawangu	Kilombero	June 2007	Rainfed lowland
07/111	Kangaga	Kilombero	June 2007	Rainfed lowland
07/112	Kisegese	Kilombero	June 2007	Rainfed lowland
07/113	India supa	Kilombero	July 2007	Rainfed lowland
07/114	Saro	Kilombero	May 2007	Rainfed lowland
07/115	Saro	Kilombero	June 2007	Rainfed lowland
07/116	Afaa Kilombero	Kilosa	April 2007	Rainfed lowland
07/117	Jaribu	Kilosa	April 2007	Rainfed lowland
07/118	Masantula	Kilosa	May 2007	Rainfed lowland
07/119	Domo la Fisi	Kilosa	June 2007	Rainfed lowland
07/120	Kisegese	Kilosa	June 2007	Rainfed lowland
07/121	Rufiji	Kilosa	April 2007	Rainfed lowland
07/120	Supa	Kilosa	July 2007	Rainfed lowland
