SCREENING BUFFELGRASS (*Cenchrus ciliaris*) FROM SELECTED PASTURE SEED FARMS IN TANZANIA FOR SEED-BORNE MICROORGANISMS: PATHOGENICITY AND EFFECT ON GERMINATION

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ABSTRACT

Buffelgrass (*Cenchrus ciliaris*) is one of the important perennial grasses in the pasture industry in Tanzania. It is drought tolerant, nutritious and has rapid growth characteristics. Three hundred sixty grams of seed samples of *C. ciliaris* collected from six selected pasture seed farms were screened for seedborne microorganisms at the African Seed Health Centre, Sokoine University of Agriculture, Morogoro Tanzania. The Blotter method, the direct plating and the Top of Paper methods were used for fungal, bacterial and seed germination tests, respectively. Fungal and bacteria pathogens of economic importance that were detected included; *Phoma* spp (28.5 %), *Curvularia lunata* (17.34 %), *Alternaria alternata* (14.09 %) *Bipolaris* spp. (12.2 %), *Acidovorax*, *Xanthomonas* and *Pseudomonas* spp. Characterization using morphological and biochemical tests including molecular techniques and pathogenicity of fungal and bacteria strains were done on buffelgrass seedlings. Results indicated that seed germination decreased (<50%) with an increase in fungal infection. Seed samples from LITI Tengeru had the lowest seed germination (8 %) and high fungal (37.8 %) incidence on their caryopses. Pathogenicity of *Bipolaris spp., Phoma spp., Pyricularia grisea, Fusarium pallidoroseum, Exserohilum rostratum, Nigrospora oryzae* and *Acidovorax, Pseudomonas* and *Xanthomonas* bacterial strains were confirmed on *C. ciliaris* seedlings. Some species of the detected fungi were found in both spikelets and caryopses. Further research is needed on losses caused by fungal infection in buffelgrass seeds.

DECLARATION

I, John Aloyce Mlay, do hereby declare to the Senate of Sokoine University of Agriculture that, this dissertation is my own original work and has neither been submitted nor concurrently been submitted for higher degree award in any other institution.

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2. **Prof. E.J. Mtengeti**

(Supervisor)

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DEDICATION

This work is dedicated to my beloved wife Dafrosa John Tarimo, my children Venitha, Magnus, Joel and Innocent. I always love you.

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LIST OF ABBREVIATIONS AND SYMBOLS

^{o}C	Degrees Centigrade
%	Percentage
ABS	Abnormal seedling
AfSHC	African Seed Health Centre
ANOVA	Analysis of Variance
cm	Centimetre
CRD	Completely Randomized Design
CV	Coefficient of variation
DAI	Days after inoculation
DASP	Department of Animal Science and Production
DM	Dry matter
DNA	Deoxyribonucleic acid
dNTP	Deoxy-ribonucleotide tri-phosphate
DS	Dead seed
FAO	Food and Agriculture Organization
H	Hour
Ha	Hectare
HR	Hypersensitive Reaction
HS	Hard seed
IM	Inert Matter
ISTA	International Seed Testing Association
KB	King's medium B
LITI	Livestock Training Institute
LRC	Livestock Research Centre
LU	Livestock Unit
MC	Moisture Content
MLFD	Ministry of Livestock and Fisheries Development
MWLD	Ministry of Water and Livestock Development
NA	Nutrient Agar
NLP	National Livestock Policy
NLRI	National Livestock Research Institute
NUV	Near Ultra Violet
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PLS	Pure Live Seed
PS	Pure Seed
RH	Relative Humidity
S.E	Standard Error
SD	Standard Deviation
SUA	Sokoine University of Agriculture
Xoc	*Xanthomonas oryzae* pv. *oryizicola*
Xoo	*Xanthomonas oryza* pv. *oryzae*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Pasture is a major source of feed for livestock in many parts of the world. In Tanzania, pasture provides over 90 percent of ruminants feed requirements (Sarwatt and Mollel, 2002). However, cultivar development and improvement in most African countries including, Tanzania is rare. Africa ranks last in the world's pasture production, cultivar improvement, seed quality control and distribution of pasture seeds (Jutzi, 1986; Lwoga *et al.*, 1984). In Tanzania, availability of high quality pasture and pasture seeds is a major production constraint (NLP, 2006). Factors known to influence pasture production include, plant species, soil fertility, weather, plant diseases, and agronomic practices.

In Tanzania, where pasture production is mostly practised in semi-arid areas, the former/early improved measures for pasture production included evaluation and promotion of drought tolerant species such as *Chloris gayana* and *Cenchrus ciliaris*, for high yields and nutritive value (Mero and Uden, 1998). However, their adoption rate has remained very slow partly due to poor availability of quality seeds in the country. Issoufou *et al*. (2008) also reported many of earlier studies by Mero and Uden (1998) and others, on buffel grass to have concentrated on estimating the nutritional value only, such as preference by animals through intake and *in vitro* organic digestibility. Other studies focused on nitrogen use efficiency (Rudmanna *et al*., 2001), variation and the relationship of several seed and spikelets related traits of buffelgrass (M'Seddi *et al*., 2003).

In areas where improved pasture measures are practiced, seed production is usually intended to make quality seeds or vegetative material available to interested farmers/producers (HSU, 1994). Key seed quality aspects include varietal purity, high germination percentage, absence of disease conditions or their causative organisms, proper moisture content and specific seed weight (Santos, 2010). At recommended seed rates, such seeds should be capable of germinating, emerging, and establishing uniformly under normal field conditions. With quality seeds, farmers are more likely to achieve an even stand of the stated cultivar without introducing weeds or diseases. This requires appropriate measurements and assurance of quality in the market such that seeds sold are accurately described. Seed quality assurance is an integral part of any effective seed supply system.

In Tanzania specialized systems for pasture seed production are lacking. Often, pasture seed production is carried out with limited inputs and the crop is harvested with limited attention to seed quality. In such cases, farmers or other opportunistic seed gatherers may hand pluck pasture seeds from roadsides, plantations or around food crop-fields. Seeds harvested in this manner are also processed, handled and stored under different ways but pooled at selling. This usually characterises the informal seed production sector in good seasons with plenty of rain that may lead to excellent flowering and seed formation but of poor seed quality.

In this system, grass seed may be hand stripped into containers or flower stalks cut with a reaping hook or sickle. Seed heads are then dried and threshed. At threshing, seeds may be swept off the ground, a method that leads to high inert matter content

(non-seed material), especially if seeds are in short supply and have a high market value. Although it is possible through the opportunist system to produce seed of high quality at low cost, it is often blended/mixed by a range of seed maturity, at harvest. Quality depends on the skill of the grower and timeliness of harvesting operations. Availability of good seed quality seeds has been listed among important factors needed to improve ruminant nutrition in Tanzania (Mwilawa, 2005).

Buffelgrass is one of the perennial grasses considered to be useful pasture plants (Grice and Martin 2005). Its rapid growth and reproduction allow it to spread and establish quickly to out-compete many native species (Jackson, 2005). In Tanzania, an increasing demand and interest on drought tolerant buffelgrass has been found to raise (Ngota, J. Personal communication, 2010) probably due to the current climate changes which seems not to affect the crop seriously. Jorge *et al*. (2008) also noted the same increase in demand and interest in buffelgrass in Ethiopia. Buffelgrass is a prolific seed producer, yielding between 490 to 2300 seeds m^{-2} Hacker and Ratcliff (1989) or 150 to 500kg/ha (Cook, 2007). It is also high in forage yield, 13,800kg/ha with no fertilizers (Ocumpaugh *et al*., 1994).

Poor seed germination has been reported by Bishaw (2003) in buffelgrass. The author noted genetical and pathological aspects to be associated with poor germination for most pasture seeds. This stresses the importance of seed health aspects to agricultural development, both food and pasture crop production. Pasture seed may be passive carrier for transmitting seed-borne pathogens which cause partial or total loss of the pasture crop. Seed-borne pathogens may be pathogenic or

non-pathogenic. Seedborne pathogens affect germination, plant vigour, and cause disease in seedlings and plants if not properly managed (Wulff *et al*, 2011). Usually seeds are said to be infected when they carry fungi, bacteria, virus or nematodes which can hinder seed germination or be transmitted to infect the resulting crop (Maude, 1996).

Fungi are considered the most important group of plant pathogens in agriculture, causing losses in both quantity and quality (Fletcher *et al*, 2006; Hajihasani *et al*., 2012). Furthermore, of all plant pathogens, fungi are reported to be responsible for the greatest damage to plants in both agricultural and natural ecosystem (Fletcher *et al.*, 2010). In grasses, most of the pathogens found in seeds cause smut, false rust, ergot, blight or leaf spot and inflorescence disease (Maritza *et al*., 2009). Important plant fungal pathogens reported to be associated with buffelgrass include, *Fusarium oxysporum, Bipolaris* spp., *Pyricularia grisea, Clavisceps, F. moniliforme, F. pallidoroseum, and Phoma* spp. (Makiela *et al*; 2003, Mathur and Mahandhar, 2003; Fridiel *et al*; 2006, Ndomba, 2009; Cameron, 2010). According to Perrot (2000) buffelgrass blight caused by *P. grisea* and ergot *(Claviceps* spp.*)* affecting seed production, are the most important diseases of buffelgrass. *F. oxysporum* has also been found in association with buffel dieback (Makiela *et al.,* 2003). Studies by Campbell and Medd (2003) also showed that post-dispersal infection of mature seeds (as would occur in seed banks) is possible and infection results in a temporary reduction in seedling growth. Therefore, the effect of seed-borne microorganisms, their effect on germination, seedling growth rate and seed pricing as related to seed quality were studied in the current research.

1.2 Justification

Poor seed germination has been observed in pasture seed farms in the country and this has contributed to lack of progress in pasture production and scarcity of quality pasture seeds. Studies on microorganisms associated with grass seeds (including buffelgrass) have shown presence of fungal infection (4.5%) (Ndomba, 2009). Attempts have been made to control fungal seed infection through treatment of seeds with chemicals (such as copper sulphate) but did not improve germination (Rukiko, 2011). It was suspected that, the observed low germination percentage was due to fungal infection. This has economic implication to farmers as they have to use high seed rates to improve initial plant population. Although, there have been minimal research on seed quality and seed health of buffelgrass in Tanzania, there is still a scarcity of published reports. Furthermore, there is paucity of information on the infection levels of seeds from various locations and their effect on yield of buffelgrass.

In Tanzania, seed technology has mainly been focused on moisture content, purity and germination percentage (William, 2006). Likewise, pasture producing farms have based their seed quality evaluations on these aspects with very little/no emphasis on seed health testing. The genera of pathogenic fungi that are most found in grass seeds include *Ustilago*, *Uromyces* and *Claviceps* which cause smut, false rusts, ergot and inflorescence diseases, respectively. Most of them reduce seed viability and cause death of seedlings at the stage of pre-emergence and post-emergence (Maritza *et al.*, 2009). However, the location of these fungi on or within buffelgrass seed structure has not been investigated. Understanding the nature of

buffelgrass seed infection by these fungal pathogens may help to determine the potential for seed transmission as well as the potential efficacy of seed treatment techniques for eradication of the fungi in infected seed and/or prevention of seed transmission. With increasing specialisation and intensification on grass seed production, routine disease and insect control programmes should now increasingly become part of grass seed management. Thus, detection, identification and control of seedborne pathogens should now be an integral part of quality pasture seed production. To address this information gap, buffelgrass seeds were screened for seed-borne microorganisms and their effects on seed germination.

1.3 Objectives

The general objective of this study was to screen buffelgrass for seed-borne microorganisms, their pathogenic potential and effect on seed germination.

1.3.1 Specific objectives

(i) To detect and identify seed-borne microorganisms in buffelgrass seeds

(ii) To determine the pathogenicity of the isolated microorganisms on buffelgrass.

(iii) To determine the effect of isolated fungal microorganisms on germination of buffelgrass seeds

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Buffelgrass Plant

Buffelgrass (*Cenchrus ciliaris*) belongs to the family *Poaceae* (*Gramineae*). It is a perennial tufted rhizomatous grass and one of the best forage grasses for semi-arid areas in the subtropics and tropics (McIvor, 2005; Makiela and Harrower, 2008). The species grows well with annual rainfall of 350-800 mm and an altitude of 1000 m (Mushtaque *et al*., 2010). The seeding rate of 9 kg/ha has been reported by ISTA (2005). It is bisexual, with bisexual spikelets and hermaphrodite florets. It has high productivity and nutritive value which makes it a grass of choice for pastoral uses (Farooq *et al.*, 2003). It is considered excellent for pasture in hot, dry areas and provides intermittent grazing during drought periods in the tropics (Singariya, *et al.*, 2011). It is also a promising grass for rehabilitation of arid rangeland and has tolerance to heavy grazing and fires (Mushtaque *et al*., 2010; M'seddi *et al.*, 2003).

The grass can be fed green, turned into silage, or made into hay and is said to increase flow of milk in cattle and impart a sleek and glossy appearance (Singariya *et al.*, 2011). This grass has excellent soil binding capacity which helps to conserve soil in desert areas (Sinha *et al.*;1996), and has also expressed maximum antibacterial and antifungal activities in humans by suppressing the growth of certain microbes (Singariya, *et al.*, 2011).

2.1.1 Feeding value

Buffelgrass withstands heavy grazing, it is also highly drought tolerant, well adapted to arid and semi-arid areas (Batra and Kumar, 2003; Cameron, 2010). It is highly palatable to all kinds of grazing animals, but the substantially high lignin content (3-5 %) reduces its digestibility (Minson and Bray, 1986). The nutritive value of buffelgrass is high with 10.7 % crude protein (Mushtaque *et al.*, 2010), and digestibility which ranges from 50 to 60 %, (Jacobs *et al.*, 2004; Cook, 2007) depending on stage of growth, cultivar, and soil fertility (incl. fertilizer use). The grass, when fed green is said to increase flow of milk in cattle and imparting a sleek and glossy appearance (Burton, 1993). Phosphorus levels are usually higher in buffelgrass than other tropical grasses and ranges from 0.15 to 0.65% in the dry matter (Cook, 2007; IF, 2010).

2.1.2 Characteristics

At least 20–25 mm of rain is required for seed germination and establishment, as buffelgrass seeds need to be moist for about 3–5 days in order to germinate. Plants can germinate from seed, mature and flower within 6 weeks of a significant less than 100 mm of rainfall (Issoufou *et al.*, 2008). Germination trend of buffelgrass seed increases with storage time while dormancy is very high at harvesting time (Cook, 2007; Ibarra *et al.*, 2004; Fig. 1). Buffelgrass seed may survive for up to an estimated 4 years in the soil, but plants can live for many years (possibly up to about 20 years) (CRC, 2008). It is very palatable when young, and remains fairly palatable at maturity. Per 100 g, the fresh plant is reported to contain on a zero-moisture basis, 11.0 g protein, 2.6 g fat, 73.2 g total carbohydrate, 31.9 g fibre and 13.2 g ash at

vegetative growth stage (Gohl, 1981). Per 100 g, hay is reported to contain, on a zero-moisture basis, 7.4 g protein, 1.7 g fat, 79.2 g total carbohydrate, 35.2 g fibre, and 11.7 g ash (Aganga and Tshwenyane, 2003). Kumar *et al.* (2005) reported Buffelgrass seed yield of 97 kg/ha under fertilizer application, and other literature reported the yield of 150-500 kg seed/ha on well established pasture depending on growing conditions and variety (IF, 2010).

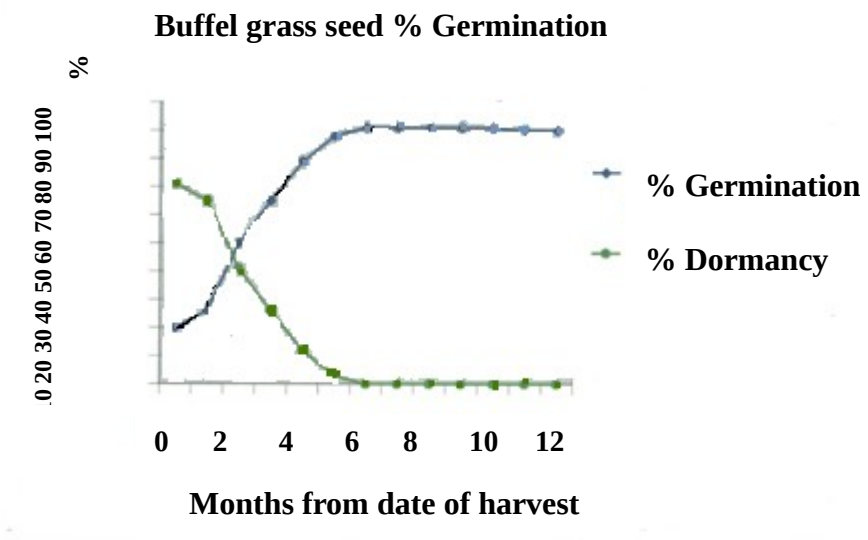

Figure 1: Buffelgrass seed germination and dormant percentage trends over time

Source: The buffel grass seed company (BSC), (2001)

2.2 Overview on Pasture Production in Tanzania

The rangeland resource of Tanzania is estimated to cover about 60 million hectares, of which about 40 million are devoted to grazing and 20 million to fallow and forestland (NLP, 2006). This resource supports about 17 million livestock units (LU).

Proper range management and disease control (tsetse) would open up more grazing land that could support over 20 million LU (Kavana, *et al.,* 2005). Attempts to improve pasture management and the use of established forages in Tanzania started in 1930s (MWLD, 2005). Major thrust has been on evaluation of different pasture species for adaptability to different agro-ecological zones, forage conservations techniques, use of browse species and the use of crop residues. In Tanzania, pasture establishment mostly remained practiced in institutional and parastatal farms; probably with very few or none in smallholder farms. Temu *et al.* (2005) reported reliance on communal land tenure system on grazinglands to have discouraged individual investment and unavailability of pasture seeds. Now, challenges are still for policy makers to enact good land tenure systems to encourage individual investment.

2.3 Seed Quality

Availability of high quality pure seeds in Tanzania has become a constraint in the production of pasture and pasture seeds (NLP, 2006). Seed quality aspects include genetics, species physical appearance, physiological properties (germination vigour), freedom from seedborne pests (seed health) and sanitary quality (ISTA, 2005). Low caryopsis index (30%), low germination (12%) and poor seed quality of buffelgrass seeds was reported by Rukiko (2011) on pasture seeds collected from four pasture seed producing farms in the country. The low seed quality observed imply that the usual sowing rate of 2 to 5 kg seed/ha described by Cameron (2010) need to be adjusted and/or can no longer be relied upon. Seeds are the primary means of elivering the potential of plant genetic resources; thus, maintaining seed quality is of

utmost importance. Maintenance of seed quality in storage from the time of production until the seed is planted is imperative to assure its planting value and avoid financial loss. The best alternative to avoid the risks associated with storage, which is deterioration of the quality of seeds, is to avoid storing the seeds under improper conditions. However, there are times when seed growers and dealers carryover seed lots across years in anticipation for better market, insure adequate supply in the following year, or other reasons. Under such circumstances the question is how to manage the seeds to maintain their quality (viability and vigor) throughout the storage period. In general, seeds maintain their quality under favorable storage conditions longer than if stored under poor conditions, e.g., high temperature and relative humidity.

A question that is frequently asked is whether good storage conditions enhance the quality of the seed? The answer is no. However, the quality of seeds can be maintained and the rate of seed deterioration can be slowed down by good storage environment. Once seeds deteriorate, their physiological quality cannot be restored because seed deterioration is inexorable and irreversible process, just like aging. Even seed enhancement techniques may allow the maximum expression of seed potential, but will not alter their basic physiological quality. Therefore, the extent and speed of drop in seed quality is largely dependent on the storage temperature, relative humidity (RH), seed moisture content, length of storage time, kind of seeds, and initial seed quality.

2.4 Seed Health

Seed health refers primarily to absence of disease causing organisms such as fungi, bacteria and viruses, and animal pests such as eelworms and insects in seed (Mathur and Kongsdal, 2003). It is an important step in the management of crop diseases (Hajihasani *et al.,* 2012). Little is known about the status of this group of pathogens in buffelgrass growing regions of Tanzania. Beckstead *et al.* (2007) described the infection process as a race for the endosperm reserves of the seed. Losses due to infection include reduced seed germination leading to pre-emergence and post-emergence seedling mortality and hence reduced seed yield and quality. According to Singh and Agrawal (2005) infected seeds can be serious focal points for disease in the field/growing crops. They also reported seedborne pathogens in general, including *Fusarium* spp. being able to survive longer in seeds under cool, dry condition than higher temperatures and relative humidity under storage.

Because of the occurrence of infected buffelgrass seed in different parts of the country and persistence of the seed-borne diseases in seed producing farms, seed health testing is increasingly becoming important in the management of seed-borne pathogens. One of the important areas is to investigate the seedborne nature of microorganism (fungi and bacteria) infecting buffelgrass. The current study focused on the effect of buffelgrass seed infection on germination of buffelgrass and its implication on pasture establishment in Tanzania.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Seed Samples

*Cenchrus ciliaris s*eed samples used in the present study were collected from 6 farms as shown in Table 1. A total of three hundred and sixty grams of buffelgrass seeds samples were collected from six pasture seed farms in Tanzania. Each sample (60g) used in the present study was from seeds harvested in the previous season (Table 1). Seed samples were collected from November 2011 to January 2012. Each collected seed sample was packed in a paper bag and labelled (variety name, collector, farm area planted, date harvested, disease recorded (if any) and date collected). Seed samples were transported to the African Seed Health Centre (A_fSHC), Sokoine University of Agriculture (SUA), Morogoro for analysis.

Table 1: Sources and dates of harvest of *Cenchrus ciliaris* seed samples used in this study

Sample no.	Farm	Location	Rainfall (mm)	Date of harvest	Altitude (m.a.s.l)*
1	Vikuge Pasture	Kibaha	900-1000	August 2011	124
2	LRC- Tanga	Tanga	1230- 1400	August 2011	6
3	NLRI	Mpwapwa	< 700	May 2011	1280
4	LRC Mabuki	Mwanza	350-850	May 2011	1166
5	LITI -Tengeru	Arusha	900- 1000	August 2011	1250
6	Mazimbu (SUA)	Morogoro	< 800	July 2010	512

* m.a.s.l = metres above sea level, LRC = Livestock Research Centre, LITI = Livestock Training Institute, NLRI = National Livestock Research Institute and SUA = Sokoine University of Agriculture.

3.2 Seed Health Testing

Seed health testing was conducted at the African Seed Health Centre, Sokoine University of Agriculture (SUA), Morogoro, Tanzania. A Completely Randomized Design (CRD), arranged in four replications was used. Experimental units were the methods used to test the seed health, viz. blotter method for detecting fungal pathogens, high constant temperature oven method for moisture content determination, top of paper method for germination tests and direct plating on agar substrate for detecting bacterial organisms. Treatments were buffelgrass seed samples collected from selected pasture farms in Tanzania.

3.3. Preparation of Working Seed Samples

The working sample was obtained by hand-halving method following the procedures described by the International Seed Testing Association (ISTA, 2005). Each of the collected seed sample was poured on a smooth clean surface, mixed thoroughly in mound with flat-edged spatula and finger. The mound was divided into two halves and each half was halved again making four portions. Each of the four portions was halved making eight portions, which were arranged into two rows of four. The alternate portions were combined and retained to get four portions. The other four portions were removed and poured back in to the original bag of the collected sample. The retained mound of the alternate portions was divided again to obtain the weight of the required working sample (6 g) as recommended by Mathur and Kongsdal (2003) and ISTA (2005) using a weighing balance type Sartorius CP1200 1S.

3.4 Determination of Moisture Content

The high constant temperature oven method (ISTA, 2005) was used to determine moisture content (MC) of seed samples. Petri dishes were dried in the oven for one Hour at 130°C and cooled. Ten grams of seeds from the submitted sample in four replicates were evenly distributed over the surface of 8.5 cm diameter petri dishes. The weight of petri dish and its cover were taken before and after filling it with seeds. Petri dishes were placed in the oven maintained at 130 ± 1°C and dried for one hour (ISTA, 2005), and after drying petri dishes were placed in a dessicator for 30 minutes to cool. Then MC (%) was calculated as $(M_2-M_3) \times 100/M_2-M_1$; where M_1 = weight of container with cover, M_2 = weight of container with cover and seeds before drying and M_3 = weight of container after drying (ISTA, 2005).

3.5 Purity Analysis

The working sample for purity analysis for buffelgrass was 6 g. Each sub-sample was analysed for the following categories: pure seed, inert matter, seed of other crops and results were expressed in percentage (ISTA, 2005).

3.6 Caryopsis Determination

Thirty seeds were randomly picked from the working sample and with the aid of magnifying lens glumes were removed using forceps/scalpels and seed grain (caryopsis) squeezed out of the florets. The proportion of caryopsis (seed grain/units) obtained from thirty buffelgrass seeds were determined to give caryopsis index in percentage.

3.7 Determination of Seed Germination

The Top of Paper method was used for germination test (ISTA, 2005; Rao *et al.*, 2006). Four hundred seeds of each buffelgrass sample representing four replications (100 seeds per replicate) were spaced uniformly (100 seeds/container) and adequately apart on three moist filter papers in well labelled plastic container with height and diameter of 10 cm x 15cm, respectively. The containers were then covered and incubated at 20 - 30°C and were checked for germination after 14 days. Thereafter, percentages of normal (NS), abnormal (ABS) seedlings and dead seeds (DS) were recorded as described by ISTA (2005). The data obtained in each category were analysed in Completely Randomized Design using SAS computer package. Least significant difference ($P \leq 0.05$) based on the different sample tested for each category were done using the General Linear Model (GLM) procedure of SAS.

3.8 Caryopsis Germination

Top of paper method and procedure for determination of caryopsis germination was done as in section 3.7 above.

3.9 Seed Health Testing for Fungal and Bacterial Pathogens

3.9.1 Detection and identification of fungal microorganisms

The standard Blotter method as described by Mathur and Kongsdal (2003) was used to detect and identify fungal microorganisms in the collected pasture seeds. Four hundred untreated seeds of buffelgrass from each seed sample were plated on three well moisten blotters in glass petri dishes (25 seeds per petri dish) in four replication of 100 seeds each. The petri dishes with seeds were incubated for 7 days at 20-25 °C under alternating cycle of 12 h near Ultra violet (NUV) light and 12h darkness

(ISTA, 2005). After 7 days, individual seeds were examined for the presence or absence of fungi (x 12, x 25 and x 50 magnification) under the stereomicroscope. The mycelia of the fungi were placed in a sterile drop of water, covered by a glass slip on a sterilized grass slide and placed on the compound microscope ((Leica® MS 5). The examinations of fungi that developed on each seed was confirmed by examining mycelium and/or conidia under different magnifications (x 10 x 20 and x 40) under a compound microscope. The fungal species present on each seed were recorded and the percentage incidence of each fungus per sample was computed. Identification of fungi was based on the type of spore growth, colour and morphological or "habit character" of fruiting bodies on seed (Habib *et al.,* 2011; Mathur and Kongsdal, 2003).

Subcultures on potato dextrose agar (PDA) slants were made for preservation of isolated fungal cultures for further analysis. Incidence (%) of seedborne pathogens in the 400 seeds per sample was calculated as described by Habib *et al.* (2011).

$$\textbf{Incidence (\%)} = \frac{\text{No. of infested seed}}{\text{Total number of seed assessed}} \times 100 \qquad \text{….. (i)}$$

The relative percentage of particular species within the genus of fungi was calculated using the Ghiasian *et al.* (2004) formula;

$$\textbf{Relative percentage (\%)} = \frac{\text{Number of fungal species isolated}}{\text{Total number of fungi isolated}} \times 100 \qquad \text{.... (ii)}$$

3.9.2 Detection of bacteria from buffelgrass seed samples

Direct plating on Nutrient Agar (NA) was used for detection of bacteria from buffelgrass seed (Mortensen, 2005). Two hundred seeds of buffelgrass were aseptically plated in eight replicates (25 seeds/petri dish) onto NA in the lamina Airflow chamber and incubated at 28.5°C for 72h. A sterile loop was used to transfer different representative bacterial colonies from NA media and streaked in triplicate onto NA (if yellow or orange) and King's B (KB) medium (if cream and white) and purified for further characterization and identification. The new plates were incubated at 27-29 °C for 12-24 h.

3.10 Purification of Bacterial Isolates

Bacterial colonies from all pasture seeds were purified for characterisation and identification by a series of single-colony transfers on King's B medium (for cream/white/gray colonies) (Kings *et al.,* 1954) and NA (yellow colonies). Purified colonies were further transferred to NA before preservation using Protect® bacteria preservers (Mortensen, 2005) for further analysis.

3.10.1 Gram staining reaction

The Gram-reaction of each isolate was determined following the staining procedure described by Mortensen (2005). Bacteria cultures purified from buffelgrass and stored at 5 ^{o}C were reactivated on NA and well grown colonies were transferred by using a sterile toothpick, mixed for at most ten seconds in a drop of 3 % KOH aqueous solution on a glass slide. The tooth pick was then raised few centimetres from the glass slide. If the strands of viscid material were observed, the tested

bacterial strain was recorded as Gram-negative. Absence of strands of viscid material was recorded as Gram-positive.

3.10.2 Kovac's oxidase test

The oxidase test was done following procedures described by Kovac's (1956) and Hildebrand and Schroth (1972). A Whatman filter paper No.1 was placed in a petri dish and 3-4 drops of fresh prepared 1% aqueous solution of tetramethyl -p-Phenylenediamine dihydrochloride was added on the centre of the filter paper. Using a platinum wire, a loopful of bacteria grown on NA or KB was streaked on the moist filter paper. Isolates which developed purple color within 10 seconds were taken as positive, purple color in 10-60 seconds were taken as slow positive and those with no color for more than 60 seconds were taken as negative for Oxidase test (Dickey and Kelman, 1988).

3.10.3 Arginine dihydrolase

The ability of pseudomonad to grow under anaerobic conditions was measured by this test, where ammonia was evolved and therefore, caused the change in pH, indicating positive reaction. The procedure described by Lelliot and Stead (1987) were used. Twenty-four-hour old bacterial cultures grown on NA media were stab-inoculated into the test tube containing 3 ml of Thornley's medium (Mortensen, 2005). All tubes were covered with 2 ml of sterile mineral oil to create anaerobic condition. Two test tubes (one without bacteria but sealed with mineral oil and the other with bacteria but without being sealed) were also included for comparison. The test tubes were incubated for three days at 27^{o}C. The change of colour to red

(alkaline) was recorded as positive for the presence of arginine dihydrolase enzyme, and lack of colour change was recorded as negative.

3.10.4 Gelatin liquefaction media

The procedure described by Mortensen (2005) was used to prepare Gelatin media. The media were stab inoculated with each bacterial isolate grown for 24-hour on NA medium and incubated at 28 ºC. After 7 and 14 days of incubation, each isolate was evaluated for gelatin liquefaction. The isolates in test tubes were kept at 4 ºC for 30 minutes and gently tipped immediately. The results were recorded as positive when the gelatin remained liquid as the tube was gently tipped and negative when the gelatin was solid (Dickey and Kelman, 1988; Egamberdiyeva, 2005).

3.10.5 Starch hydrolysis

Taxonomic characterization of certain bacteria with ability to hydrolyse starch was used to differentiate bacteria in this test. The 24-hour –old-bacterial cultures grown on NA were streaked on starch agar medium (starch soluble, 20g; Peptone, 5g; Beef extract, 3g; agar, 20g in 1 liter distilled water with P^H 7 and autoclaved at 121ºC for 15 minutes) in a zig zag manner to evaluate their ability to hydrolyze starch (amylase production). The plates were incubated at 28ºC and for 3 days starch hydrolysis was observed by flooding the plates with Lugol's iodine solution for 30 seconds. The appearance of clear zone around the line of growth of each isolate indicated starch hydrolysis (Aneja, 1996), and recorded as positive reaction. Appearance of reddish colored zone indicated that there was a partial hydrolysis of starch to dextrin, while a negative reaction gave starch staining blue-black.

3.10.6 Nitrate reduction

Nitrate reduction test was used to determine the ability of the isolates to reduce nitrate to nitrite. The procedure described by Fahy and Persley (1983) was used. A heavy loopful of growth of 24 hour-old-pure culture was inoculated by stabbing and sealed with 3 ml-sterilized molten agar to avoid false positives and incubated at 28 ^{o}C. A non inoculated test tube was also included. Observations were made after 7 days of inoculation. Three drops of reagent A (starch iodide solution) together with three drops of reagent B (hydrochloric acid solution) were added to each test tube and results were recorded. A change of colour of the reagent to blue-black was an indicator for nitrate reduction (positive), no change in colour was considered as a negative reaction, implying that, nitrate was not reduced or was partly or totally reduced beyond nitrite (Dickey and Kelman, 1988).

3.10.7 Potato soft rot

The test was done as described by Lelliot and Stead (1987). Washed round potato tubers were surface disinfected in alcohol 75 % and briefly flamed. The disinfected potato tubers were aseptically cut into slice of 7-8 mm thick. The slices were placed in a sterile petri dish filled with 3-4 mm of distilled water. A small groove was made in each potato slice and smeared with bacterial growth of 24 hour-old-culture grown on NA medium. Un-inoculated control was included. The petri dishes were incubated in darkness at 26°C for 24h. Symptoms were tested by drawing an inoculating wire loop across the surface to determine whether the slice has rotten beyond the inoculation point. Negative reaction was considered when slight or no rot at the inoculating point was observed.

3.10.8 Hypersensitivity reaction

Tobacco (*Nicotiana tabacum*) and sweet pepper (*Capsicum annum*) plants were used in this test as procedures described by Mortensen (2005). An aqueous suspension of 24 hour-old-bacterial cultures were prepared in sterile distilled water. Sufficient inoculum was injected and infiltrated with a syringe without a needle to the intercellular space of leaf blade of respective plants. The injection was done in more than one leaf and one section of a leaf and the control was arranged in opposite side of the main vein. Labelling of the inoculated areas was done using adhesive labels. The plants were incubated at 22-25 °C, with about 80-85 % relative humidity. Rapid collapse and water-soaking of inoculated tobacco of host tissue in the infiltration area within 24 hours or utmost 48 hours for inoculated pepper followed by a dry, light–brown of localized necrosis within 3 days indicated positive reaction and the bacterium was likely a pathogen for another host.

3.10.9 Detection of bacteria in buffelgrass seeds using polymerase chain reaction (PCR)

Bacterial cultures on NA were harvested from the colonies of 48h as described by Mbega *et al.* (2012) and DNA extraction was conducted using procedures described in the DNeasy Blood and Tissue kit protocol handbook (Qiagen, 2006). The extracted DNA was tested using PCR primers amplifying a 402bp fragment of xanthan synthesis pathway gene, *gum*DPrimers: X-gumD-fw 5’GGCCGCGAGTTCTACATGTTCAA and X-gumD-rv 5’CACGATGATGCGGATATCCAGCCACAA) for spacer region of *Xoo/Xoc*, respectively (Mbega *et al.*, 2012). The amplification was performed in eppendorf

mastercycler gradient PCR machine (GenAmp® 9700 PCR System); using procedures described by Mbega *et al.*(2012). A 25-µl reaction mixture was used for the multiplex PCR reaction (1.5 µl of DNA template, 24 µl of 10 mM dNTP, 9 µl of 25 mM $MgC1_2$, 30 µl of 10 xTaq polymerase buffer, 1.5 µl each primer (10 pmols each) and 1.5 µl (0.5units) Taq DNA polymerase (Fermentas, inc.)). The reaction involved an initial de-naturation of 5 min at 95 ^{0}C followed by 30 cycles of de-naturation for 95 °C for 10s, annealing at 56 °C for 1sec, elongation at 72 °C for 10 sec. the final extension was 72 °C for 3 min.

Eight microlitre of each amplified PCR product was fractionated on 1.5 % agarose gel electrophoresis in 0.5X TBE (Tris-borate EDTA) buffer for 45 minutes. The agarose gel was pre-stained with ethidium bromide (12 µl) and was visualized under UV light then photographed with Kodak camera (Black and white).

3.11 Effect of Fungal Microorganisms on Seed Germination

3.11.1 Artificial inoculation of buffelgrass seeds with seedborne pathogenic fungal conidia

Buffelgrass seeds were surface sterilized with 1% sodium hypochlorite for 10 min and then rinsed twice with sterile distilled water. The conidia were harvested from 7days-old-culture of detected fungal species on PDA plates with sterile distilled water. Conidial concentration in suspension was adjusted to 10^4 conidia/ml. The disinfected seed units were immersed in suspension for 30 min and then spread on tissue paper. The seed were air dried at room temperature. Plates incubated with surface-sterilized seeds without an added test fungus served as control.

The Top of Paper and Blotter methods as described above were used for germination of the seed caryopses and fungal on spikelets, respectively. Germinated seeds were retained for the full 14 days in the container with their coleoptiles unclipped to evaluate infection levels on germinated seeds, as evidenced by the appearance of pathogen stromata. The stereo microscope was used to examine presence of fungi on all separated seed parts after seven days of incubation. Data on percentage germination of normal, abnormal seedlings, hard seeds, dead seeds and fungal infection load were recorded on both caryopses and spikelets.

Data collected were subjected to general linear modal (GLM) and Least Means of observed parameters and Least Significance Difference (LSD) determined by using SAS (2004) Statistical Package and P values ≤ 0.05 were considered statistically significant.

3.12 Pathogenicity of Fungal and Bacterial Isolates on Buffelgrass Seedlings

Pathogenicity test was done to determine whether or not a suspected pathogen would cause disease symptoms in the host from which it was isolated. This test was done to confirm the initial presumptive diagnosis (Lelliot and Stead, 1987).

3.12.1 Pathogenicity of fungal strains

The buffelgrass plants used in this experiment were grown from NLRI Mpwapwa seeds on sterilized soil in pots of 20 cm x 18.5 cm. Surface sterilized (1 % NaOHCl) seeds were sown according to the procedure described by Campbell and Medd

(2003). Five seeds per pot were used. Plants were thinned to four plants per pot 21 days after germination. All pots were placed on a greenhouse bench at 15 to 25°C Watered and fertilized as required.

3.12.1.1 Inoculum preparation

A small mycelial plug from a stock culture was aseptically transferred to fresh modified V_8 agar (200 mL of V_8 juice, 800 mL water and 14 g agar) (Dingha and Sinclair, 1995). The plates were incubated for 10 days under 25 °C, 12 h/12 h light/darkness, where adequate colony growth was observed. For each fungal isolate, 10-day-old culture on NA was added to 10 mL SDW and spores scrapped off using a bent glass-rod. The resulting conidia suspensions were filtered using cheese cloth to remove NA and spore suspension adjusted to desired concentration 1 $x10^8$cfu/mL by using Haemocytometer.

Seedlings at four leaf growth stage in four replicates of 4 seedlings/pot were sprayed-inoculated with the suspension till run off using a 1000 ml gun sprayer. Inoculated plants were then covered with polyethylene sheets for 24 h to maintain humidity (Jugah *et al.*,2007). Control plants were sprayed with SDW and maintained under the same conditions. Disease incidences were recorded 7, 14, 21 and 28 days after inoculation (DAI) using the methods of Kadir and Charudattan (2000).

Disease assessment was based on the number of plants affected among the total inoculated (disease incidence), expressed as the percentage of diseased plants and plant reaction to disease based on disease severity (area of plant tissue that is

diseased) (Jugah *et al.*, 2007). Disease progress was assessed on the inoculated plants in each pot by estimating the disease development on 4 lower leaves. The disease development was expressed as disease incidence using procedure developed by Kleczewski and Flory (2010) of disease rating where: 0 = no disease, 1 = 1to 5 % of leaf surface area with lesions; 2 = 6 to 10 %; 3 = 11 to 25 %; 4 = 26 to 50 % and 5 = > 50 %. Disease severity was quantified by assigning a rating to each leaf of every plant. Then to provide a single disease severity measurement for each plant, the converted mean rating was arranged for all 4 leaves from each plant. In order to calculate means and variance for these nonparametric data, rating values were converted for each leaf to the midpoint of the percent leaf area with lesions (e.g. rating 2 = 8 %).

Data on diseased plants collected were subjected to General Linear Modal (GLM) and Least Significance Difference (LSD) determined by using Statistical Package at $P \leq 0.05$ (SAS, 2004).

Four leaves from each treatment were sampled at 28 DAI in order to determine the germination of conidia in blotter test as in the previous experiment. The symptomatic leaf tissue was surface sterilized in 0.1 % NaOCl for one minute then rinsed three times in sterile distilled water (SDW), blotted dry with sterilized paper and ten pieces (5 per petri dish) were plated out on three layer of moisten filter papers then incubated for 7 days under 25 °C, 12 h/12 h light/darkness, where adequate colony growth was observed. Detection and identification was carried out to confirm Koch's postulates.

The experiment was performed once using a completely randomized design with four replications. All data were subjected to the standard SAS (2004) procedure. Least Significance Difference (LSD) was done if treatments showed significant differences.

3.12.2 Pathogenicity tests for bacterial strains

Strains were revived from beads streaked on NA in a laminar air flow cabinet. The petri dishes were incubated at 28.5 °C for two days; all bacterial strains were revived using the same method. Bacterial suspension was prepared on fresh inoculum, where a 48 h loopful of bacteria culture was added in 10 ml of SDW, and then thoroughly vortexed. More SDW was added to make 100 mL of suspension. Plants were subjected to pathogenicity test using the clip and foliar spray inoculation methods. In clip method, a pair of sterilized scissors was dipped in the bacterial inoculum (10^7-10^8 cfu/ml). Leaves of all four plants in a pot were grasped in one hand and the top 5-6 cm of four leaves were clipped off simultaneously. In folia spray inoculation method, bacterial inoculum (10^7-10^8 cfu/ml) was sprayed using hand atomizer sprayer onto the leaf surface of buffelgrass seedlings. Control plant leaves were sprayed and clipped off using sterilized distilled water for both methods. The inoculated seedlings were covered in polyethylene bags for 48 h and incubated for 7 days in the screenhouse. Seven days after inoculation, the plants were observed daily for appearance of disease symptoms and the final data were recorded after 28 days of inoculation. Percent disease incidence was calculated using the procedures of Gnanamanickam *et al.*, (1999). Lesion lengths were measured at 7, 14, 21 and 28 days after inoculation.

Disease incidence (%) = $\frac{\text{lesion length}}{\text{Total length}}$ x 100 ……....(iii)

3.12.3 Re-isolation of bacteria from infected buffelgrass leaves

The leaves of buffelgrass inoculated by clip method showing the symptoms of bacterial infection were used for isolation of the bacteria for confirmation. The infected clipped leaves were cut using a pair of scissors from the plants in the screenhouse and taken into the laboratory. These were then washed with sterilized distilled water 2-3 times. Isolation of the bacteria was done using direct plating of infected leaves on NA medium. Plates were incubated at 28 °C and monitored between 48 to 72 h for appearance of bacterial colonies. For the other buffelgrass seedlings inoculated by spraying method showing the visual disease symptoms, the same procedure was followed.The colonies that grew on the media were purified and grown on NA (yellow) and KB (cream or white) media then incubated for 24 h at 28 °C. Colonies that appeared were examined if were similar to the bacterial isolates used for inoculation.

3.13 Buffelgrass Seed Cost and Seeding Rate in Relation to Seed Quality from Selected Pasture Seed Farms in Tanzania.

Recorded results from germination and purity were used to calculate the actual seed rate and compare it with the ISTA (2005) recommended seed rate (9 kg/ha). The selling price per kilogram weight from each seed sample from each farm was calculated and compared with their respective germination and purity percentage. The most economical seed sample with minimum seed rate, high pure live seed and germination percentage were determined and new seed cost per kg proposed according to data obtained.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Seed Health Testing

4.1.1 Seedborne fungi detected in buffelgrass seed samples

Using the Blotter Method for fungal detection, results (of seed health testing) showed that buffelgrass seed samples were contaminated by different fungi (Table 2). The pathogenic fungi with their incidence were *Phoma spp* (28.5 %), *Curvularia lunata* (Plate 1A) (17.34 %), *Alternaria alternata* (Plate 1B) (14.09 %), *Bipolaris* spp. (Plate 1C) (12.2 %) *Fusarium pallidoroseum* (8.4 %) and *Exserohilum rostratum* (7.3 %). Others included *Nigrospora oryzae* (Plate 1D) (1.08 %), *F. moniliforme* (2.2 %), *F*. spp. (4.1 %) and *Pyricularia grisea* (4.9%) (Table 2 and Fig. 2). Presence of fungi in buffelgrass seeds from different pasture farms imply that the health quality of the produced seeds was poor and that these disease causing pathogens may be disseminated with seeds and cause diseases in other fields (Mukhtar, 2009). Ocamb and Alderman (2004) confirmed *Fusarium* spp associated with tall fascue grass to decrease seed germination. The use of pathogen free seeds minimises the possibilities of dispersing the pathogens with the seeds (Safdar *et al*., 2009).

The pathogens detected from buffelgrass seeds used in this study have been found in association with different cereal seeds (Fukhrunnisa *et al*., 2006; Niaz and Dawar, 2009). Manyangarirwa *et al.* (2009) reported *F. pallidoroseum, F. moniliforme,* and *Phoma* sp. to be seed transmitted causing direct damage to the seeds through toxins or physical invasion or indirect seed damage through heating due to respiration of the

fungi. Agarwal and Sinclair, 1997; Thomsen and Schmidt, 1999; Amadi and Adeniyi, 2009 reported similar results.

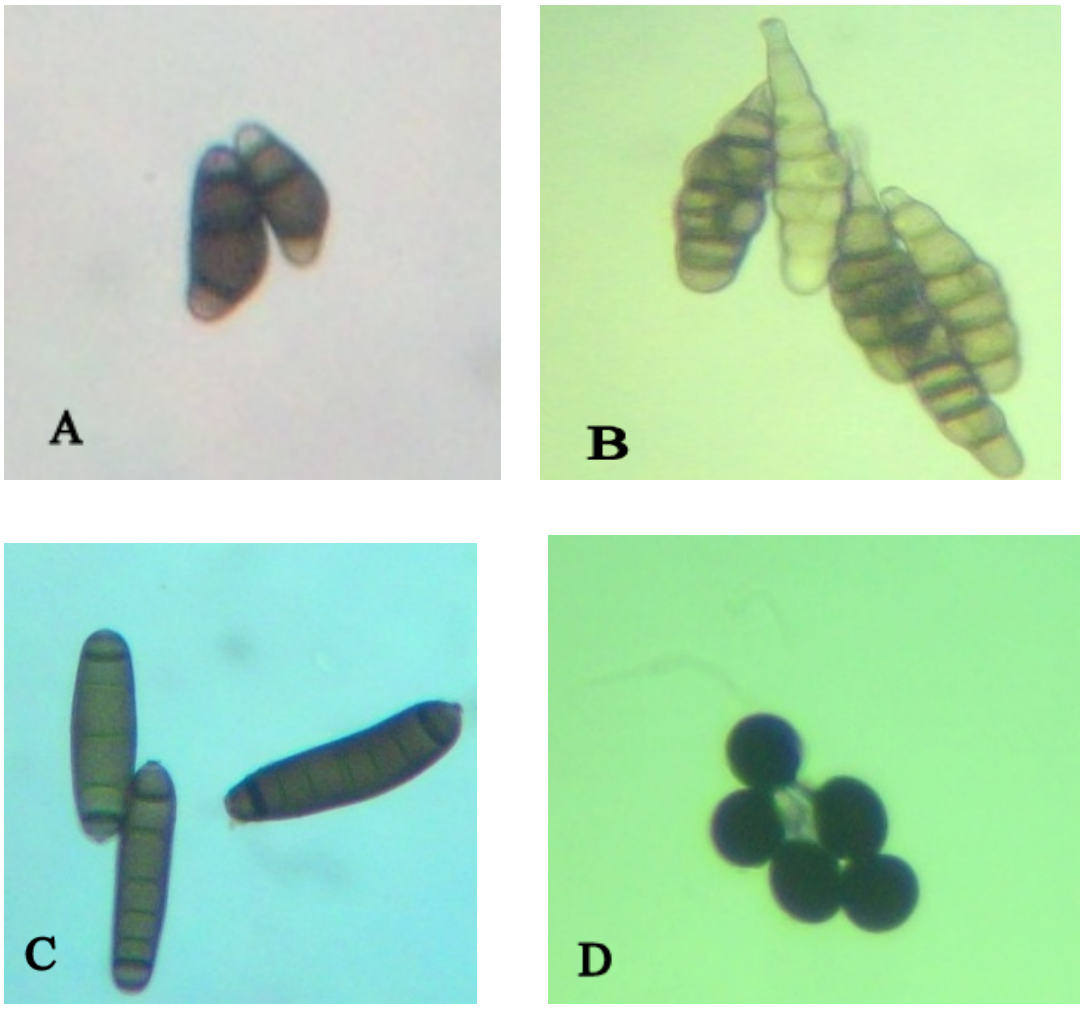

Plate 1: Conidia of fungi detected on buffelgrass seeds collected from selected pasture seed farms in Tanzania

A = *Curvularia lunata*, B = *Alternaria alternata*, C = *Bipolaris* spp and D = *Nigrospora oryzae* (x 40)

Table 2: Incidence of fungal microorganisms in buffelgrass seeds collected from pasture seed farms in Tanzania

Seed Farm	Fungal Incidence [a] (%) by										
	Aa	**Bs**	**Ph**	**Pg**	**Cl**	**Fp**	**Ex**	**Fs**	**F m**	**No**	
LITI Tengeru	4.5	2.0	6.5	0.5	6.5	8.0	0.5	0.0	0.0	1.5	
LRC Mabuki	4.5	2.0	4.0	0.0	2.0	4.0	4.0	1.0	0.0	0.0	
Mazimbu	1.0	0.5	0.5	0	4.0	0.0	0.5	0.0	2.0	0.5	
NLRI Mpwapwa	6.5	11	35	1.5	15	2.0	5.0	1.5	0.0	0.0	
LRC Tanga	0.5	1.0	1.0	0.0	1.0	1.5	0.0	4.0	2.0	0.0	
Vikuge	9.0	5.5	5.5	7.0	3.5	0.0	13.5	1.0	0.0	0.0	
Total	26.0	22.5	52.5	9.0	32.0	15.5	8.4	7.5	4.07	2.0	= 184.5[c]
Fungi[b] (%)	**14.09**	**12.2**	**28.46**	**4.88**	**17.34**	**8.4**	**7.32**	**4.07**	**2.17**	**1.08**	

[a] = percent incidence based on 400 seeds tested using the standard Blotter method, [b] = fungi (%) = sum of individual species /overall total (c) Aa = *Alternaria alternata*, Bs= *Bipolaris* spp., Ph= *Phoma* spp., Pg = *Pyricularia grisea*, Cl= *Curvularia lunata*, F.p = *Fusarium pallidoroseum*, Ex= *Exserohilum rostratum*, Fs = *Fusarium* spp., Fm = *F. moniliforme*, No = *Nigrospora oryzae*

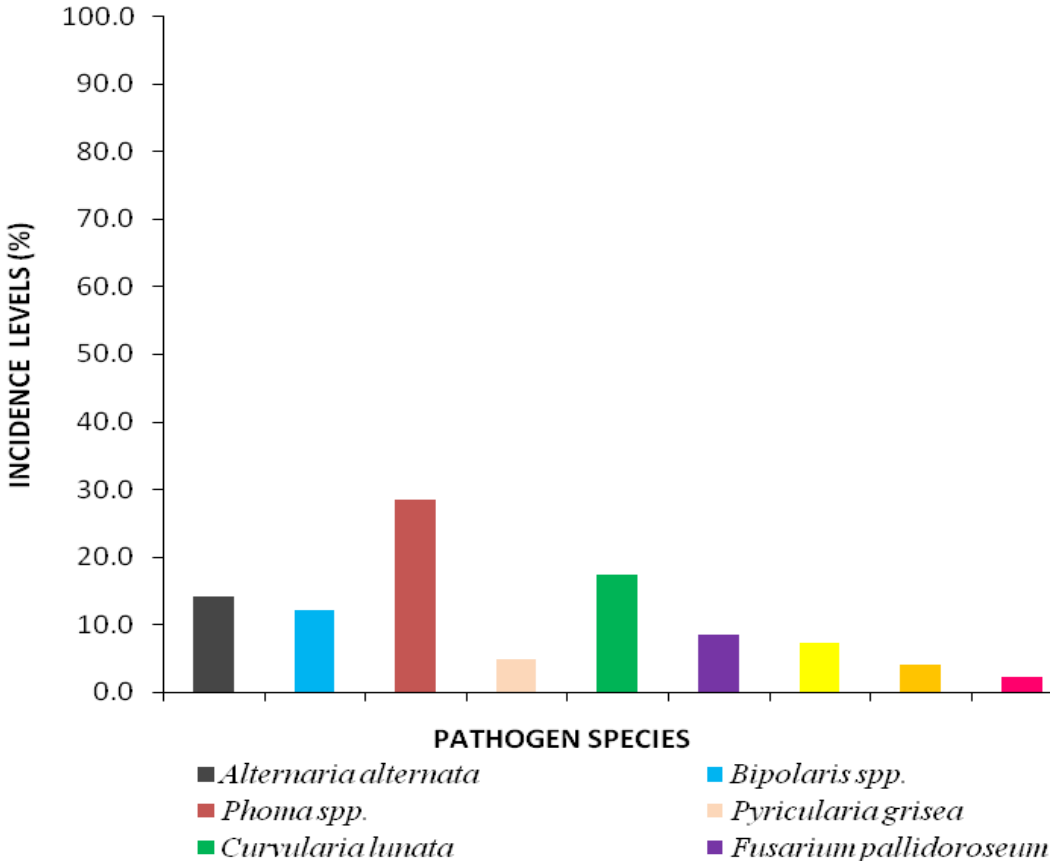

Figure 2: Incidence (percentage) of fungi detected in the buffelgrass seed sample collected from selected pasture farms in Tanzania

4.1.2 Relative percentage of fungi in pasture seed farms

The results also showed that, seed infection levels were the highest in buffelgrass seeds collected from NLRI Mpwapwa (77.5 %), followed by Vikuge pasture farm (35 %), LITI Tengeru (30 %), LRC Mabuki (21.5 %) and LRC Tanga (11 %). Seeds from Mazimbu (SUA) had the lowest seed infection (9.5 %) (Fig. 3). These results indicated that, customers purchasing buffelgrass seeds from NLRI Mpwapwa get seeds with a large number of seedborne fungi compared to the customers purchasing seed from Mazimbu farm and vice versa. In addition, weather conditions at Mpwapwa

favour fungal growth and seed infection and may not be conducive for pasture seed production.

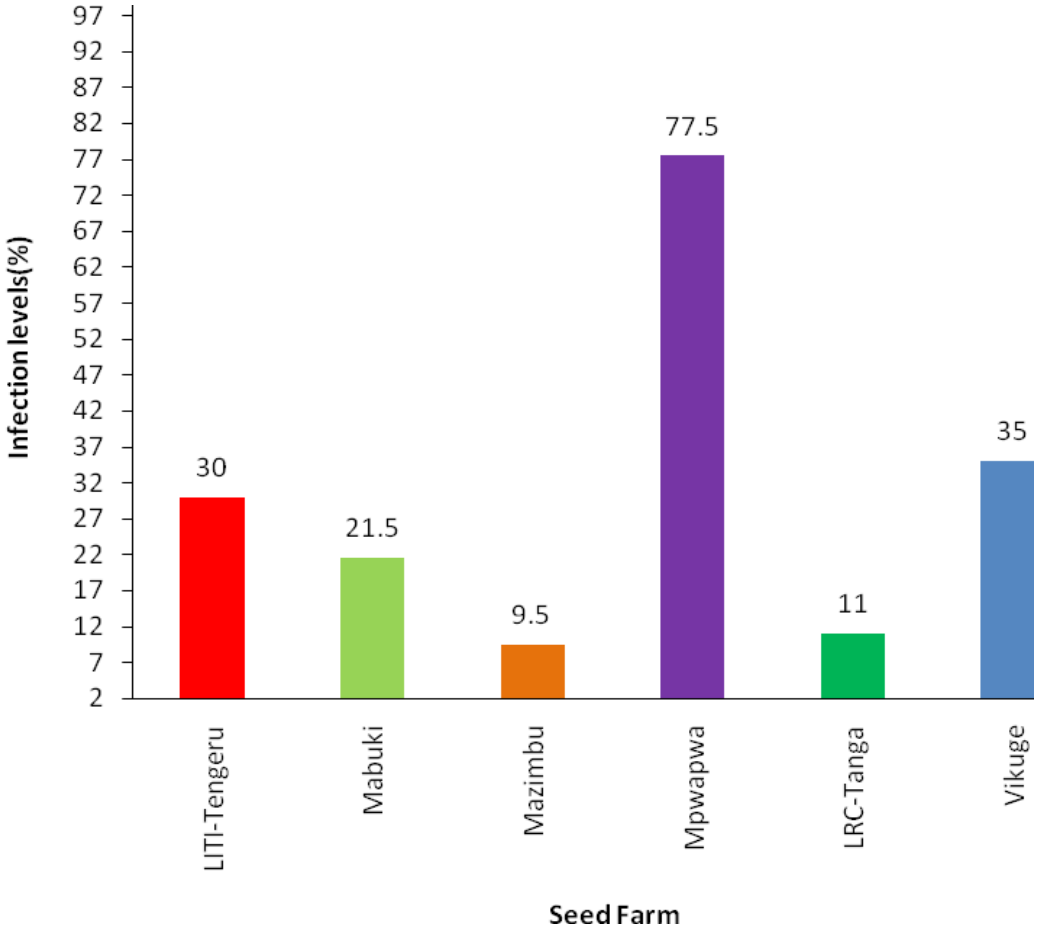

Figure 3: **Fungal infection levels (percentage) in pasture farms surveyed in this study in Tanzania**

Other fungal species detected in pasture seed during the study included *Actinomycetes*, *Asperigillus*, *Cladosporium*, *Epiccocum*, *Penicillium* and *Rhizopus* (Table 3). These saprophytic fungi are known to produce toxic substances that affect the quality of seeds (Adam, 1977; Amadi and Adeniyi, 2009).

Table 3: Other micro-organisms detected from pasture seed samples used in this study

Farm	Micro-organisms detected
NLRI Mpwapwa	*Actinomycetes spp* . *Asperigilus* spp *Rhizopus* spp.
Mazimbu	*Actinomycetes spp Asperigilus flavus Cladosporium spp. Epicoccum spp .Penicillium spp. Rhizopus* spp.,
LRC Mabuki	*Asperigilus* spp. Bacterial ooze *Penicillium* spp., *Rhizopus* spp.* Smuts *
Vikuge	*Aspergillus* spp., Smut, *Rhizopus* spp. and *Penicellium spp.*
LITI Tengeru	*Aspergillus* spp., Bacterial ooze *Rhizopus* spp.*
LRC Tanga	Bacterial ooze

* Prevalent (>65%), LITI = Livestock Training Institute, LRC= Livestock Research Centre and NLRI= National Livestock Research Institute.

4.1.3 Incidence of fungal and bacterial diseases under field conditions

The results of field survey in three pasture farms which were found to have un-harvested grasses indicated presence of mixed infection of fungal and bacterial diseases. The incidence of buffelgrass leaf spot disease was more than 50 %. The fields had mixed infection (leaf spot, blights and streaks) (Plates 2 and 3). Cooper (2006) mentioned *Xanthomonas* and *Pseudomonas* genera to be responsible for nearly all of bacterial leaf spot. For example, through visual observation at Mpwapwa, Vikuge and Mazimbu farm, buffelgrass fields when visited during April, May and July were found with mixed bacterial and fungal leaf spot infection (>65 %) (Plates 2 and 3) (Mlay, J. Personal visit, 2012).

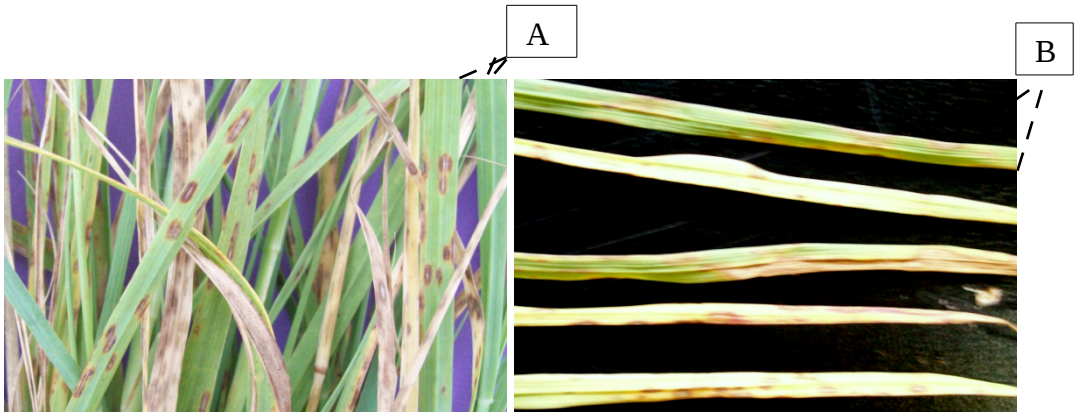

Plate 2: Buffelgrass leaf infections as assessed from National Livestock Research Institute Mpwapwa and Mazimbu pasture farms in Tanzania

A = chocolate Leaf blight/spot from NLRI Mpwapwa and B= leaf streak/spot, mixed leaf infection from Mazimbu farm (SUA).

Plate 3: Mixed bacterial and fungal buffelgrass leaf infection (A and B) from field samples collected from Vikuge and Livestock Research Centre Mabuki pasture farms, respectively

4.2. Bacterial Species Detected on Buffelgrass Seeds

Using the direct plating method, 60 bacterial isolates from 6 pasture seed samples were detected. Eight bacterial isolates were selected based on the morphological

characteristics and biochemical similarities in the ability of the species to use glucose as a carbon source, the specific oxygen requirements for growth (Christopher and Bruno, 2003) and comparisons of species to known bacterial characteristics as described by Mortensen (2005). Results based on biochemical and physiological tests indicated isolates with yellow bacterial colonies (Plates 4) were preliminarily identified as *Xanthomonas oryzae* pv. *oryzae and X. oryzae pv oryzicola*, and the cream bacterial colonies (Plate 5) as *Pseudomonas glumae* and *Acidovorax avenae* (Table 4).

The results imply that buffelgrass had mixed infection. The *Xanthomonas* spp. have a wide range of host including monocotyledons and dicotyledonous (Ndomba, 2009). Yellow and cream 24 h-old-colonies of bacteria (Plate 4 and 5) were grown on NA and KB, respectively. Yellow bacterial colonies were circular and viscid. They were negative for Gram reaction test, Kovac's Oxidase (except one was variable) and nitrate reduction tests. The potato soft rot tests also gave negative results implying that they were not soft rotting bacteria (Cooper, 2006). Hypersensitive reaction on tobacco and sweet pepper was negative for all bacterial isolates (Table 4). The bacterial isolates were also variable on starch hydrolysis, positive to gelatin liquefaction and pathogenicity test on buffelgrass seedlings (Table 4).

The cream bacterial isolates were positive for gelatin liquefaction, nitrate reduction and variable on starch hydrolysis test (Table 4).

Table 4: Biochemical and morphological characteristics of bacterial isolates detected from pasture seed samples used in this study

Sample	colour	Pot	Gram	Ox	Arg	St	Nitr	HR/TP	Pig	Gel.
S_1	Yellow	-	-	-	+	-	-	-/-	-	+
S_2	Yellow	-	-	+	-	v	-	-/-	-	+
S_{2c}	Cream	-	-	-	-	-	+	-/-	-	+
S_3	Cream	-	-	-	-	v	+	-/-	-	+
S_4	Yellow	-	-	-	+	-	-	-/-	-	+
S_5	Yellow	-	-	+	-	v	-	-/-	-	+
S_6	Yellow	-	-	-	+	-	-	-/-	-	+
S_{6c}	Cream	-	-	-	-	v	+	-/-	-	+

-Ve = Negative, +Ve = positive, v = variable, Pot =potato soft rot, gram = Gram reaction, Ox= Oxidase test, Arg= Arginine dihydrolase, St= Starch hydrolysis, Nitr = Nitrate reduction, HR= Hypersensitivity reaction, T/P = tobacco/pepper, colour= colony colour pig= Pigment production on KB medium, Gel= Gelatin liquefaction

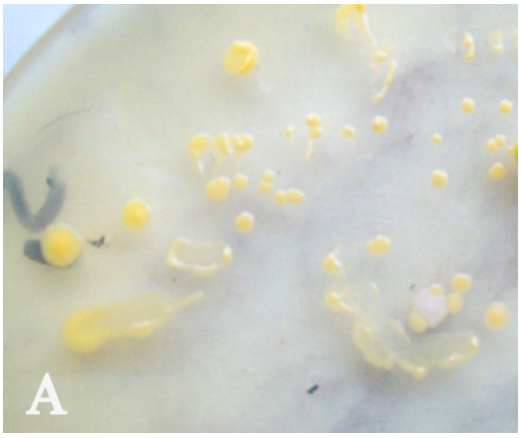

Plate 4: Purified yellow bacterial colonies (A) grown on Nutrient Agar medium from National Livestock Research Institute Mpwapwa buffelgrass seeds using the Direct plating method

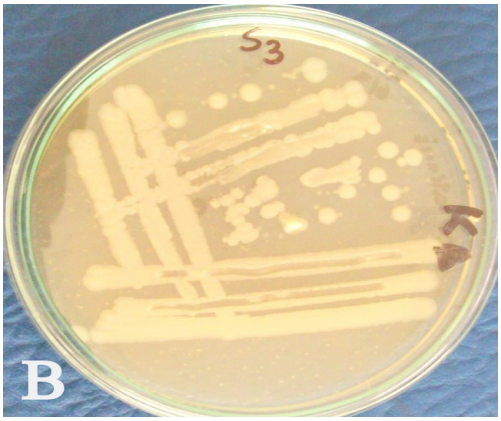

Plate 5: Purified cream bacteria colonies (B) on King B medium from Mabuki buffel grass seeds tested using the Direct plating method

4.2.1 Hypersensitive reaction on tobacco and sweet pepper

Results for hypersensitivity reaction (HR) of the bacterial isolates on tobacco (*Nicotiana tabacum*) and sweet pepper (*Capsicum annum*), indicated that none of the bacterial isolates induced HR. Such results implied that the bacteria were not pathogenic or presence of *hrp* genes. Fett and Jones (1995) reported *h*ypersensitive *r*esponse and *p*athogenicity (*hrp*) genes to control both pathogenicity and the ability to cause the HR. The inability of bacteria to elicit HR may be due to presence of the *hrp* genes. Mutation of (*hrp*) genes has been reported to be the cause of failure to induce infection by bacteria (He *et al*., 1993, Huang *et al*., 1995, Bobosha, 2003 and Samudrala *et al*., 2009). Zou *et al.* (2006) reported these *hrp* genes to be involved in induction of HR in host and non-host plants and the pathogenicity of susceptible plants. The plant pathogenic bacteria that are mutant for *hrp* gene effector protein have been found to be non pathogenic to compatible hosts (Collmer *et al*., 2000; Tang *et al*., 2006).

4.2.2 Identification of bacteria using Polymerase Chain reaction (PCR)

The results showed that, three out of eight bacterial isolates isolated from buffelgrass seeds were amplified by XanF7/R7 primers targeting *Xanthomonas* and produced a product size of 402 bp (Plate.6). Such results clearly indicated that the amplified organisms were xanthomonads. It was not possible to differentiate different species of xanthomonads using these primers. No amplification was observed in a sterile distilled water control or in other bacteria isolated from buffelgrass seeds. The DNA from other two yellow and the cream pigmented bacterial isolates were not amplified implying that they were not xanthomonads. These results concur with the study by Sakthivel *et al*., (2001) when genomic DNA of *X. o.* pv *oryzae* was used as a template (Table 5). Seeds of many plant species are reported to contain compounds that inhibit PCR and can lead to amplification failure (Ha *et al*., 2009). Presence of inhibitors may interfere with the cell lysis or capture components necessary for DNA extraction (Clarissa *et al*., 2010). The failure of DNA from the yellow colonies thought to be xanthomonads in the biochemical test might have also been due to presence of PCR inhibitors in plants.

Low time priming during the amplification of DNA is reported to cause locations of potential base mismatches or overlapping bands in PCR (Roux, 1995). The causes of band overlapping in lane 4 and 5 (Plate 6) might have been due to failure of PCR to amplify under optimum conditions, leading to generation of multiple undefined and unwanted products, even to the exclusion of the desired products.

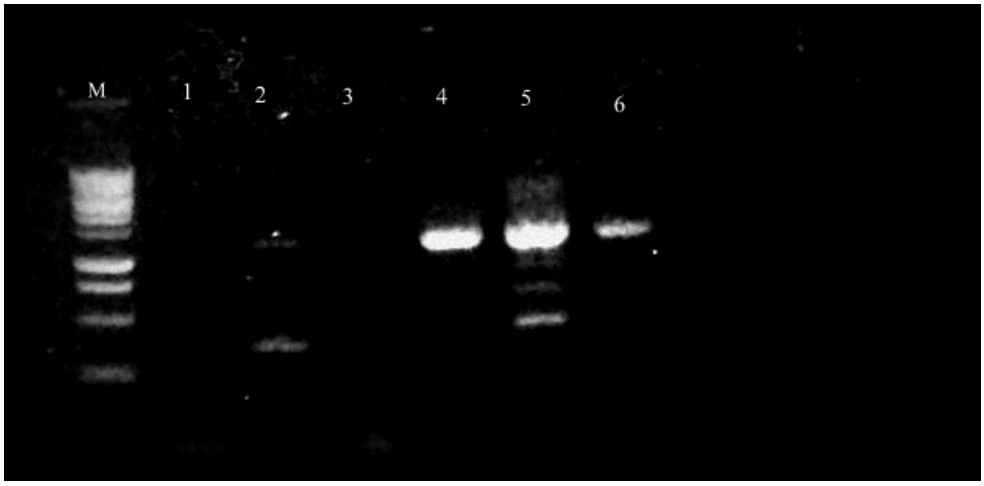

Plate 6: Gel electrophoresis products as amplified by the XanF7/R7 primer pair of bacteria isolated from buffelgrass seeds collected selected pasture farms in Tanzania

Lane M = 1kb molecular weight marker, Lane 1 = negative control and 3 = No amplification, 2, 4, 5 and 6 = showing amplification products of approximately 402 bp size of bacterial isolates identified from collected buffelgrass seeds

Thus, from this work we conclude that *Xanthomonas* spp. is the primary causal pathogen for buffelgrass leaf blight as previously described under section 4.2**.**

Table 5: Detection of bacteria using polymerase chain reaction (PCR) on buffelgrass seed samples collected from selected pasture seed farms in Tanzania

Farm	Sample Identity	Colony colour	PCR result
NLRI Mpwapwa	S_1	Yellow	Positive
Mazimbu	S_2	Yellow	Weak positive
Mazimbu	S_{2c}	Cream	Negative
LRC Mabuki	S_3	Cream	Negative
Vikuge	S_4	Yellow	Positive
LITI Tengeru	S_5	Yellow	Negative
LRC Tanga	S_6	Yellow	Positive
LRC Tanga	S_{6c}	Cream	Negative

LITI = Livestock Training Institute, LRC= Livestock Research Centre and NLRI= National Livestock Research Institute.

4.2 3 Pathogenicity of fungi and bacteria isolated from buffelgrass seeds

4.2.3.1 Fungi

Results of pathogenicity tests of fungi isolated from buffelgrass seeds in the screen house are shown in Table 6. The highest disease incidence (40 %) was observed on plants sprayed with *Bipolaris* spp., and *P. grisea* followed by *N. oryzae* (37.5 %), *Phoma* spp. (35 %), and *F. pallidoroseum* (32.5 %). The lowest disease incidence was observed on plants sprayed with *Exserohilum rostratum* (27.5 %). There were no significant differences (P≤0.05) in pathogenicity among the isolates 7 to14 days after inoculation (DAI). Significant differences (P ≤ 0.001) between disease incidence was observed 21 DAI on the plants sprayed with different fungal pathogens (Table 6). The results also showed that the pathogenicity of different fungi on buffelgrass plants on the 28 DAI was not significantly different (P≤0.05).

Sanogo and Moorman (1993) reported that low density of pathogenic fungi may lead to low inoculum potential, hence failure of infected plants to show significant symptoms of infection even though the pathogen may be present in their cells or tissue. The results indicated that, the trend of disease appearance on inoculated seedlings might have been affected by initial density of pathogenic fungi. Of the inoculated fungi only *P. grisea* has been reported on buffelgrass to cause leaf spot (Perrot and Chakraborty, 1999 and Cook, 2007). Thus this is the first report to show that *Bipolaris* spp., *Nigrospora oryzae*, *Phoma* spp., *Fusarium pallidoroseum* and *Exserohilum rostratum* are major seedborne fungi associated with buffelgrass seeds in Tanzania.

Table 6: Frequency of occurrence of fungal disease on buffelgrass seedlings at 7 to 28 days after inoculation from selected pasture farms in Tanzania

	Disease incidence			
Isolates	**7DAI[g]**	**14DAI**	**21DAI**	**28DAI**
Bipolaris spp	10.0[a]	10.0[a]	22.50[b]	40.0[a]
Exserohilum	10.0[a]	10.0[a]	20.0[b]	27.5[a]
F. pallidoroseum	10.0[a]	15.0[a]	27.5[a]	32.5[a]
Nigrospora oryzae	0.0[a]	10.0[a]	20.0[b]	37.5[a]
P. grisea	0.0[a]	10.0[a]	20.0[b]	40.0[a]
Phoma spp.	10.0[a]	15.00[a]	22.5[b]	35.0[a]
SDW	0.0[a]	0.00[a]	0.00[c]	0.00[b]
Mean	5.71	10.00	18.93	30.36
F test	Ns	Ns	*	Ns
$LSD_{0.05}$	12.84	15.05	4.81	15.47
CV	152.75	102.35	17.29	34.66
S.E (±)	4.36	5.12	1.64	5.26

[g] = Days after inoculation, *Means in the same column with same letter are not significantly different at* P≤0.05. SDW = sterilized distilled water, CV = coefficient of variation, LSD = Least Significance differences, S.E = Standard Error, Ns = not significant and * = significant

4.2.3.2 Disease severity of different fungi inoculated on buffelgrass seedlings

The results showed that, the highest disease rating (2.5) and (2.4) was recorded in plants inoculated with *Phoma and Bipolaris* isolates on the 7, 21 and 28 days after inoculation (DAI), respectively (Fig 4;). Results showed an alternating disease progress on infected buffelgrass leaves on 7, 14 and 21 DAI. The lowest leaf spot disease severity on 28 DAI was shown by *E. rostratum*. There was a gradual increase in disease severity from 7 to 28 DAI. As the disease progressed, the area around the discrete lesions turned yellow. Tips and edges of infected leaves turned dark green to brown giving the leaf folded appearance.

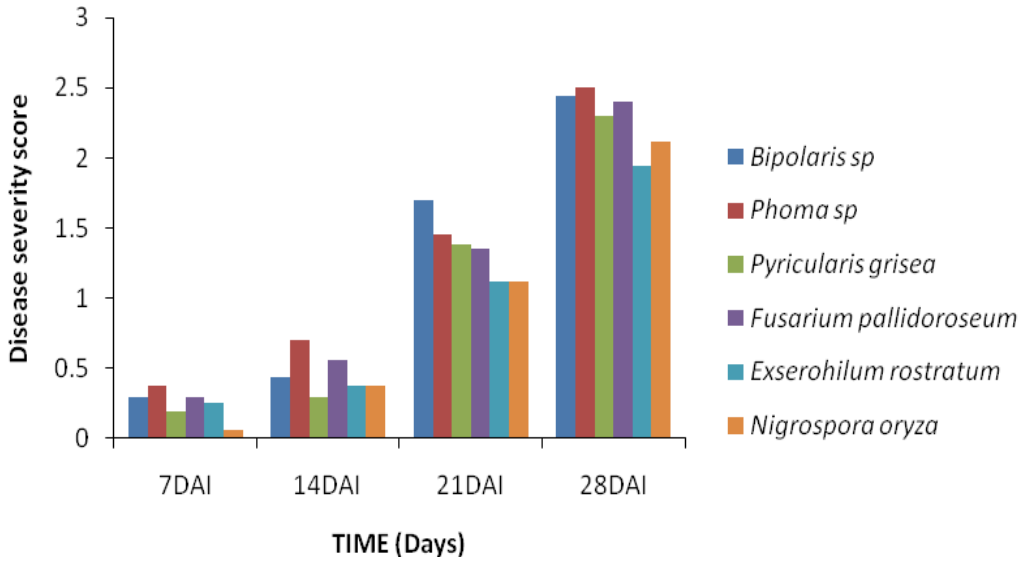

Figure 4: Disease severity rating on buffelgrass leaves 7 to 28 days after inoculation

4.2.3.3 Frequency of occurrence of infection symptoms on different fungi inoculated buffelgrass seedlings

The disease symptoms of fungal infection on inoculated buffelgrass seedling were as indicated in Table 7. The highest disease symptoms (15.50 %) by fungal infection was caused by *Phoma* and *Bipolaris* spp. followed by *Pyricularia grisea* and *F. pallidoroseum* (13.0 %) at 28 days after inoculation (DAI).The results indicated that there were no significant differences (P≤0.05) between plants inoculated with different fungal isolates at 7 and 14 DAI (Table 7). There was a slow or late leaf spot disease development at 7, 14 and 21 DAI. This might be caused by uncontrolled changes of temperature and humidity at the screen house during the study. Brecht (2005) reported an increased amount of leaf spotting and leaf tip necrosis of *Bipolaris hawaiiensis* and *Curvularia lunata* on bermudagrass when inoculated at 30 and 25 °C than at 20 °C.

Table 7: Fungal disease symptoms observed on buffelgrass seedlings at 7 to 28 days after inoculation

Isolates	7 DAI[k]	14DAI	21DAI	28DAI
Bipolaris spp	1.50[a]	2.25[a]	8.00[a]	15.50[a]
Exserohilum	1.50[a]	1.50[ab]	4.25[bc]	8.00[b]
F. pallidoroseum	1.50[a]	2.25[a]	3.00[c]	13.00[ab]
Nigrospora oryzae	0.00[a]	1.50[ab]	4.25[bc]	11.75[ab]
P. grisea	0.00[a]	1.50[ab]	5.50[abc]	13.00[ab]
Phoma spp.	1.50[a]	2.25[a]	6.75[ab]	15.50[a]
SDW	0.00[b]	0.00[b]	0.00[d]	0.00[c]
Mean	0.86	1.61	4.53	10.96
$LSD_{0.05}$	1.92	2.21	2.89	7.31
F test	Ns	Ns	***	**
CV	152.75	93.33	43.37	45.96
S.E (±)	0.65	0.75	0.98	2.48

Means within the column with the same letters are not significantly different at (P≤ 0.05) based on GLM procedure, ns = not significant, ** = very significant *** = extremely significant and [k] = Days after inoculation

The duration of surface wetness or high humidity in most terrestrial plants has also been reported to determine leaf spot disease development (Luo *et al*., 2001; Khazanda *et al*,. 2002; Magarey *et al*., 2005; Jugah *et al*., 2007; Satish e*t al*., 2010; Kleczewski and Flory, 2010; Harmon *et al.*, 2011). Further studies are thus, suggested to be done under more controlled environment. In this experiment, a single spray application with fungal inoculum on buffelgrass seedlings did not result in plant death. Infected plants recovered from initial damage and produced new foliage. The summary of leaf disease symptoms produced by fungi on buffelgrass seedlings used in the study is as shown in Table 8.

Table 8: Disease symptoms observed on buffelgrass seedlings inoculated with different fungi used in this study

Fungi	Symptoms
Bipolaris spp.	Leaf spot damping off, leaf blight, leaf spot
Phoma spp.	Leaf spots, shrink, loss in germination
Fusarium pallidoroseum	Leaf blight, leaf spot
Pyricularia grisea	Gray leaf spot and dieback,
Exserohilum rostratum	Dieback, leaf streak, leaf spot on older leaves,
Nigrospora oryzae	Chocolate brown spots, brown lesion

4.2.3.4 Pathogenicity of bacterial isolates on buffelgrass seedlings

Results showed that, all bacterial isolates that were positive for biochemical, physiological and PCR tests were pathogenic (Plate 7 and 8) on buffelgrass seedling (Table 9). Results obtained from aggressiveness of the bacterial isolated used in this study were all positive. These results show that there were significant differences ($P \leq 0.001$) between lesion length produced by different bacterial isolates at 7 and 14 days after inoculation on buffelgrass seedlings (Table 9). There were no significant differences ($P \leq 0.05$) on disease symptoms between buffelgrass seedlings inoculated with different bacterial isolates at 21 to 28 days after inoculation. The initial symptoms were leaf curling near the cut-off portion. Results indicated that, the seedlings produced water soaked lesions on the clipped ends and yellow discolouration which extended downwards (Plate 7 A and B). Such characteristics were similar to those described by Ali *et al.* (2009) in rice and Bradbury (1986) in *Clitoria tenatea*. The symptoms were also typical of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) which cause bacterial blight of rice (Neergaard, 1977; Ali *et al*., 2009). Sullivan

et al. (2011) reported buffelgrass to be alternative host of *Xoo* of rice which agrees with the current findings.

Plate 7: Disease symptoms caused by bacterial blight isolates on buffelgrass seedlings two weeks after inoculation using the clip method

T = blight lesions on different pot A and B

The largest disease lesion (1.28 cm) was observed on the buffelgrass plant inoculated with the bacterial isolate S_6 obtained from LRC Tanga followed by isolate S_1 (1.26 cm) from NLRI Mpwapwa at 21 DAI (Table 9). Likewise at 28 DAI the longest disease lesion (1.37 cm) was caused by bacterial isolates S_6. In addition, the control plants which were inoculated with SDW did not show disease symptoms.

Table 9: Lesions length caused by bacterial blight isolates on buffelgrass leaves at 7 to 28 days after inoculation using the clip method

	Lesion length (cm)				
Sample no.	**7DAI**	**14DAI**	**21DAI**	**28DAI**	**Sample source**
S_5^g	0.59^c	0.87^b	0.99^a	1.34^a	LITI Tengeru
S_6	1.18^a	1.26^a	1.28^a	1.37^a	LRC Tanga
S_2	0.68^c	0.93^b	1.16^{ab}	1.34^a	Mazimbu
S_1	0.97^b	1.14^a	1.26^a	1.35^a	NLRI-Mpwapwa
S_4	0.6^c	0.99^b	1.12^{ab}	1.21^a	Vikuge
Mean	0.80	1.04	1.16	1.32	
F test	***	***	Ns	Ns	
$LSD_{0.05}$	0.18	0.14	0.25	0.32	
CV	12.49	7.61	12.23	13.29	
S.E (±)	0.06	0.04	0.08	0.1	

Means within the column with the same letters are not significantly different at (P≤ 0.05) based on GLM procedure, ns = not significant, *** = extremely significant, +ve = positive aggressiveness, ^{g} = Bacterial isolate; $S_{1,}$ = isolate from Mpwapwa, $S_{2,}$ = isolate from Mazimbu, S_4 = isolate from Vikuge S_5 = isolates from LITI Tengeru and S_6 = isolates from LRC Tanga.

Bacterial blight on rice is favoured by warm temperatures (25 to 32ºC), high humidity (60 %), rain and deep water for at least two weeks (Saettler, 1989; Sullivan *et al.*, 2011). In this study, disease symptoms (Plate 8) were observed 14 DAI. This slow infection due to *Xanthomonas* spp. was also reported by Akhtar and Bhutta (2002) on paddy, wheat and cotton in Pakistan and was caused by fluctuating weather conditions. Pathogenicity results of isolates ($S_{2c,}$ S_{3c} and S_{6c}) on buffelgrass seedling are shown on Table10. The longest leaf lesion length (13.23 cm), was caused by S_{3c} isolate followed by S_{6C} (12.13cm) and S_{2c} (7.99cm). There were no significant differences on buffelgrass (P≤0.05) disease symptoms between seedlings inoculated at 7, 14, and 21 DAI (Table 10).

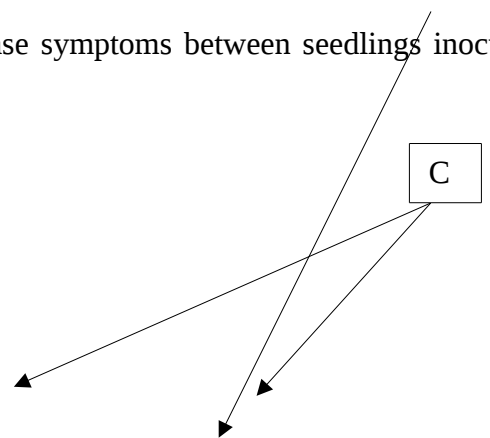

Plate 8: Disease symptoms caused by bacterial streak isolates using Spray method on buffelgrass leaves two weeks after inoculation

C = streak lesions

However, isolates (S_{3c}) from LRC Mabuki and (S_{6c}) from LRC Tanga were significantly different ($P \leq 0.05$) from those obtained from Mazimbu 28 DAI. The inoculated leaves indicated distinct, brown lesion with prominent haloes 28 DAI. Plants inoculated with sterile distilled water (negative control) did not develop any disease symptom.

The bacterial strains S_{2c} and S_{6c} caused small, water soaked and translucent to light yellow brown banded lesions running along the leaf veins. The lesions then developed to brown /chlorotic halo. The symptoms were similar to those caused by *A. avenae* reported on millet and rice by Nelson (2009). *Acidovorax avenae* is also reported to infect buffelgrass plants (Song *et al*., 2004).

Table 10: Disease lesions caused by bacterial streak isolates on buffelgrass seedlings 7 to 28 days after inoculation using spray method

	Lesion length (cm)				
Sample no.	**7DAI**	**14DAI**	**21DAI**	**28DAI**	**Sample source.**
S_3	2.08[a]	3.47[a]	11.38[a]	13.23[a]	LRC Mabuki
S_{6c}	1.79[a]	3.12[a]	9.49[ab]	12.13[a]	LRC Tanga
S_{2c}	1.70[a]	2.65[a]	5.84[b]	7.99[b]	Mazimbu
Mean	1.86	3.08	8.92	11.12	
F test	Ns	Ns	Ns	*	
$LSD_{0.05}$	1.10	1.50	4.86	1.98	
CV	29.72	24.38	27.28	13.45	
S.E (±)	0.32	0.43	1.4	0.86	

Means followed by the same letter within a column are not significantly different (P≤ 0.05) according to LSD test. Ns = not significant, * = significant, 2c = isolates from Mazimbu, S_{3c}= isolates from LRC Mabuki and 6c = isolates from LRC Tanga.

These bacterial isolates S_{2c} (from Mazimbu) and S_{6c} (from Tanga) might have been *A. avenae* as were previously confirmed by the preliminary biochemical tests. The symptoms caused by bacterial isolate S_3 (which preliminarily characterized as *Pseudomonas*) on buffel grass were difficult to differentiate with those caused by *A. avenae.* Song *et al.* (2004) reported failure of the two pathogens to produce distinct symptoms under field condition. Shakya *et al*. (1995) reported of plants surviving infection in the seedling stage to harbour the bacterium without showing symptoms as the Acidovorax avenae is reported to be located between the glumes and pericarp or deeper in seed.

4.2.3.5 Detection of bacteria from infected buffelgrass leaves

To confirm that bacterial symptoms which developed on the inoculated buffelgrass plants were due to bacterial strain inoculated, infected leaves were plated as previously described. The results of purified colonies had similar characteristics as those of bacterial strains inoculated (Table 11). The strains were negative to Gram reaction, Oxidase and potato soft rot tests and were pathogenic on buffel grass seedlings. Based on these results, *Pseudomonas glumae and A. avenae* were

confirmed to be causal agents of leaf spot. *Pseudomonas* spp. has also been reported to be the causal agent of necrotic spot on various organs of plant species including cowpea (Bradbury, 1986). *Acidovorax avenae* is also reported to cause brown streaks on leaves of rice and pathogenic to a wide host range such as barley, maize and millet (Kadota, 1996; Yuan, 2004).

Table 11: Characteristics of re-isolated bacteria from inoculated buffelgrass plants

Isolate identity	Initial colony colour	Re-isolation (DAI)	Final colony colour	Gram/ Oxidase test	Arg. Test	Nitr. Test	Gel. test	St. test	Initial isolate
S_1^k	Yellow	21	Yellow	-ve/-ve	+ve	-ve	+ve	+ve	Present
S_4	Yellow	21	Yellow	-ve/-ve	+ve	-ve	+ve	-ve	Present
S_6	Yellow	21	Yellow	-ve/-ve	+ve	-ve	+ve	-ve	Present
S_2	Yellow	21	Yellow	-ve/+ve	-ve	-ve	+ve	V	Present
S_5	Yellow	21	Yellow	-ve/-ve	-ve	-ve	+ve	V	Present
S_3	Cream	21	Cream	-ve/-ve	-ve	+ve	+ve	V	Present
S_{6c}	Cream	21	Cream	-ve/-ve	-ve	+ve	+ve	V	Present
S_{2c}	Cream	21	Cream	-ve/-ve	-ve	+ve	+ve	-ve	Present

-ve = negative, +ve = positive, v= variable, *Arg= Arginine dihydrolase, St= Starch hydrolysis, Nitr = Nitrate reduction,DAI* =days after inoculation, ^{k}= bacterial isolates; S_1, = isolate from Mpwapwa, S_2 and 2c, = isolate from Mazimbu, S_4 = isolate from Vikuge S_5 = isolates from LITI Tengeru and S_6 and 6c = isolates from LRC Tanga, S_3= isolates from LRC Mabuki.

4.2.3.6 Re-isolation of fungi from buffelgrass infected leaves

The results for the recovery of fungi on buffelgrass plants previously inoculated with the fungal pathogens are shown in Table 12. The blotter method was used for fungal re-isolation from symptomatic leaves. Using the compound microscope and procedure developed by Mathur and Kongsdal (2003), the results confirmed the presence of *Bipolaris* spp., *Phoma* spp., *Fusarium pallidoroseum***,** *Exserohilum rostratum*, *Pyricularia grisea* and *Nigrospora oryzae*.

Table 12: Fungal microorganisms re-isolated from inoculated buffelgrass plants following pathogenicity tests

Isolate identity	Name of inoculated fungi	Re-isolation (DAI)	Final re-isolates conidia	Referral template	Initial isolate
F_1[g]	*Bipolaris* spp.	28	*Bipolaris* spp. conidia	Mathur and Kongsdal,(2003)	Present
F_2	*Phoma*	28	*Phoma*	Mathur and Kongsdal,(2003)	Present
F_3	*Pyricularia grisea*	28	*Pyricularia grisea*	Mathur and Kongsdal,(2003)	Present
F_4	*Fusarium pallidoroseum*	28	*Fusarium pallidoroseum conidia*	Mathur and Kongsdal,(2003)	Present
F_5	*Exserohilum rostratum*	28	*Exserohilum rostratum* conidia	Mathur and Kongsdal,(2003)	Present
F_6	*Nigrospora oryzae*	28	*Nigrospora oryzae* conidia	Mathur and Kongsdal,(2003)	Present

[g] = isolate identity, DAI = days after inoculation

4.3 Effect of Fungal Infection on Seed Germination

4.3.1 Germination percent of buffelgrass seeds

Results for germination test conducted using the Top of Paper method indicated that the number of normal buffelgrass seedling was significantly different ($P \leq 0.001$) between the seed farm samples tested (Table 13). Buffelgrass seed samples collected from Vikuge pasture farm had the highest number of normal seedlings (36 %) followed by seed samples from Mazimbu pasture farm (27 %), LRC Mabuki (24.5 %) and NLRI Mpwapwa (10.5 %) (Table13). Buffelgrass seed samples collected from LRC Tanga did not germinate.

Table 13: Percentage germination of buffelgrass seeds collected from selected pasture seed farms in Tanzania

Farm	Germination[f] (%)			
	Normal Seedlings	Abnormal Seedlings	Hard seeds	Dead Seed
NLRI Mpwapwa	10.5[b]	1.50[b]	17.50[ab]	70.50[b]
Mazimbu (SUA)	27.0[a]	1.5[b]	3.50[c]	68.00[b]
LRC Mabuki	24.5[a]	2.5[ab]	19.50[a]	53.50[c]
Vikuge	36.0[a]	5.5[a]	13.50[ab]	45.0[c]
LITI Tengeru	8.0[b]	2.5[ab]	20.5[a]	69.0[b]
LRC Tanga	0.0[b]	0.0[b]	10.25[bc]	89.75[a]
Mean	17.67	2.25	14.12	65.96
$LSD_{0.05}$	12.14	3.11	7.52	13.90
F test	***	*	**	***
CV %)	46.25	93.11	35.86	14.19
S.E (±)	4.09	1.05	2.53	4.68

Within a column means with the same letters are not significantly different at P≤ 0.05 based on Least Significant Difference (LSD), [f] = Represents percentage from 400 seeds used in germination test. * = significant, ** = very significant and *** = extremely significant. LRC = Livestock Research Centre, LITI = Livestock Training Institute, NLRI = National Livestock Research Institute and SUA = Sokoine University of Agriculture

These results show that germination was low in all pasture seed farms. Seed dormancy may be one of the causes of such results because seed samples used in this study were collected soon after harvest. Therefore, storage of buffelgrass seeds may be required for breaking seed dormancy (Gobius *et al*., 2001). Palma-Rivero *et al.* (2000) obtained a lower germination of buffelgrass seeds (17.8%) and (24.7%) after 4 and 9 months of seed storage, respectively. Yadav *et al*. (2001) reported slightly higher (32.4%) buffelgrass seed germination after storing the seeds for one year compared with Kizima *et al*. (2012) who reported the germination of 10 % in buffelgrass seeds that were directly tested soon after harvest.

Seed germination of buffelgrass has been reported to improve by 70 % after storing under dry conditions for two years (Aganga and Tshwenyane, 2003; Parihar and Pathak, 2006; Mganga *et al*., 2010). The current germination results on the number of

abnormal seedlings also showed that the seed samples collected from Vikuge (5.5 %) were significantly different (P≤ 0.05) from those collected from Mazimbu and NLRI Mpwapwa both of which had (1.5 %) of abnormal seedlings (Table 13). Mukhtar (2009) reported the seed quality and seed germination vigour of buffelgrass seedlings to be adversely affected by fungal infection (e.g. *Alternaria* spp).

The results also showed that the number of dead seed was significantly different (P ≤ 0.001) between seed samples collected from different seed farms. Seeds collected from LRC Tanga had the highest percentage of dead seeds (89.75 %), followed by NLRI Mpwapwa (70.5 %), LITI Tengeru (69 %) and Mazimbu, SUA (68 %). Such results implied that other factors rather than seed dormancy may contribute to poor germination observed in this study. The most important factor which contributed to the high number of dead seeds might be infection by seed-borne pathogens (Plate 9 & 10), which have been reported to cause seed decay and deterioration in buffelgrass (Ndomba, 2009; Habib *et al.*, 2011).

A

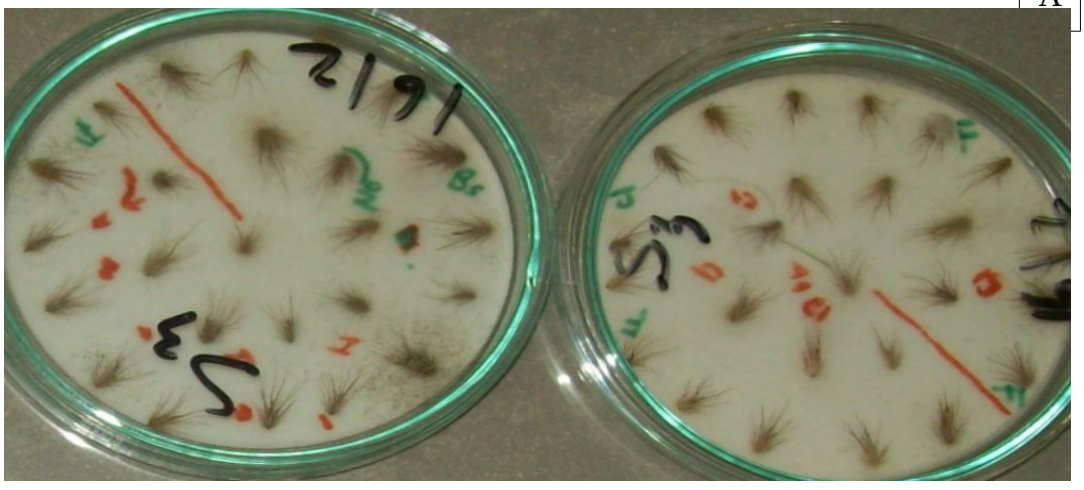

Plate 9: Different fungal species (A) growing on buffelgrass seed collected from LRC Mabuki, detected using Blotter method

Plate 10: Fungi (B) growing on buffelgrass seed collected from NLRI Mpwapwa detected using Blotter method

The results also indicate that there were significant differences ($P \leq 0.001$) between the number of hard seeds recorded for different pasture seed farms. Pasture seed samples from LRC Mabuki had the highest (19.5 %) number of hard seed (Table 13). The lowest number of hard seeds (3.5 %) was recorded in pasture seed samples collected from Mazimbu SUA farm. The results implied that a big number of pasture seeds collected from pasture seed farms used in this study did not germinate. Low seed viability might have been due to seed dormancy and improper storage conditions. Freshly harvested buffelgrass seeds have been reported to have a high level of dormancy (Cook, 2007). Germination percent has been reported to improve when buffelgrass seed were stored 6 to 18 months after harvest (Factsheet, 2012 and Fig. 1). This post-harvest dormancy is a typical seed behaviour which occurs also in seeds of *Hordeum* spp. *Triticum* spp. and *Festuca rubra* L. (Palma-Rivero *et al*., 2000). Butler (1985) found that the dormancy of *Cenchrus ciliaris* caryopsis is caused

mainly by the presence of inhibitors in the seed wrappings (bristles, glumes, lemma and palea). Harty *et al.* (1983) and Gonzalez *et al.* (1994) also observed a similar behaviour in the seeds of *Brachiaria* and *Panicum maximum*.

4.3.2 Caryopsis index of buffelgrass seeds

The results of the pure seed unit (Springer and Dewald, 2001) index or caryopsis are shown in Table 14. The buffelgrass seeds from Mazimbu SUA and LRC Mabuki had the highest caryopsis index (50 %) followed by NLRI Mpwapwa (33.3 %), LRC Tengeru (26.7 %) and LRC Tanga (20 %) (Table 14). The lowest caryopsis index was recorded in seeds from Vikuge (6.7%). Such results imply that many of the spikelets from each seed sample were empty. Parihar and Pathak (2006) reported poor seed setting in spikelets to be susceptible to weather condition prevailing at the time of anthesis and grain formation. The empty spikelets obtained might have resulted from the effect of weather conditions during seed setting and microorganism infections. Franco *et al.* (2007) also reported microorganism (fungal) infection to prevent maturation of rice grains for about 50 % leading to unfilled rice spikelets.

Table 14: Caryopsis indices for buffelgrass seeds from selected pasture farms in Tanzania

FARM	Number of seeds	Caryopses in seeds	Caryopsis index (%)
NLRI-Mpwapwa	30	10	33.3
Mazimbu	30	15	50.0
LRC-Mabuki	30	15	50.0
Vikuge	30	2	6.7
LITI-Tengeru	30	8	26.7
LRC-Tanga	30	6	20.0

NLR = National Livestock Research Institute, LITI = Livestock Training Institute and LRC = Livestock Research Centre.

The low caryopsis index (of not more than 50 %) obtained in this study indicated that viability of buffelgrass seeds in the selected pasture seed farms were below 50 %. This has a negative implication to farmers as they need to buy more seeds for sowing to compensate for the seeds that will not germinate/unfilled thus, increasing the cost of pasture establishment.

4.3.3 Purity of buffelgrass seed samples

The results for seed purity determined based on procedures described by ISTA (2005) are shown in Table 15. The highest proportion of pure seed was recorded from seed samples collected from LRC Tanga (96.7 %.), LRC Mabuki (95.1%) and NLRI-Mpwapwa and LITI Tengeru (75.4 %). These results imply that most of the seed purity was within the FAO (2006) minimum recommended range for quality declared seeds of buffelgrass (90 %) except those from the two pasture farms above. The highest percentage of inert material was in seed samples from LITI Tengeru (23 %), followed by NLRI Mpwapwa (21 %) and Vikuge (6.6 %). Presence of high proportion of inert material in the seed entail more cost to farmers who purchase these seeds. Likewise, inert matter may harbour seedborne pathogens (Loch and Boyce, 2001), which may also contribute to reducing germination of buffelgrass.

Table 15: Percentage purity of buffelgrass seeds collected from selected pasture seed farms in Tanzania

Farm	Purity (%)[h]		
	Pure Seed	Inert Matter	Other crop seed
NLRI Mpwapwa	75.4	21.3	1.6
Mazimbu	93.4	06.6	0
LRC Mabuki	95.1	3.3	0

Vikuge	93.4	6.6	0
LITI Tengeru	75.4	23.0	0
LRC Tanga	96.7	3.3	0
Mean	88.3	10.7	Na

[h] = represent percentage of 6 g of buffelgrass seeds used (ISTA, 2005), LRC = Livestock Research Centre, LITI = Livestock Training Institute, NLRI = National Livestock Research Institute and SUA = Sokoine University of Agriculture.

4.3.4 Moisture content of buffelgrass seed samples

Results of moisture content (MC) of buffelgrass seed samples are shown in Table 16. Seed samples collected from LRC Mabuki had the highest MC (9.7 %) followed by seeds from LRC Tanga (9.4 %). Seed samples from NLRI-Mpwapwa had the lowest MC (5.1 %). A similar MC range was observed by Kizima *et al*. (2012) on buffelgrass seed collected from Mazimbu (5.6 %), Vikuge (5.2 %) and LRC Tanga (5.0 %). Such results indicated that, the seed samples used in this study had acceptable MC range i.e. below 12 % (Elias *et al*., 2006). Chin and Hanson (1999) recommended the MC of as low as 5% or below (8%) to be important for viability of several forage species including buffelgrass. Low moisture content increases storage time and longevity of forage seeds regardless of age (Elias *et al*., 2006 and ESGPIP, 2010). Anjorin and Mohammed (2009) reported MC of the seed, prevailing temperature, storage period and degree of seed invasion with the pathogen as factors influencing the development of seed-borne fungi.

Table 16: Moisture content (percentage) of buffelgrass seeds collected from selected pasture seed farms in Tanzania

Farm	Moisture Content (%)[a]
NLRI Mpwapwa	5.1
Mazimbu	8.1
LRC Mabuki	9.7

Vikuge	6.9
LITI Tengeru	7.6
LRC Tanga	9.4
Mean	7.8

[a] = Represents percent of 10 g (ISTA, 2005) in three replicates of buffelgrass seeds used in moisture determination, LITI = Livestock Training Institute, LRC = Livestock Research Centre and NLRI = National Livestock Research Institute.

4.3.5 Germination of infected buffel grass seed caryopsis

Germination results of seed unit/caryopses infected with fungal pathogens detected in this study are as shown in Table 17. The results indicated that, there were significant differences ($P \leq 0.05$) in germination of caryopsis from Vikuge (40.75 %) and those from NLRI Mpwapwa (12.75 %), LITI Tengeru (23.58 %), Mazimbu (31.75 %) and LRC Mabuki (33 %). The seed units/caryopses from Vikuge pasture farm were relatively high in seed infection; however, germination of their caryopsis was significantly higher than those from other farms (Table 17). This indicated that poor germination of buffelgrass seeds might be associated with fungal infection in their seed units/caryopsis. Cram and Fraedrich (2005) and Haikal (2008) reported the ability of pathogenic fungi to infect seeds internally and destroy the endosperm and the embryo or contaminated the seeds and affect seedling germination and development of plants.

The results of germination of buffelgrass caryopsis indicated that removing caryopsis from spikelets, improves the seed germination compared to seeds with spikelets (Tables 2 and 17). These results concur with those of Parihar and Pathar (2006) and Gleiser *et al.* (2004) who reported the germination of 86 % and 92 % when caryopsis were removed from the spikelets in buffelgrass and *Brachiaria ruziziensis*,

respectively. The improvements in the germination of the caryopsis might have been due to rapid absorption of moisture when planted. Such results are in conformity with those of Loch (1993) who reported that chaffy husk in buffelgrass has a marked influence on moisture relation around the caryopsis during germination. Olivier (2009) also recommended the removal of switchgrass seed coat to improve their germination. Seed with spikelets may acts as reservoir of seedborne pathogens. Seedborne pathogen infestation can reduce sanitary quality of seeds leading to decreased seed germination (Mbega, 2007; Dawar *et al.*, 2007).

Table 17: Buffelgrass caryopsis germination percent from selected pasture seed farms in Tanzania

Farm	Germination (%)			
	Normal Seedlings[f]	Abnormal seedlings	Hard seed	Dead seed
NLRI-Mpwapwa	12.75[d]	6.25[a]	20.0[b]	61.00[ab]
Mazimbu	31.75[b]	6.25[a]	13.20[ad]	48.75[c]
LRC Mabuki	33.00[b]	3.00[b]	7.25[d]	56.75[b]
Vikuge	40.75[a]	6.25[a]	11.75[cd]	41.25[d]
LITI Tengeru	23.25[c]	4.50[ab]	15.0[bc]	57.25[b]
LRC Tanga	0.00[e]	0.00[c]	34.50[a]	65.50[a]
Mean (%)	23.58	4.38	16.96	55.08

F test	***	***	***	***
$LSD_{(0.05)}$	7.09	1.89	6.58	6.22
CV	20.26	29.14	26.12	7.61
S.E (±)	2.39	0.62	2.2	2.1

Means within the column with same letter are not significantly different (P≤0.05), [f] = Based on 400 seeds, ns = not significant, * =significant, ** = very significant and *** = highly significant, CV= coefficient of variation, S.E = standard error, LSD = Least Significance difference, LITI = Livestock Training Institute, LRC= Livestock Research Centre and NLRI= National Livestock Research Institute.

Spikelets from Vikuge had the highest fungal incidence (55.3 %) followed by Mabuki (18.4 %) and Mazimbu SUA (13.2 %) (Table 18). Thus the removal of spikelets from caryopses reduced fungal infestation. Similar findings have been reported by Tariq *et al*. (2005) in soybean seed and Rasheed *et al*. (2004) in groundnut seed. Beckstead *et al*. (2010) also reported slow germinating or dormant seeds of *Bromus tectorum* due to infection by pathogens.

4.3.6 Fungal species located on buffelgrass seed caryopsis and spikelets

The results indicate that fungal infection located on the caryopsis of buffelgrass included *Verticillium* spp. which had the highest incidence (41 %) (Plate 11; Table 18). This fungal load on caryopsis was almost twice as much compared to that on the spikelets (21.1 %) (Table 19). The results also show that caryopses of buffel grass seeds from LITI Tengeru had the highest seed infection (37.8 %), followed by those from Vikuge (27.5 %) and Mabuki (24 %). Other fungal species found on buffel grass caryopsis included *F. pallidoroseum* (14.2 %), *Curvularia lunata* and *Exserohilum rostratum* (13.7 %). *Pyricularia grisea*, *Phoma* spp. and the common storage fungal seed contaminants namely *Penicillium*, *Actinomycetes*, *Rhizopus* and *Aspergillu*s were observed on spikelets. High incidence of *Rhizopus* was detected in

seed samples collected from LRC Mabuki and this could be attributed to high temperatures and high relative humidity which favours its sporulation and growth.

Table 18: Frequency of occurrence of associated fungi on buffel grass caryopsis from selected pasture farms in Tanzania

	Caryopsis[a] fungal frequency (%)							
Farm	Aa[b]	Cl	Fp	Vc	Ex	No	Total	Frequency (%)
NLRI Mpwapwa	0	0	0	0	0	0	0	0.00
Mazimbu	0	0	0	0	0	0	0	0.00
LRC Mabuki	0	0	0	56	0	0	56	24.03
Vikuge	0	16	0	24	24	0	64	27.47
LITI Tengeru	16	0	24	16	8	24	88	37.77
LRC Tanga	0	16	9	0	0	0	25	10.73
Total	16	32	33	96	32	24	233	
Frequency (%)	6.9	13.7	14.2	41.2	13.7	10.3		

[a] = 400 hundred seeds per sample were tested using blotter method. [b] Aa = *Alternaria alternata*, Cl =*Curvularia lunata*, Fp= *Fusarium pallidoroseum*, Vc = *Verticillium* sp, Ex =*Exserohilum rostratum*, No = *Nigrospora oryzae*, NLR = National Livestock Research Institute, LITI = Livestock Training Institute, LRC = Livestock Research Centre

Table 19: Frequency of fungal microorganisms on buffel grass seed spikelets from selected pasture farms in Tanzania

	Fungal frequency (%) on spikelet							
Farm	Aa	Ph	Cl	Vc	Pg	Ex	Total	% Total Frequency
NLRI Mpwapwa	0	16	8	0	0	0	24	7.89
Mazimbu	16	16	8	0	0	0	40	13.16
LRC Mabuki	0	0	0	56	0	0	56	18.42
Vikuge	0	16	56	8	84	4	168	55.26
LITI Tengeru	8	0	0	0	0	8	16	5.26
LRC Tanga	0	0	0	0	0	0	0	0.00

Total	24	48	72	64	84	12	304[b]
Frequency (%)	7.9	15.8	23.7	21.1	27.6	3.9	

Aa = *Alternaria alternata*, Cl =*Curvularia lunata*, Fp= *Fusarium pallidoroseum*, Vc = *Verticillium* sp, Ex =*Exserohilum rostratum*, Ph = *Phoma* sp, Pg= *Pyricularia grisea* and No =*Nigrospora oryzae*. Fungal (%) = sum of individual fungal spp/b. NLR = National Livestock Research Institute, LITI = Livestock Training Institute, LRC = Livestock Research Centre

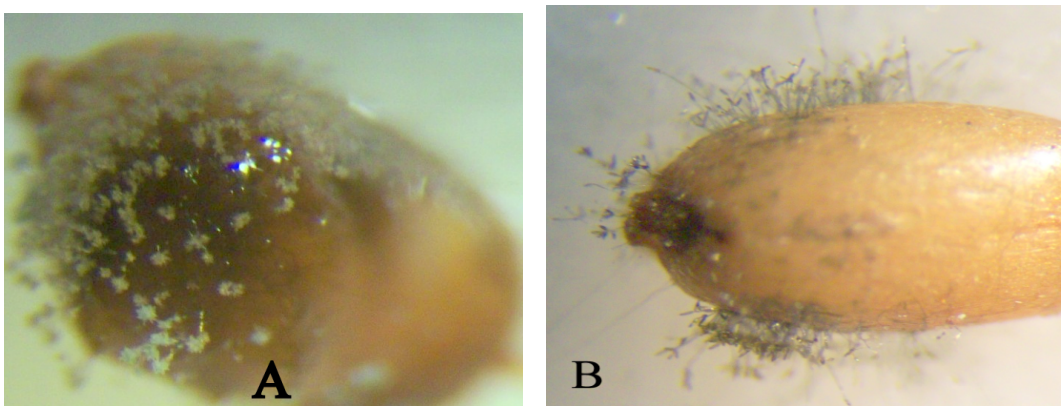

Plate 11: Fungal species detected on buffelgrass caryopsis from Livestock Research Centre Mabuki using Blotter method

A =*Verticillium* spp., B = *Bipolaris* spp. mycelia on buffel grass caryopsis

4.4 Buffelgrass Seed Cost and Seeding Rate in Relation to Seed Quality from Selected Pasture Seed Farms in Tanzania

The results of pure seeds (75.4 to 96.7 %) and normal germination of between 8 to 36 % (as previously reported in Table 13) were used to determine the new seed cost and seeding rate (Table 20). Results of pure live seed (PLS) as described by Houck (2009) and Bosworth, (2012) were calculated in order to determine actual seed rate after poor seed germination on buffel grass were obtained in the present study. The results show that the highest seeding rate for buffelgrass adjusted based on quality was on seeds

from LITI Tengeru (149 kg/ha), followed by NLRI Mpwapwa (108 kg/ha), LRC Mabuki (38 kg/ha), Mazimbu (SUA) (35.7 kg/ha) and Vikuge farm (26.8 kg/ha) (Table 20). Table 20 shows that, as the PLS value declines the seed rate increases markedly.

Such results implied that adjusted seed rates of buffel grass were to be determined for each farm instead of the previous 9 kg/ha recommended by ISTA (2005). Based on the adjusted seed rates under this study and the former cost per ha (Tsh. 90 000.00 and 135 000.00), the actual cost of buffel grass seeds per kilogram were to be re-adjusted. The results showed the highest cost per kilogram (Tsh./kg) to be in pasture seeds from Vikuge (Tsh. 3 361.97), followed by Mazimbu SUA (Tsh. 2 521.70), LRC Mabuki (Tsh. 2 329.80) and NLRI Mpwapwa (Tsh.829.00) (Table 20). LITI Tengeru seeds were to be sold at Tsh.603.00 per kilogram based on the adjusted seed rate instead of Tsh. 10 000.00 per kilogram. The increment in the seed rates also affected the cost of seeds, from which livestock keepers would not afford to purchase and establish their pasture farms if previous price were to remain. However, CRC (2001) recommended increment in the sowing rates to compensate for the low seed quality. The increase in seed rate in this study might have been contributed by microorganism contamination, harvest of immature seeds (some empty florets), inert matter inclusions (Table 13), improper seed handling and storage conditions. Therefore the required seed rates for each farm would be an alternative choice before solutions to these factors are obtained. This suggest the need for further investigation on seed quality attributes to be carried in order to increase the percent of PLS available in pasture seed farms and so as to attract many stakeholders who would wish to invest in pasture production.

Table 20: Buffelgrass seed cost and seeding rate in relation to seed quality values from selected pasture farms in Tanzania.

Species	NLRI	Vikuge	Mazimbu	LRC	LRC	LITI
Buffelgrass	**Mpwapwa**	**Farm**	**(SUA)**	**Mabuki**	**Tanga**	**Tengeru**
Price (Bulk)/ kg	10 000	15 000	10 000	10 000	10 000	10 000
Seed rate (kg/ha)[a]	9	9	9	9	9	9
Cost/ha (Tsh.)	90 000	135 000	90 000	90 000	90 000	90 000
% Purity	0.754	0.934	0.934	0.951	0.967	0.754
% Germination	0.11	0.36	0.27	0.245	0	0.08
PLS	0.0829	0.3362	0.2522	0.233	0	0.0603
Re-calculated Seed rate (kg/ha)	108.56	26.77	35.69	38.63	na	149.25
Re-calculated price/kg	829.03	3 361.97	2 521.70	2 329.80	na	603.00

PLS= Pure live seed, PLS = %germination*%purity, New Seed rate = recommended rate/total PLS.[a] = based on ISTA, (2005), na = not available, Tsh. = Tanzanian shilling, and New seed price/kg = (cost/ha)/new seed rate

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The study indicates that buffelgrass seed tested were variably contaminated with the seed-borne microorganisms. The fungal pathogens with the highest seed infection were *Curvularia lunata* (18.4%), followed by *Bipolaris* spp. (13.8%) and *Alternaria alternata* (14.8%). Other fungal species identified in the seed tested with moderate infection included *Phoma*, *Exserohilum*, *Fusarium*, *Pyricularia* and *Nigrospora* species. Also storage fungi detected in the buffelgrass seed samples used in this study included *Actinomycetes, Asperigillus*, *Rhizopus*, *Cladosporium* and *Penicillium* species, whilst the bacterial species belonged to the genera of *Xanthomonas, Pseudomonas* and *Acidovorax.* There was also a highly to moderately variability of buffelgrass seed-borne microorganism infection among different farms studied. It is concluded that before selling buffelgrass seed for establishment; seed health measures/procedures should first be considered so as to reduce transmission of seed-borne microorganisms to other areas.

This study has also shown that removing of spikelets from the caryopsis showed increased buffel grass seed germination from 36 % to 40.7 % implying that spikelets contributed to the inhibition and reducing seed germination. The study also showed the buffel grass seed rates (or actual seed rates) at different pasture farms to be low due to seed-borne microorganism infection and high when purity and germination of the seed were compared with their pure live seed values. In order to compensate for

the factors reducing seed germination such as microorganism infection, dormancy and empty seeds, a new seed rate of 149.25 kg/ha was considered to be the most appropriate in buffelgrass seeds samples collected. With the view of the results obtained in the study it is concluded that seed rate to be increase well above the recommended rate of 9 kg/ha.

5.2 Recommendations

This study recommends;

(a) Further seed health analysis to be done on predominant seedborne microorganisms identified for other pasture species across the country so that disease mapping can be done and updated regularly targeting potentially important pathogens. Seed health assays must continue so as to improve bulk seed production and discard seeds that are suspected and often shown to be sources of inoculum for disease epidemics. More experiments need to be conducted on the on effective control measures of seedborne microorganisms to reduce the incidence of the buffelgrass diseases.

(b) Measures to be taken in all pasture seed farms to ensure that good cultural practises are adhered to in order to minimize the incidence of pathogens, and ensure that clean and healthy pasture seeds are produced. This will contribute to reducing the level effect of microorganisms in buffelgrass seed and their effect on seed germination.

(c) Proper processing of seed before storage to remove inert materials that will lead to higher seed quality and more uniform seed delivery during sowing, as well as compensatory effect of lowering seeding rates and costs per hectare should be arrange and emphasised in existing price-sensitive markets of buffelgrass in the country.

(d) Establishment of pathogen inoculum thresholds and development of standardized assays that will allow seed produced in the country to be monitored for some minimum level of health quality.

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APPENDICES

Appendix 1: ANOVA tables for buffelgrass seed Germination tests

1. Dependent Variable: NS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	3707.333333	741.466667	11.10	<.0001
Error	18	1202.000000	66.777778		
Corrected Total	23	4909.333333			

R-Square	Coeff Var	Root MSE	NS Mean
0.755160	46.25529	8.171767	17.66667

2. Dependent Variable: ABS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	67.5000000	13.5000000	3.08	0.0352
Error	18	79.0000000	4.3888889		
Corrected Total	23	146.5000000			

R-Square	Coeff Var	Root MSE	ABS Mean
0.460751	93.10967	2.094968	2.250000

3. Dependent Variable: HS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	836.875000	167.375000	6.52	0.0013
Error	18	461.750000	25.652778		
Corrected Total	23	1298.625000			

R-Square	Coeff Var	Root MSE	HS Mean
0.644432	35.85740	5.064857	14.12500

4. Dependent Variable: DS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4778.208333	955.641667	10.91	<.0001
Error	18	1576.750000	87.597222		
Corrected Total	23	6354.958333			

R-Square	Coeff Var	Root MSE	DS Mean
0.751887	14.18977	9.359339	65.95833

Appendix 2: ANOVA tables for disease severity rating on buffelgrass plants 7 to 28 days after inoculation

1. Dependent Variable: 7DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.46428571	0.07738095	1.62	0.1896
Error	21	1.00000000	0.04761905		
Corrected Total	27	1.46428571			

R-Square	Coeff Var	Root MSE	D7AI Mean
0.317073	101.8350	0.218218	0.214286

2. Dependent Variable: 14DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.11607143	0.18601190	2.72	0.0411
Error	21	1.43750000	0.06845238		
Corrected Total	27	2.55357143			

R-Square	Coeff Var	Root MSE	14 D AI Mean
0.437063	66.59776	0.261634	0.392857

3. Dependent variable: 21DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	7.14620000	1.19103333	31.81	<.0001
Error	21	0.78632500	0.03744405		
Corrected Total	27	7.93252500			

R-Square	Coeff Var	Root MSE	21D AI Mean
0.900873	16.71746	0.193505	1.157500

4. Dependent variable: 28DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	18.74107143	3.12351190	20.09	<.0001
Error	21	3.26562500	0.15550595		
Corrected Total	27	22.00669643			

R-Square	Coeff Var	Root MSE	28 D AI Mean
0.851608	20.16728	0.394342	1.955357

Appendix 3: ANOVA tables for fungal disease symptoms observed on buffelgrass seedlings at 7 to 28 days after inoculation.

1. Dependent Variable: 7DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	15.42857143	2.57142857	1.50	0.2265
Error	21	36.00000000	1.71428571		
Corrected Total	27	51.42857143			

R-Square	Coeff Var	Root MSE	7DAI Mean
0.300000	152.7525	1.309307	0.857143

2. Dependent Variable: 14DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	15.42857143	2.57142857	1.14	0.3727
Error	21	47.25000000	2.25000000		
Corrected Total	27	62.67857143			

R-Square	Coeff Var	Root MSE	14DAI Mean
0.246154	93.33333	1.500000	1.607143

3. Dependent Variable: 21DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	163.7142857	27.2857143	7.05	0.0003
Error	21	81.2500000	3.8690476		
Corrected Total	27	244.9642857			

R-Square	Coeff Var	Root MSE	21DAI Mean
0.668319	43.36670	1.966989	4.535714

4. Dependent Variable: 28DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	716.214286	119.369048	4.83	0.0030
Error	21	518.750000	24.702381		
Corrected Total	27	1234.964286			

R-Square	Coeff Var	Root MSE	28DAI Mean
0.579947	45.33035	4.970149	10.96429

Appendix 4: ANOVA tables for disease lesions length caused by bacterial isolates on buffelgrass leaves at 7 to 28 days after inoculation using clip method

1. Dependent variable: 7DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.80517333	0.20129333	19.93	<.0001
Error	10	0.10100000	0.01010000		
Corrected Total	14	0.90617333			

R-Square	Coeff Var	Root MSE	7 D AI Mean
0.888542	12.48949	0.100499	0.804667

2. Dependent variable: 14 DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.31029333	0.07757333	12.43	<.0007
Error	10	0.06240000	0.00624000		
Corrected Total	14	0.37269333			

R-Square	Coeff Var	Root MSE	14 D AI Mean
0.832570	7.615071	0.078994	1.037333

3. Dependent variable: 21DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.16576000	0.04144000	0.1639	2.04
Error	10	0.20280000	0.02028000		
Corrected Total	14	0.36856000			

R-Square	Coeff Var	Root MSE	21 D AI Mean
0.449750	12.23435	0.142408	1.164000

4. Dependent Variable: 28DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.05117333	0.01279333	0.41	0.7960
Error	10	0.31020000	0.03102000		
Corrected Total	14	0.36137333			

R-Square	Coeff Var	Root MSE	28 D AI Mean
0.141608	13.29579	0.176125	1.324667

Appendix 5: ANOVA tables for disease lesions caused by bacterial isolates on buffelgrass seedlings at 7 to 28 days after inoculation using spay method

1. Dependent variable: 7DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	2	0.24246667	0.12123333	0.40	0.6881
Error	6	1.82693333	0.30448889		
Corrected Total	8	2.06940000			

R-Square	Coeff Var	Root MSE	7D AI Mean
0.117168	29.72020	0.551805	1.856667

2. Dependent variable: 14DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.01580000	0.50790000	0.90	0.4548
Error	6	3.38260000	0.56376667		
Corrected Total	8	4.39840000			

R-Square	Coeff Var	Root MSE	14 D AI Mean
0.230948	24.37805	0.750844	3.080000

3. Dependent variable: 21DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	47.10906667	23.55453333	3.98	0.0794
Error	6	35.51553333	5.91925556		
Corrected Total	8	82.62460000			

R-Square	Coeff Var	Root MSE	21 D AI Mean
0.570158	27.28544	2.432952	8.916667

4. Dependent variable: 28DAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	45.70246667	22.85123333	10.22	0.0117
Error	6	13.41473333	2.23578889		
Corrected Total	8	59.11720000			

R-Square	Coeff Var	Root MSE	28 D AI Mean
0.773082	13.44654	1.495255	11.12000

Appendix 6: ANOVA tables for buffelgrass Caryopsis germination tests

1. Dependent Variable: NS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4494.833333	898.966667	39.37	<.0001
Error	18	411.000000	22.833333		
Corrected Total	23	4905.833333			

R-Square	Coeff Var	Root MSE	NS Mean
0.916222	20.26187	4.778424	23.58333

2. Dependent Variable: ABS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	126.3750000	25.2750000	15.55	<.0001
Error	18	29.2500000	1.6250000		
Corrected Total	23	155.6250000			

R-Square	Coeff Var	Root MSE	ABS Mean
0.812048	29.13725	1.274755	4.375000

3. Dependent Variable: HS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1823.708333	364.741667	18.59	<.0001
Error	18	353.250000	19.625000		
Corrected Total	23	2176.958333			

R-Square	Coeff Var	Root MSE	HS Mean
0.837732	26.12292	4.430011	16.95833

4. Dependent Variable: DS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1529.833333	305.966667	17.43	<.0001
Error	18	316.000000	17.555556		
Corrected Total	23	1845.833333			

R-Square	Coeff Var	Root MSE	DS Mean
0.828804	7.606539	4.189935	55.08333