THE OCCURRENCE OF SEED-BORNE BACTERIA AND FUNGI ON COMMON BEAN (*Phaseolus vulgaris* L.) UNDER DIFFERENT FARMING ECOSYSTEMS IN MUTASA DISTRICT, MANICALAND PROVINCE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP PRODUCTION

BY

LAZARUS I. NHAMARARE

FACULTY OF AGRICULTURE AND NATURAL RESOURCES

AFRICA UNIVERSITY

MUTARE

2015

ABSTRACT

Seed quality is the basic important factor for bean crop production. Seed-borne diseases affect seed quality and viability. A study was conducted to assess the occurrence of seed-borne fungal and bacterial diseases on common bean landraces in three ecological regions of Mutasa district. Eight seed lots representing each landrace were obtained from farmers during the field survey for laboratory and field assessments. The blotter test method was used to analyse fungal pathogens. Bacterial pathogens were analysed using the growing-on test and the liquid assay method. Germination tests were conducted using ISTA standards. The detached leaf inoculations, spraying of live plants in a greenhouse and field plot assessments were used to evaluate Angular leaf spot disease tolerance in bean landraces. Data were analysed using Genstat 5, release 3.2. Of the thirty respondents in the farming systems survey, 53.3% of the farmers grew beans in summer, 66.7% during early winter and only 4% grew the crop in early summer. All the farmers used farm retained bean seed. The kidney class of sweet bean is the only bean class grown in Mutasa district. In all the eight seed samples, the pathogenic fungi recorded were *Fusarium moniliforme*, *Botryodiplodia theobromae*, and *Collectotrichum lindemuthianum*. The percentage of seed samples infected by fungi was *Fusarium moniliforme* (100%), *Botryodiplodia theobormae* (12.5%), and *Collectotrichum lindemuthianum* (12.5%). The infection of samples by storage fungi was *Aspergillus flavus* (75%), *Cladosporium* sp. (100%), Aspergillus *niger* (50%) and *Rhizopus* sp. (75%). There were significant differences ($p<0.05$) in normal germination rate between Red speckled, Black speckled, Honzo sweet (long) and Honzo sweet (small and round) seeds. No bean landraces were tolerant to Angular leaf spot from laboratory and field plot assessments. *Xanthomonas axonopodis* pv. *phaseoli* was isolated from samples of Honzo sweet (s&r) and Moza. No landraces were tolerant to bacterial blight in the field assessment. From the results, farmers should grow more than one class of beans to reduce disease incidence through a diversified gene pool of bean varieties. Retained seed should be treated with fungicides such as captan or copper oxy-chloride to suppress seed-borne fungi and bacteria.

DECLARATION

I……. I do hereby declare that this is my own original work accepted by Africa University, Mutare, Zimbabwe in partial fulfilment of the requirements for the degree of Master of Science in Crop Production and has not been submitted to any university for the award of any degree. All the work that was written by other authors and used in the present thesis is fully acknowledged.

…….....

Student's signature

Date ……

Approved for submission by:

Name of supervisor

.....

…….

Signature

Date ……

COPYRIGHT

Correct citation: Nhamarare Lazarus I., 2015. The occurrence of seed-borne bacteria and fungi on common bean (*Phaseolus vulgaris* L.) under different farming ecosystems of Mutasa District, Manicaland Province. MSc. dissertation, Africa University, Mutare, Zimbabwe.

ACKNOWLEDGEMENTS

Many thanks go to Dr. W. Manyangarirwa for his effort in supervising my work. I want also to appreciate the effort from Mr. J. Tabarira (Crop science technician), Mr. M. Mtetwa and my classmate Mr. M.S. Ntambo in data analysis. Lastly, my great thanks go to the Food science assistant technician, horticulture section personnel and Crop science assistant technician in providing me with the necessary support for my experimental work. Also to remember are Mr. J. Mlambo, Mr. B. Mawonazvawa and Mrs. R. Mabika who helped in data collection from the farmers.

DEDICATION

In memory of my late father Mr. Steven Nhamarare.

“Father this was always your dream”

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF PLATES

Page

LIST OF APPENDICES

Page

CHAPTER 1

INTRODUCTION

1.0 Background

Common beans (*Phaseolus vulgaris* L.) are used as a food legume in many parts of the world. Common bean provide proteins (Arulbalachandran and Mullainathan, 2009) and is also rich in vitamins, minerals and dietary fibre. The immature pods (also known as green beans, snap beans, French beans or string beans are also an important food source around the world

(Ndegwa, Muchui, Wachiuri and Kimamira, 2006). Common bean protein is high in lysine which is relatively deficient in maize, cassava and rice thus beans makes a good complement to these staples in the diet. In Africa bean products are consumed at various stages of plant development; they offer a staggered and prolonged food supply in the form of leaves, green pods, fresh grain, as well as dry grains. Protein is expensive and improved bean yields will supply a cheap source of protein to cash limited farmers.

Dry beans have immense health benefits being rich in protein (about 22%) and providing a good source of iron and zinc (iron and zinc are both key elements for mental development). Moreover, bean consumption reduces colon and breast cancer, and heart diseases (US Dry Bean Council, 2011). Regular consumption of common bean and other pulses is now being promoted by health organizations since it reduces the risk of diseases such as cancer, diabetes or coronary heart diseases (bean is low in fat and cholesterol free) (Leterme and Munoz, 2002).

Common bean apart from being a food legume also plays a vital role in soil fertility amendment practices of low input farming systems in Africa (Legesse, Kumussa, Assefa, Taha, Gohena, Alemaw, Mohhamed and Terefe, 2006). Legume crops are important for their nitrogen fixing capabilities (Amannuel, Kiihne, Tanner and Vlek, 2000) and can be used in crop rotation systems to improve soil conditions. Nitrogen fixation by legume crops offers an alternative to nitrogen fertilisers which may present a serious environmental problem (Brentrup, Küsters, Kuhlmann and Lammel, 2001). With the increasing interest in organic farming that requires minimum or zero use of chemical pesticides, bean varieties that are resistant to diseases are in high demand.

Common bean (*Phaseolus vulgaris* L.) is one of the major cash crops for smallholder farmers in Zimbabwe. Besides promoting food, health and nutritional security, beans provide a steady and lucrative source of income for many rural people (FAO, 2011). It has considerable local economic significance. Most bean production takes place in intercropping systems with maize. The understanding of the quality of seed produced and testing of practices that help produce seeds of acceptable quality under different intercropping systems is a sound strategy for the assurance of the supply of high quality seeds for the smallholder farmers (African Scholarly Science Trust, 2014).

In the smallholder farming sector, the majority of farmers get their seeds mainly from open markets that include on-farm saved seeds, seed exchanges among farmers at agricultural shows and seed technology fairs among other sources. In Zimbabwe the main sowing time for common bean is from November to December (Wortmann, Kirby, Eledu, and Allen, 1998).

The rain-fed crop is the major crop since irrigation water is scarce among the smallholder sector. Some farmers in small scale irrigation schemes plant beans from February -March and from August-September under full irrigation. The incidence of seed-borne diseases is very high during summer as rainfall provides favourable conditions for pathogen survival and multiplication. Pathogen load and type varies among natural regions as influenced by rainfall amount and temperature.

The farmer is confronted with disease control challenges since disease load increases during this main sowing period and labour bottlenecks are a constraint as the crop is regarded as a women's crop.

About 74% of bean area in Eastern Africa and 57% of bean area in Southern Africa (Wortmann *et al.*, 1998) are grown under multiple cropping systems. Bean is mainly grown as an intercrop or mixed crop with maize. Intercropping has been found to increase seed-borne disease incidence (African Scholary Science Trust, 2014). Intercropping may complicate the application of necessary disease management strategies since weeding of the maize crop may not be stopped during wet weather conditions to reduce spread of such diseases like Common bacterial blight. The lack of knowledge on the appropriate chemicals for disease control is a major challenge to disease management. The flexibility of chemical use in maize- bean intercrops may also pose a challenge to disease management. Variations in climatic conditions are expected to affect disease occurrence in the three agro-ecological regions under study.

The main production constraints for bean are poor agronomic practices, soil infertility, lack of improved cultivars, moisture stress, weed competition, insect pests and diseases (Wortmann *et al*., 1998). Angular leaf spot and Anthracnose are major fungal diseases in Africa (Buruchara *et al*., 2010). Insect pest and disease problems present major constraints to agricultural productivity of the common bean, particularly in the tropics (Graham and Vance, 2003). Angular leaf spot (ALS) caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris is a serious disease of economic importance that occurs in tropical and subtropical countries (Buruchara *et al*., 2010). The disease occurs worldwide but it is essentially a disease of the tropics and subtropics.

In Zimbabwe, Angular leaf spot and Anthracnose have been seen to have a high relative importance while Common bacterial blight was of low relative importance (Wortmann, Kirby, Eledu, and Allen, 1998) Angular leaf spot is the most important biotic constraint in eastern and southern Africa. It is favoured by moist, warm conditions with abundant inoculum supply (Saettler, 1994).

Anthracnose is a fungal seed-borne disease caused by *Colletotrichum lindemuthianum*. The fungus is harboured under the seed coat making it difficult to treat with seed treatments. Disease infection is favoured by cool and wet conditions. The spores are dispersed by natural rain or splashing of irrigation water. The fungus overwinters on crop residues or seeds (Helene and Legard, 1991). Anthracnose is relatively more important at higher altitudes than at lower altitudes.

The major bacterial diseases include Common bacterial blight, bacterial wilt, halo blight and bacterial brown spot.

The losses from Common bacterial blight have been noted to reach 40% (Finisia, 2003). Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli*, is a serious seed-borne disease. The bacterium overwinters in the seed or crop residue and persists for long periods on seed. The bacterium is spread readily by wind, rain, irrigation splashing, feeding insects or contact with contaminated machinery. Disease infection and development is favoured by wet and warm weather (Helene and Legard, 1991).

Dry beans are affected by weed competition. Early weed growth reduces bean yields by competing for moisture, and nutrients. Weeds increase harvest losses and reduce bean quality. Small scale farmers use hand weeding methods (Whingwiri, Mashingaidze and Rukuni, 1991).

1.1 Statement of the problem

Beans are used as a food legume in many parts of Zimbabwe. Common bean (*Phaseolus vulgaris* L.), is one of the major cash crops for smallholder farmers in Mutasa District. The legume provides proteins, vitamins, minerals and dietary fibre. Bean yields are low among the smallholder farmers in Zimbabwe despite the availability of new technologies on the market; inorganic fertiliser, inoculants and availability of improved varieties. Smallholder farmers use retained/farmer saved stocks as their main seed source and this reduces bean productivity. The seed is usually infected by seed- borne diseases which cause reduced bean germination, poor crop stands and low yields. The major seed-borne diseases of dry beans in Zimbabwe are Angular leaf spot, Anthracnose and Common bacterial blight. The occurrence of these diseases can be influenced by cropping practices and agro-ecological regions.

The control of these biotic constraints to bean production would improve bean yields and hence in-turn enhance nutritional (proteins, vitamins and minerals) and income benefits for smallholder farmers.

1.2 Justification for the study

Seed is the most important input for crop production. Pathogen- free healthy seed is needed for desired plant populations and good crop harvest. On common bean numerous plant pathogens are seed-borne and they can cause high crop losses through reduced plant growth and crop productivity (Dawson and Bateman, 2001; Islam, Masum and Fakir, 2009). The fluctuations in the germination and emergence of legume seeds that are incurred in the field are often a result of the use of poor quality seeds, poor storage conditions and increased incidence of seed-borne fungal and bacterial infection (African Scholarly Science Trust, 2014).

Seed-borne diseases affect the growth and productivity of crop plants. The presence or absence of seed- borne pathogens determines the quality of seed (Weber, Hrynczuk, Runowska, and Kita, 2001; Dawson and Bateman, 2001).

Studies done in Ethiopia revealed that sole cropping (75%) and intercropping (59%) have significant differences in incidence of Common bacterial blight (African Scholarly Science Trust, 2014).

This study would help in identifying seed-borne fungi and bacteria in Mutasa ditrict and this would provide a basis for the recommendation of specific control strategies and cropping practices to control diseases and avoid yield losses from seed-borne fungal and bacterial diseases. If this study is not done farmers will continue losing yields from seed-borne diseases for instance up to 50% form ALS.

1.3 OBJECTIVES OF THE STUDY

1.3.1 Overall objective:

To assess the occurrence and transmission of seed-borne fungi and bacteria on bean seed from three agro-ecological regions of Mutasa district.

1.3.2 Specific objectives:

i) To characterise the major bean farming ecosystems in Mutasa district.

ii) To identify Angular leaf spot tolerant bean landraces in Mutasa district.

iv) To identify bacterial blight tolerant bean landraces in Mutasa district

1.4 Research Questions

i) What are the characteristics of the major bean farming ecosystems in Mutasa district?

ii) Do the differences in farming ecosystems significantly affect the occurrence of seed-borne bacteria and fungi on common beans in Mutasa District?

iii) Are there any bean landraces tolerant to Angular leaf spot and bacterial blight in Mutasa District

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Common bean (*Phaseolus vulgaris* L.) belongs to the leguminosae family, genus *Phaseolus* and species *vulgaris*. Common bean is an important crop for women and are primarily responsible for its production. Dry beans are important in the diets of rural families. Some diseases are of localized importance, but others such as angular leaf spot and bean stem maggot are major constraints in most production areas (Wortmann et al., 1998). Common bean supply 32 % and 65% 0f the energy and protein respectively to Rwandan diet (CIAT, 2004). Bean also contributes about 30% of dietary energy in the maize-based cropping systems of Eastern and Southern Africa.

2.2 Distribution of common beans

The distribution of beans in Africa is heavily dependent on rural population density and mean temperature during the growing season. Bean production intensity is greatest in areas of high population density, where farms are small and few significant sources of dietary protein exist.

2.3 Uses of common beans

The consumption and contribution of common bean to human nutrition in Eastern and Southern Africa is relatively high, due to high population; growing at rate of 2.2-2.6 percent per year and the low incomes.

The domestic use of beans as food for human consumption and seed ranges from 70-100 percent of production depending on country. For instance in Kenya domestic use is estimated at 100 percent, where domestic consumption demand often exceeds domestic production. It is lowest in Ethiopia, where production is mainly for export.

The consumption per person is estimated at 14 kg per year in Kenya, but it can be as high as 66 kg/yr in western Kenya (Spilsbury, Jagwe and Wanda, 2004; Buruchara, 2007). Beans are also used in improving soil fertility in crop rotations in poor cropping systems in Zimbabwe.

2.4 Diseases of common beans

Common bean is affected by bacterial, viral and fungal diseases. Angular leaf spot (*Phaeoisariopsis griseola* (Sacc.) Anthracnose (*Collectotrichum lindemuthianum*), Cercospora leaf spot (*Cercospora* spp), Alternaria leaf spot (Alternaria spp), Powdery mildew (*Erysiphe polygoni*), White mould (*Sclerotinia sclerotiorum*) and Rust (*Uromyces appendiculatus)* are the major fungal diseases. Common bacterial blight, Halo blight (*Pseudomonas syringae* pv. *phaseolicola*) and Bacterial brown spot (*Pseudomonas. syringae* pv. *syringae*) and bacterial wilt (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*) are major bacterial diseases. Seed-borne fungal diseases include Anthracnose, Angular leaf spot, Alternaria leaf spot and Charcoal rot/Ashy stem blight. White mould is a seed-lot contaminant. Common bacterial blight, bacterial brown spot, bacterial blight and halo blight are seed-borne bacterial diseases.

Angular leaf spot, Anthracnose and Common bacterial blight are serious seed-borne diseases of economic importance in Africa (Helene and Legard, 1991; Buruchara, Mukankasi and Ampofo, 2010)

The damage caused by seed-borne pathogens includes seed abortion, shrunken seeds, seed rot, seed necrosis, seed discoloration, reduced germination and vigour (Shetty, 1992). Infected seed is a major source of primary inoculum. Diseased seed is also an important source of local and long distance seed-borne disease spread (Saettler, Schaad, and Roth, 1995). The majority of farmers in the small scale farming sector in Zimbabwe are unable to buy certified or treated seed, fungicides or bactericides, due to cash constraints. Healthy seed refers to seed that is free from pathogens like fungi, bacteria and viruses, and animal pests (nematodes and insects) (International Seed Testing Association (ISTA), 1999).

2.5 Major seed-borne fungal diseases of common bean

2.5.1 Angular leaf spot

The disease occurs worldwide but it is essentially a disease of the tropics and subtropics (Mahuku, Jara, Cuasquer and Castellanos, 2002). ALS is the second important factor after nitrogen deficiency which is causing production losses in southern Africa estimated at 93.5 thousand tonnes per year (CIAT, 1998). Bean yield losses of 10 to 80% by ALS in tropical and subtropical countries have been reported by Jesus, Vale, Coelho, Zambolin, Costa and Berghamin –Filho (2001).

Contaminated seed is one major source of inoculum and the fungus is usually associated with the helium.

Seed contamination can be either external or internal (Saettler, 1994). Infection and disease development are favoured by humid conditions and moderate temperatures (20-25^{o}C) (Buruchara *et al.*, 2010).

Moisture is one single most important factor governing the development of ALS epidemics and it is a pre-requisite for infection, synnemata formation, and sporulation. Stroma formation, spore release and dissemination, and disease development can occur under relatively dry conditions (Schwartz and Pastor-Corrales, 1989).

Infection and disease development can occur over a wide temperature range (16-28^{o}C.) with the optimum temperature being 24^{o}C. Common bean plants are susceptible to fungal infection throughout the whole growing season. Fluctuating temperature, relative humidity and sunlight usually favour disease development under field conditions (Schwartz and Pastor-Corrales, 1989).

2.5.2 Symptoms

Severe ALS symptoms in the field are not usually observed until soon after flowering or as plants reach maturity.

The period from infection to the appearance of visible symptoms ranges from 4 to 9 days depending on ambient temperature, bean variety, and age of the plant tissues. Angular spots appear on the foliage. The lesions are initially tannish –gray but later become dark –brown or black. The lesions increase in size and may coalesce and large portions of the leaf become infected and chlorotic. If humidity is high, the under surface of the leaves may have a black felt-like appearance due to sporulation.

The lesions are initially confined to tissue between main veins, giving an angular appearance. On some varieties a yellow halo may surround the spots and eventually the entire leaf becomes yellow before aborting prematurely. Lesions can be seen on the underside of the leaf and appear slightly paler than those on the upper leaf surface. Angular leaf spots on stems and petioles are elongated, and dark brown (Buruchara *et al*., 2010).

2.5.3 Disease management

2.5.3.1 Cultural control

Crop rotation of at least two years between bean crops, planting in well-drained soil, removal of infected crop debris by ploughing and planting pathogen-free seed can reduce disease incidence (Helene *et al*., 1991).

The disease can also be controlled by using tolerant cultivars. Disease tolerance is the most economic means to control the disease because the fungus is available in the field at almost any time of the year. Tolerant genotypes in one location may be susceptible in another because the fungus exhibits high pathogenic variability (Nietsche, Borém, Carvalho, Paula Junior, Ferreira, Barros and Moreira, 2001). Growing of bean variety mixtures including resistant ones help to reduce the spread of infection (Buruchara *et al.*, 2010). In Zimbabwe, commercial varieties which offer some tolerance to this disease include Cardinal and Red speckled sugar bean.

2.5.3.2 Chemical control

Disease control was best obtained and yields were highest when 0.13 g/L of benomyl was used and the plants sprayed at intervals of four weeks.

The use of Maneb, Copper oxy-chloride, and Bordeaux mixture is recommended. Economic disease control has been achieved from the use of foliage sprays for instance Mancozeb, 20 days after planting (Schwartz and Pastor-Corrales, 1989). The use of benomyl at 6 g/ kg seed and a captan-zineb (3.7g/kg seed) combination applied in water-based slurry (0. 11 g/ ml) can effectively eliminate the fungus from contaminated seed. Seed treatment with benomyl prior to planting will significantly have less leaf infection (Schwartz and Pastor-Corrales, 1989). Bean seed can be dressed with suitable fungicides such as Mancozeb, Benzimidazole, Thiabendozole, Trifloxystrobin and Azoxystrobin (Buruchara *et al.*, 2010).

2.6 Anthracnose

Anthracnose is one of the most important diseases of economic importance of beans in the world. The disease causes severe damage on susceptible bean cultivars and when badly contaminated seed is planted and favourable conditions prevail during the growing seasons. It causes greater crop losses in temperate and subtropical zones than in the tropics (Pastor-Corrales and Tu, 1989). The imperfect stage is commonly seen. Early leaf senescence and plant death, shrunken seed and an increase in the amount of diseased seed on the seed coat reduce yield (Pastor-Corrales and Tu, 1989). High lesion density is associated with premature yellowing and discolouration of leaves (Bassanezi, Amorin, Bergamin Filho, Hau and Berger, 2001).

Collectotrichum lindemuthianum is a seed-borne fungus which can survive from one season to another in crop debris from infected plants and diseased seed. Anthracnose is widespread and one of the most important diseases of bean worldwide.

It is prevalent in regions with frequent rainfall and more destructive under cool to moderate temperatures with high relative humidity (Buruchara *et al.*, 2010)

Infected seeds and crop debris are the major sources of infection. The fungus is spread by rain splash, wind-blown rain and movement of insects, animals and human beings especially when plant foliage is moist. Frequent rainy weather increases disease occurrence and severity

The most important symptoms of bean anthracnose appear on pods. Lesions on pods are sunken and are encircled by a slightly raised black ring surrounded by a reddish border. When infection is severe, young pods may shrivel and dry prematurely (Buruchara *et al.*, 2010).

The spores of the fungus can be spread from seedlings by splashing rain, animals and humans from infected plants to healthy plants. Splashing rain also moves the disease further up the plant and onto the pods. The fungus may get to the seed by penetrating through the pod causing discoloration and distortion of the seed. The pathogen may also enter into the seed through the seed coat and become firmly established within the seed. Such infected seed, when planted, serve as the source of infection to the succeeding crop (Buruchara, 2010).

2.6.1 Symptoms

The disease affects all parts of the bean plant, depending upon time of infection and source of inocula. The initial symptoms appear as a dark brown to black lesions along the veins on the underside of the leaves and leaf petioles and stems may also show this symptom. Sunken cankers also appear on the developing hypocotyls.

The most striking symptoms of *Collectotrichum lindemuthiunum* infection appear on pods. Pod lesions are sunken and are bordered by a slightly raised black ring surrounded by a reddish border. Under heavy infestation, young pods may shrivel and dry prematurely (Buruchara *et al.*, 2010).

Salmon coloured ooze on lesions and the veins on lower leaf surfaces turn black. Elongate and sunken lesions appear on stems, leaf petioles and veins on under-side of leaves. Infected seeds are discoloured and might have sunken lesions. During moist weather, a gelatinous mass of pinkish spores may also develop on infected plant parts (Hagedorn and Inglis, 1986).

2.6.2 Disease management

2.6.2.1 Cultural control

The fungus has a limited host range and dry beans are the most important crop that is affected by this pathogen. Thus growers are provided with numerous options for selecting alternate crops for inclusion in long term rotations. Crop rotation for 2-3 years with non-host crops (e.g. maize) and planting disease-free seed can achieve effective control of the fungus. Affected plants should be rogued out and buried. Infected crop debris should be incorporated by deep ploughing soon after harvesting (Buruchara *et al.*, 2010). In Africa, some small scale farmers practise crop rotations, use fungicides, use certified seed and plant tolerant cultivars.

2.6.2.2 Chemical control

Seed treatment with fungicides like copper oxy-chloride and captan can reduce transmission of anthracnose to the seedlings.

Unfortunately, seed treatment will not eliminate the disease from heavily infected seed. Many foliar fungicidal sprays can also decrease the spread of the disease. The application of the foliar fungicides can reduce losses in seed yield and quality. The timing of application is a critical factor in determining the effectiveness of the spray application in reducing disease severity and seed infection. Spray applications at the early or late bloom stage can minimize yield losses and reduce seed infection. Seed treated with the three commercial formulations of *Bacillus subtilis* and one of *Pseudomonas chlororaphis* and *Curtobacterium* sp. had a significant disease-suppressive effect resulting from seed-borne infections by *Collectotrichum lindemuthianum* (Tinivella, Hirata, Celan, Wright, Amein, Schmitt, Koch, Wolf, Groot, Stephan, Garibaldi and Gullino, 2009). The disease can be controlled by using protectant fungicides or systemic fungicides such as Kocide (Buruchara *et al.*, 2010).

2.7 Charcoal rot/Ashy stem blight/Crown rot (*Macrophomina phaseolina*) (Tassi) Goid

The disease is caused by the fungus *Macrophomina phaseolina* (Tassi) (Goid) and occurs worldwide. Charcoal rot is more prevalent and destructive to beans that are subjected to drought and warm temperatures. It is a major disease in warm bean growing areas (Hagedorn and Inglis, 1986).

The fungus attacks *Phaseolus vulgaris* L., *P. lunatus* L., *Glycine max* L., *Zea mays* L., sorghum, and other crops (Schwartz and Pastor-Corrales, 1989) as *Brassica napus* L. (Khangura and Aberra, 2009), strawberry (Koike, 2008), *Beta vulgaris* L. (Karadimos, Karaoglanidis, 2002), *Helianthus annuus* L. (Gulya, Krupinsky, Draper and Charlet, 2002.), *Gossypium hirsutum* L. (Baird and Brock, 1999) and Cassava (Msikita, James, Wilkinson, and Juba, 1998), white clover and alfalfa (Pratt, McLaughlin, Pederson and Rowe, 1998). The fungus produces black globose pycnidia that contain large, colourless, one-celled, fusiform conidia which are pointed at one end and rounded at the other end. Sclerotia and pycnidia are also produced on infected plants (Schwartz and Pastor- Corrales, 1989). The disease may be seed-borne thus can spread long distances. Local transmission is by air-borne pycnidia and/or pycnidiospores and by movement of sclerotia or pycnidia in plant debris or infected soil (Hagedorn and Inglis, 1986). *Macrophomina phaseolina* thrives best in warm temperatures ranging from 24-27^{o}C.

2.7.1 Symptoms

The fungus firstly produces black, sunken, cankers on the stem which have a sharp margin and often contain concentric rings. The plant's growing tip may be killed or the stem broken where it is weakened by the canker.

Infection may continue into the hypocotyl and root region or the primary leaf petioles. Older seedling and plant infections may cause stunting, leaf chlorosis, premature defoliation, and plant death (Schwartz and Pastor-Corrales, 1989).

When infection goes up the plant, the entire stem may become affected causing wilting and premature defoliation and death. Stem blight may be more prominent on one side of the plant. Many black sclerotial bodies and/or pustule-like pycnidia form within diseased tissues. The pycnidia are usually found on lesions with characteristic greyish-tan (ashen) colour hence the name 'Ashy stem blight' (Hagedorn and Inglis, 1986).

2.7.2 Disease management

2.7.2.1 Cultural control

The planting of clean seed, treating seed with chemicals such as Benomyl at 1 kg/ha, and sanitation or deep-ploughing of plant debris containing pycnidia and sclerotia can control the disease. Organic soil amendments and high soil temperatures and moisture may reduce sclerotia levels (Schwartz and Pastor-Corrales, 1989). The use of 4-5year rotations with non-susceptible crops is advisable. Resistant varieties when available for instance 'Negrito' can control the disease. Many cultivars are susceptible (Hagedorn and Inglis, 1986).

2.7.2.2 Chemical control

Seed treatment with Captan can completely eradicate seed-borne infection without any adverse effect on seed germination (Frison, Bos, Hamilton, Mathur and Taylor, 1990).

2.8 Major seed-borne bacterial disease of common bean

2.8.1 Common bacterial blight

A number of bacterial diseases affect common bean and these include common bacterial blight (CBB) *Xanthomonas axonopodis* pv. *phaseoli*, halo blight (*Pseudomonas syringae* pv. *phaseoli*cola) and bacterial brown spot (*Pseudomonas syringae* pv. *syringae*).

Common bacterial blight can cause up to 40% yield loss (Opio *et al*., 1996; Finisia, 2003) and is still considered a major constraint to dry bean production in many African countries particularly Uganda, Zambia, Zimbabwe and South Africa (Gilbertson and Maxwell 1992; Fourie 2002; Lak, Shamsbakhsh and Bahar, 2002; Harveson 2009; Zamani, Bahar, Jacques, Lak, Akhavan, 2011 ; Karavina, Mandumbu , Parwada and Zivenge, 2011). The disease is widespread throughout Africa's bean growing areas, and it is favoured by warm to high temperatures and high humidity (Buruchara *et al.*, 2010). In Zimbabwe, the disease has been noted in both the smallholder and large-scale commercial farming sectors in Natural Farming Regions II, III and IV (Giga, 1989).

Common bacterial blight is caused by *Xanthomonas axonopodis* pv. *phaseoli* (also known as *Xanthomonas campestris* pv. *phaseoli*) and it's variant; *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (Schaad, Vidaver, Lacy, Rudolph and Jones, 2000; Vauterin, Rademaker and Swings, 2000). The two variants are identical in terms of their epidemics and disease cycle but colonies of *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* are distinguished by a distinct brown colour in media containing tyrosine, 2 to 9 days after inoculation (Goodwin and Sopher, 1994).

The bacteria can be found on the leaves of many plants, but only causes disease on common bean.

Disease infection occurs when the inoculum is deposited onto foliage by splashing water, aerosols, or from contaminated seed, and multiplies to form large populations.

The bacteria enter into plants through natural openings (stomata, lenticels) and wounds on leaves and stems. Infection occurs most readily during warm (28 -32ºC), wet weather, especially hard, wind- driven rains. Bacteria are transmitted within and among fields by splashing water, aerosols, and on contaminated equipment and workers. The bacterium survives between susceptible hosts in and on weeds, infested crop debris and seed. Bacterial cells are also readily transmitted mechanically, particularly when plants are wet. Insects may transmit the bacterium from plant to plant (Kaiser and Vakili, 1978; Lindemann and Upper, 1985).

The bean stem can be penetrated via the stomata, vascular system of the leaf and from infected cotyledons (Kaiser and Vakili, 1978). Bacterial cells can also gain entry into the seeds via the vascular system or through the pedicel. Infection of the young plant occurs when internally infected seed germinates and the bacterium is moved from the seed to the seedling (Saettler 1989a; Gilbertson and Maxwell, 1992). Generally the pathogen causes very severe disease under high rainfall and humidity and warm conditions (25-35°C). Maximum disease development occurs around 28°C (Gilbertson and Maxwell, 1992).

The survival of *Xanthomonas axonopodis* pv *phaseoli* in the soil or plant debris is influenced by geographical area, climate, cultural practices, host genotypes, and bacterial strains (Karavina, Tigere, Chihiya, 2008).

The bacterium may survive in crop debris in the soil from season to season (Arnaud-Santana and Pena-Matos, 1991; Gilbertson and Hagedorn, 1990). In Zimbabwe the bacteria can overwinter between crops in crop residues; thus residues can be considered as sources of inoculum for CBB (Karavina *et al.*, 2008). *Xanthomonas axonopodis* can also thrive and multiply as an epiphyte or resident on the shoot surfaces of weed hosts, mainly members of the legume family without showing symptoms (Pernezny and Jones, 2002). A large number of foliar spread bacterial pathogens are able to survive and multiply on aerial parts of plants without any visible symptoms (Andrews and Harris, 2000). The symptomless period can lead to a huge bacterial population that will enhance disease epidemics to develop later when more favourable environmental conditions occur and also found *Xanthomonas axonopodis* from bean residues kept in the greenhouse for 12 months in Zimbabwe (Wilson, Hirano, Lindow, 1999; Karavina *et al.*, 2008). Opio, Teri, and Allen (1994) reported that the bacteria thrived for more than 18 months in dried foliage kept in the laboratory.

2.8.2 Symptoms

Water- soaked spots appear on the underside of leaves. The leaf spots enlarge and coalesce to form large brown irregular lesions surrounded by a narrow yellow zone. Lesions may begin to coalesce, and the yellowing of leaves become more general. Stems may rot at the first node where cotyledons were attached causing the plant to break (Hagedorn and Inglis, 1986).

Affected pods have sunken circular spots which are water-soaked initially but later dry, with a reddish brown narrow border.

During wet conditions, yellow slimy bacterial exudates ooze out of the lesions and hence form a crust in the process. Infected pods may shrivel and infected seed may rot (Buruchara *et al.*, 2010).

Early pod infection results in shrivelled seeds. The pathogen may cause yellow to orange discoloration under the seed coat of infected seeds. A stem girdling or joint rot occurs above the cotyledonary node of plants grown from infected seeds. Yield losses can range from 20 to 40% depending on the year. The disease causes reduction in seed size and quality.

Bacterial blight is considered mainly as a foliar disease, but symptoms can also be observed on stems, pods and seeds. Symptoms on these plant parts initially appear as small water-soaked spots, which then enlarge and become necrotic and are usually bordered by a yellow zone in case of leaf spots (Gilbertson and Maxwell, 1992; Harveson, 2009).

Individual lesions may coalesce causing plants to look burned and spots on pods are usually circular and brownish red. Yellow to brown spots develop on infected seeds and they show weak vigour and germination (Gilbertson and Maxwell, 1992). Seeds may sometimes show no visible symptoms and can germinate vigorously while they support large symptomless epiphytic communities of the pathogen (Akhavan, Bahar, Saeidi and Lak, 2009).

2.8.3 Common bacterial blight disease management

2.8.3.1 Cultural control

Numerous disease management strategies can be used to control Common bacterial blight. The saving of seed from previously infected fields for re-use should be avoided. Bean fields should not be worked when plants are wet. Infested bean debris should be completely and thoroughly incorporated and covered into the soil soon after harvest to encourage decomposition. Rotating beans with non-host crops such as small grains for at least two to three years help to manage the disease. Fields where the disease is a problem should not be planted to beans again until infected debris are completely decomposed. This can be achieved by following a minimum of 2-year rotation out of beans (Helene and Legard, 1991).

Overwintering of pathogen populations that cause common bacterial blight can be reduced by using at least a three-year bean crop rotation. Effective sanitation practices with bean residues to reduce the amount of inoculum that can affect neighbouring bean crops in the following season and future bean crops in the same field. Bacterial pathogens survive longer on crop residues left on the soil surface than on residues incorporated into the soil. The residues should therefore be incorporated into the soil soon after harvest to promote quick decomposition. It is vital to remove and destroy all volunteer bean plants (Wohleb and du Tolt, 2011).

Tolerant cultivars can be used since bean cultivars differ widely in their susceptibility to the pathogen (Helene and Legard, 1991; Wohleb and du Tolt, 2011). In Zimbabwe some commercial varieties such as Cardinal and Red speckled sugar bean have some tolerance.

The use of certified seed is a recommended disease management strategy. The best control of CBB is prevention. The chances of acquiring seed-borne inoculum are significantly reduced by using certified bean seed produced in disease free areas. Bean growers should not use retained seed, and as well they should avoid using non-certified seed. If non-certified seed must be used such fields should be isolated from fields planted to certified seed to reduce disease transmission from the contaminated fields (Helene and Legard, 1991).

Seed treatment with streptomycin reduces bacterial contamination on the seed coat but will not control systemic infections in the seed (Wohleb and du Tolt, 2011). Spraying of plants with Streptomycin and Copper sulphate kills the bacteria (Buruchara *et al.*, 2010).

Cleaning of tractors and other equipment before moving them from one bean field into another prevents unintended spread of the inoculum. Soil cultivation or any traffic through fields, including foot traffic for manual field inspections, when the foliage is wet should be avoided (Helene and Legard, 1991; Wohleb and du Tolt, 2011). The spread of bacterial pathogens is more likely to occur when bean plants are wet. The wounding of bean plants particularly mechanical injury enhances disease attack. Bean fields should not be cultivated when crop stands are tall enough that equipment passing through the fields will damage plants (Wohleb and du Tolt, 2011).

Weeds and volunteer beans should be removed early in the succeeding season. Affected bean straw should not be spread on new fields to be used for bean production. Planting of beans close to recently infected blighted fields should be avoided at all cost.

The pathogen is unable to survive in soil without bean residues. Bean residues left on the soil surface do not decay quickly, allowing the bacteria to survive longer (Helene and Legard, 1991).

The use of overhead irrigation can be avoided where possible since it increases secondary spread of the pathogen compared to furrow or drip irrigation (Helene *et al*, 1991; Wohleb and du Tolt, 2011). Where overhead irrigation is used plant surfaces should be allowed to dry completely between irrigation shifts. If feasible farmers should irrigate in the morning so that the canopy dries out in the afternoon instead of remaining wet all night. The longer the time the leaves remain wet, the higher the risk of infection (Wohleb and du Tolt, 2011). Re-using irrigation water should be avoided because bacterial pathogens can be transmitted in water (Wohleb and du Tolt, 2011).

2.8.3.2 Chemical control

Use copper-based bactericides such as copper hydroxide and Copper oxy-chloride to reduce bacterial growth on foliage and to reduce transmission of bacteria to healthy foliage and pods. Copper-based bactericide applications are most effective as prophylactic measures to protect the plants when conditions are conducive to disease development. The first application should be done before symptoms appear.

Thereafter, repeat applications at 7 to 14 day intervals. The bactericides eradicate the pathogen once infection has occurred (Wohleb and du Tolt, 2011).

Copper-based bactericides can reduce epiphytic populations of bacterial pathogens on bean foliage, and also reduce disease severity when applied as a preventative measure. The compounds cannot eradicate the pathogens once the plants are infected.

Persistent wet weather can increase bacterial populations quickly and this makes it difficult to arrest them unless many applications of copper-based bactericides are made. Thus because of these reasons, copper-based bactericides are not highly recommended (Helene and Legard, 1991).

2.9 Dry bean production in Zimbabwe

In Zimbabwe there are five major agro-ecological regions notably, I, II, III, IV and V. Natural region II is sub-divided into IIa and IIb based on the frequency of dry spells during the rainy season and the total number of rainy pentads experienced in the regions (FAO, 2006).

Natural region I receive more than 1000 mm per annum in areas lying below 1700 m altitude, and more than 900 mm per annum at greater altitudes. Some precipitation normally occurs in all months of the year. Temperatures are usually comparatively low and the rainfall is consequently highly effective. In Natural region IIa, rainfall is confined to summer months and is moderately high (750-1000 mm per annum). This sub-region normally enjoys reliable conditions, rarely experiencing severe dry spells in summer.

Sub-region IIb is subjected either to rather more severe dry spells during the rainy season or to the occurrence of relatively short rainy seasons. Natural region III receives 650-800 mm per annum. Much of the rainfall is accounted for by infrequent heavy falls and temperatures are generally high. The region is also subject to fairly severe mid- season dry spells. Natural region IV experiences per year 450-650 mm per annum and is subjected to periodic seasonal droughts and severe dry spells during the rainy season. In Natural region V, rainfall is too low and erratic (FAO, 2006).

Dry bean production is feasible in most parts of the country. Optimum temperature for crop growth is 16ºC to 24ºC.

The crop is normally grown as rain-fed during summer in areas with average annual rainfall ranging from 500 to 1000mm. Excessive rain increases the incidence of fungal diseases. The crop is also grown in winter around late May in small irrigation schemes around the country particularly in the lowveld. Crops planted earlier than January usually succumb to diseases (Whingwiri *et al.*, 1992). The bulk of dry bean production in Zimbabwe is in the small-scale farming areas (Communal and Resettlement) (Whingwiri *et al.*, 1992).

The major constraint to low production is the unavailability of adapted varieties that are tolerant to diseases and insect pests. The crop is usually grown as a sole crop especially in small-scale irrigation schemes. Common bean is intercropped with maize and other cereals in some communal areas especially in Honde Valley. Some important diseases of beans in Zimbabwe include Anthracnose, Common bacterial blight, Halo blight, Bean common mosaic virus, Rust, Damping-off and root rots.

Disease severity is increased by the use of uncertified and untreated farmer saved seed (Whingwiri *et al.*, 1992).

In Zimbabwe dry bean is normally grown as an intercrop or mixed crop with cereals particularly maize during summer and the winter crop is grown as a sole crop in small irrigation schemes.

Studies have shown that bean seeds produced in an intercropping system with maize and sugarcane had low germination capacity and vigour. Some studies have indicated that seedling growth rate, shoot length and seed sizes were unaffected when produced under maize- bean intercropping systems (African Scholarly Science Trust, 2014).

Seeds obtained from sole crops were found to be different from those obtained from intercrops in physical purity and percentage of pathogen infected seeds.

However, the physiological quality related characteristics were similar between the two cropping systems. In studies done in Ethiopia standard germination of seeds of both intercrops and sole crops showed a wide range of variation with an average of 75 and 84% for sole crop and intercrop originated seeds, respectively (African Scholarly Science Trust, 2014). Intercropping promotes slow air movement in the field creating humid and warm conditions conducive for disease development.

Some management practices done at different crop growth stages of the component crops in an intercropping system may affect both yield and quality of seeds produced under adverse competition for growth resources.

Overhead sprinkler irrigation is more conducive to disease spread and pressure than furrow irrigation for such diseases like Angular leaf spot. Sprinkler irrigation has effects similar to rain as it can lower leaf and canopy temperature, increase relative humidity, and may prolong dew periods in the field. Overhead irrigation can also enhance splash dispersal and it may remove spores from the atmosphere and deposit them on susceptible plant parts. Favourable weather conditions and frequency of irrigation play a vital role in such situations. The use of furrow irrigation systems in low rainfall areas has been found to restrict the spread of splash-dispersed pathogens. Large epidemics of Common bacterial blight have frequently occurred under sprinkler systems causing huge yield losses (Osdaghi, Shams-bakhsh, Alizadeh and Lak, 2010). Overhead irrigation provides a means for bacterial dispersal, unlike furrow irrigation (Harveson, 2009).

Overhead irrigation systems seem to favour pathogen dissemination from colonized to healthy leaves as the key factor in increasing the total field epiphytic population size of *Xanthomonas axonopodis*. Sprinkler irrigation provides a layer of water on the host plant surface, which creates an ideal multiplication site for the microbial planktonic community on plant leaves. Epiphytic population size can increase when the plant surfaces are wet (Beattie and Lindow, 1995).

The presence of a film of water on leaves also helps to promote nutrient release from plant foliage, which can enhance the availability of nutrient sources for the epiphytic bacterial community (Hirano and Upper, 1983).

Small scale farmers in Mutasa use overhead irrigation system particularly those in small irrigation schemes like Manunure. Some irrigation schemes use flood irrigation systems.

The predominant bean class mostly grown is the Kidney type comprised mainly by sweet beans for instance sugar beans and landraces. Also white haricot is grown at a very low proportion. Most of the bean types grown are susceptible to Common bacterial blight, Angular leaf spot, Rust, Damping-off and Anthracnose diseases (Central High Plains Dry Bean and Beet Group, 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 Survey of farmers' fields/households in Mutasa district

A structured questionnaire (Appendix 2) was administered in the three ecological regions under study. The questionnaire captured information such as source of seed used by the farmer, problematic diseases in the area, strategies used to control the diseases of bean, landraces grown, method of bean crop production, method of irrigation and the cropping system used. A total of 30 respondents were interviewed

with ten (10) respondents from each agro-ecological zone. Three agro-ecological regions were picked at random using the simple random sampling method. The questionnaire was administered by visiting individual households.

3.2 Ethical Consent of the Respondents in the Survey

Before administering the questionnaire in the survey, all participants in the survey received adequate explanations on their ethical rights in the survey. It was also disclosed that the information gathered was confidential and they could choose to answer or not answer questions as they wished. All the data collected was done so after the explicit consent of the individual interviewed.

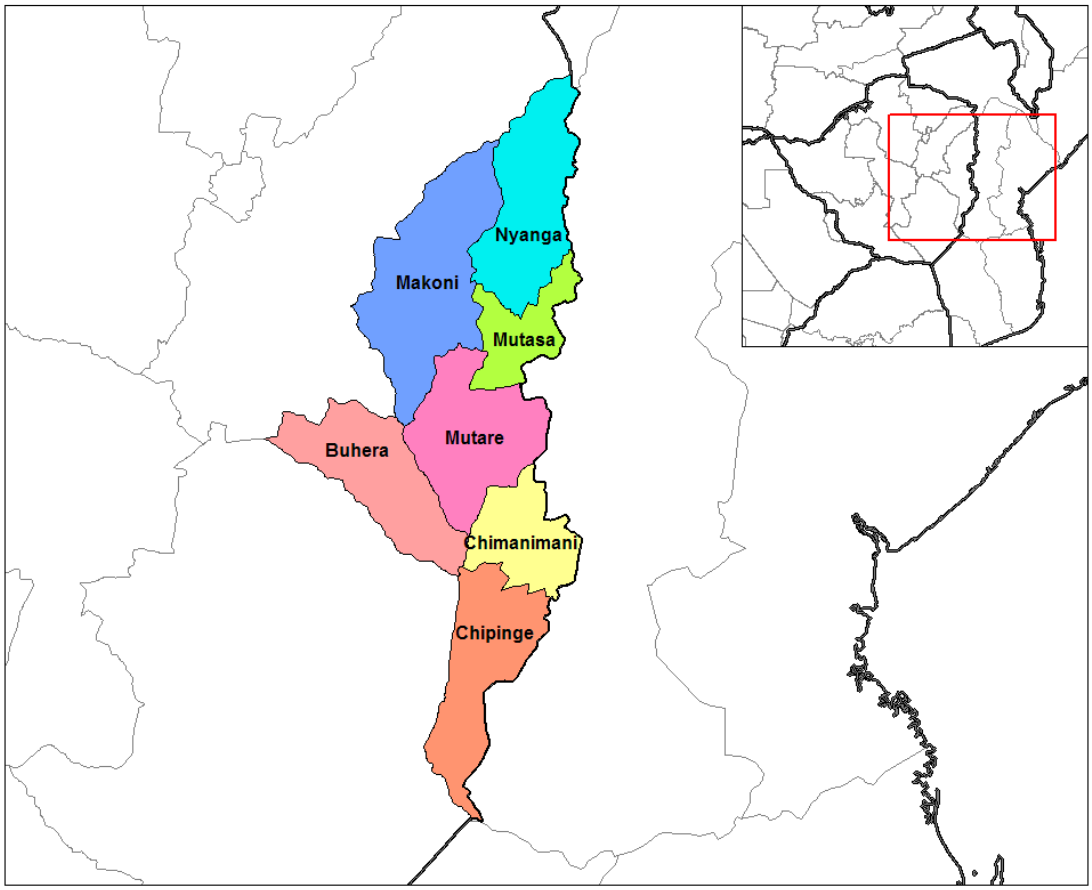

Figure 1: Map of Manicaland Province of Zimbabwe showing the location of Mutasa District.

3.3. Bean seed samples collection

The thirty one (31) wards in Mutasa district were grouped into their respective agro-ecological regions. Three wards were selected at random using the simple random sampling method from the three agro-ecological regions. From the selected wards, major beans producing areas were identified.

Ten farmers were selected at random from each area as respondents. Seed samples were collected from Samanga (natural region I), Honzo (natural region IIa) and Tsonzo (natural region IIb).

The altitudes for Samanga area range from 1267 to 1338m, Honzo area, 950 to 1150m and Tsonzo area, 1400 to 1430m. The annual rainfall for the study area is presented in appendix 1.

The seed samples were collected from farmer retained bean seed stocks harvested during the 2013/14 season in October 2014 before the main planting season.

Approximately one and half kilogram of seed was collected for each identified landrace from the ten interviewed farmers. The seed samples were kept in khaki envelopes and stored under room temperature in the laboratory at Africa University. The landraces were characterised into market classes using guidelines developed by the Central High Plains Dry Bean and Beet Group of the USA (2004).

3.4 Seed health analysis for seed-borne fungal infection

The purpose of the seed health analysis was to determine seed-borne fungal pathogens on the seed of each landrace. The seed samples were analysed using the blotter test method. The blotter method is deemed not expensive (use of filter papers) and easier and does not require aseptic techniques. The method reduces the probability of contamination of dishes by profuse growth of saprophytes and external microbes, which can interfere with identification of pathogens (Saettler *et al*., 1995; Kutywayo, 2000).

Standard samples of 400 non-sterilised seeds were plated in batches of 10 seeds per petri dish on 3 layers of water soaked paper according to the protocol developed by ISTA, (2007).The Completely Randomized Design (CRD) was used for seed health analysis.

3.4.1 Plating of seeds

Sterilized 90mm diameter petri dishes were used for plating seeds. A set of three blotter papers were picked by a pair of forceps and dipped in distilled water and then placed in the petri dish.

The seeds were poured on to a clean surface on a table and samples for plating were taken at random using a pair of forceps. Ten (10) bean seeds were plated per petri dish. Four hundred (400) seeds, comprising of hundred (100) seeds per replicate were used for each sample ISTA, (2007). The date of plating, accession number and replicate number were written on each petri dish cover for identification.

3.4.2 Examination of incubated seeds and recording of infections

After seven days of incubation, the petri dishes were grouped per replicate (10 dishes). Each seed was examined under a stereomicroscope to observe habit characters of each fungus. Habit characters were used to identify the fungi that grew on the seeds. Preparation of slides with fruiting structures and spores of fungi were made and examined under a compound microscope to confirm fungal identities by consulting mycological literature. Individual fungal counts from each dish were entered on a working record sheet immediately after examination of each dish according to the protocol by ISTA, (2007).

3.5 Seed health analysis for seed-borne bacterial infection

The seeds were analysed for bacterial infection using the protocol according to Mortensen (2000).

3.5.1 Visual examination/Dry Inspection of Seed

The seeds were inspected visually under normal daylight for butter -yellow discoloration around the hilum as this is associated with *Xanthomonas* spp. infection (Mortensen, 2000). The visual scoring scale of 1-5 was used to score bacterial discolouration on seeds according to Mortensen (2000) protocol.

3.5.2 Liquid assays, destructive method

3.5.2.1 Sample suspension preparation

Seed samples from the three selected wards were used for the assay. Intact seeds (150g) were weighed using a balance scale and placed into a 1 litre conical flask. The weighed seeds were washed thoroughly using running tap water to remove soil and

other debris. Thereafter, 300ml of sterile distilled water was poured into each conical flask. An Aluminum foil was used to cover the top of the conical flask and the seeds were soaked for 12 hours.

After 12 hours of soaking, the flasks were shaken manually to have a uniform mixture of the bacterial suspension. Six glass tubes per sample (2 replications per dilution) were sterilised first before use. One millilitre of suspension was drawn from each sample in the conical flask using a 1ml pippette and then poured into a glass tube labelled original. Some 0.1 ml was drawn from the original glass tube (undiluted) using a 200 micron pippette with a fresh tip and then poured into a separate glass tube (labelled 10^{-1}) containing 0.9 ml of distilled water i.e. 10 times dilution. Then 0.1ml of mixture from the diluted (10^{-1}) glass tube was drawn using a 200 micron-litre syringe with a fresh tip and then empted into another glass tube containing 0.9 ml distilled water thus diluting it 100 times.

Some 0.1ml from the 10^{-2} glass tube was drawn using a pippette with a fresh tip and then put into the third glass tube containing 0.9 ml distilled water thus 1000 times dilution.

Fifty micro-litres (50µl) were drawn from each glass tube (10^{-1}, 10^{-2}, 10^{-3}) for each sample and then spread evenly over nutrient agar (two replicates each) using a sterile triangle bent glass rod. The same sterile glass rod was used for spreading the diluted mixtures from one sample and then sterilised with alcohol and placed over a flame on an alcohol lamp before spreading other mixtures from different samples. After three days of incubation, the petri dishes were inspected for development of *Xanthomonas axonopodis* colonies.

3.5.2.2 Examination and recording of assayed seeds-bacterial colonies

After three days of incubation, the dishes were inspected for bacterial colony development. Colour of the colonies was used to distinguish between different bacterial colonies. *Xanthomonas axonopodis* was identified as yellow round colonies. The number of the *Xanthomonas axonopodis* colonies per petri dish was counted and recorded per each dilution.

3.5.3 Seedling grow -out test/assay or Growing –on test

Seedling grow-out is one of the most applicable and widely used seed detection assays (Capoor, Rao, and Sawant**;** 1986; Lee *et al*., 1990; Yang *et al*., 1997) but for successful implementation, infected seedlings must display obvious and characteristic symptoms. The seedling grow-out assay is a direct measure of the seed's ability to transmit a disease to the seedling.

Three replicates of seed samples were planted into black plastic pots under greenhouse. The Completely randomised design was used to set the experiment. After two weeks, seedlings were examined for the development of typical symptoms of *Xanthomonas axonopodis* infection. The primary leaves were inspected for disease symptoms.

3.6 Evaluation of bean landraces for tolerance to Angular leaf spot disease

3.6.1 Fungal inoculation using the detached leaf method.

Inoculation procedure

Some affected bean leaves were used as sources of inoculum. *Phaeiosariosis griseola* synnemata (greyish –silvery) from lesions on affected leaves were identified

under a stereomicroscope. A scarpel blade was used to scrap off the spores from the leaves and put on a slide. The slide was examined on a compound microscope to confirm typical *P. griseola* spores. The leaf samples were put into a plastic bag and tap water was added.

The sample was shaken for 120 seconds in a Stomacher blender at normal speed to dislodge the spores from the leaves into the water. The concentration of fungal spores in the prepared inoculum was evaluated by preparing a slide and examining it for spores under a compound microscope. The plants were inoculated at first trifoliate leaf stage.

Primary detached leaves from Giant, Black speckled sweet bean, Honzo (long kernel) sweet bean, Honzo (small and round) sweet bean, Moza and Red speckled sweet bean landraces were dipped into the *Phaeoisariopsis griseola* inoculum.

The wetted leaves were placed on top of moistened filter paper (4 sheets) in petri dishes and incubated for seven days to allow symptom development. Each landrace was replicated thrice. Control leaves were dipped in sterile distilled water.

3.6.1.2 Spraying live plants method

Three plants per landrace were sprayed with Angular leaf spot inoculums prepared in the same manner as the one used for the detached leaf method. The plants were sprayed at the first trifoliate stage and incubated for seven days. The control plants were sprayed with sterile distilled water.

3.6.2.3 Examination and recording of symptoms on incubated leaves and plants

Typical laboratory symptoms of *P. griseola* infection were inspected on the sprayed bean plants after 7days of inoculation on all the landraces. The leaves were checked for dark –brown or black lesions on the upper and lower surfaces of inoculated leaves. Each replication was recorded separately using a standardised CIAT scale of 1-9.

3.7. Evaluation of landraces to natural disease infection in the field.

A field experiment was conducted to determine the effect of natural environment on disease development on seven landraces at Africa University Farm during 2014/2015 season.

Seven bean landraces namely Pfumisai. Moza, Honzo sweet (s&r), Honzo sweet (long), Red speckled sweet, Black speckled sweet and Giant were planted at Africa university farm to evaluate their reaction to natural disease infection. Each landrace was replicated thrice in a completely randomised design.

Compound D was applied by banding at a rate of 600 kgs per hectare. The planting spacing was 50 cm x 15 cm. Each replicate was planted in a 3 m row. A Completely randomised design was used.

3.7.1 Evaluation and recording of natural disease infection in the field

The landraces were examined for typical fungal and bacterial disease symptoms particularly Angular leaf spot and Common bacterial blight. Three plants for each landrace were picked at random in each replication and examined visually. The disease symptoms were evaluated using a standardised CIAT scale of 1-9.

3.8 Evaluation of landraces for seed germination-ISTA Germination test

The germination rate of the landraces was evaluated according to the ISTA standards (ISTA, 1999). Seeds were sown in sterile sand medium. Four replicates of 25 seeds each were sown per landrace in wooden trays. Seeds were sown to a depth of 2 cm. Seeds were placed on top of the sand and then pushed into the soil using the back of a pencil to a depth of 2 cm before covering. A Completely randomised design was used.

3.8.1 Evaluation and recording of germinated seeds/seedlings

The seeds were evaluated after ten days of sowing. The seedlings were grouped into four categories i.e. normal, abnormal, fresh and dead (decayed) seeds.

Seedlings without cotyledons or damaged terminal buds or bent over hypocotyls or cracked hypocotyls or short and thick hypocotyls or missing primary leaves or shoot and root system trapped in seed coat or seedlings with under developed rooting systems among other attributes were regarded as abnormal (ISTA, 2007).

3.9 Data analysis

Analysis of variance (ANOVA) was carried out using Genstat 5, Release 3.2. Data on seed health analysis and germination test was transformed before analysis.

CHAPTER 4

RESULTS

4.1 Survey of farming systems

The majority of farmers from the three agro-ecological regions grew their bean crop during early winter and a small number grew beans in early summer most probably due to lack of irrigation water (Table 4.1). All farmers in the area used retained seed and sole cropping is the major cropping practice being utilised especially for the winter and early summer crops. In natural region I, 80% of the farmers grow beans as an intercrop in summer while 40% grow the crop as a sole crop in early winter. In

natural region IIb, 100% of the farmers grow beans as an intercrop in summer and 60% grew beans as a sole crop in early winter. All Farmers in natural region IIa grew beans as a sole crop in early winter. Beans were rotated with maize and only 30% of the farmers in natural region I practised rotation, 100% practised rotation in natural region IIa and 50% in natural region IIb (Table 4.1).

Table 4.1. Agronomic practices for farmers categorised by natural region

		Natural region		
		I	IIa	IIb
Practice		n=10	n=10	n=10
Time of planting: i. Summer	a)Sole crop	0	0	0
	b)Intercrop	80	0	100
ii. Early winter	a)Sole crop	40	100	60
	b)Intercrop	0	0	0
iii. Early summer	a)Sole crop	10	100	0
	b)Intercrop	0	0	0
Rotation(with maize)		30	100	50
Use of overhead irrigation		70	0	100
Use of retained seed		100	100	100

n=number of respondents in sample

Table 4.2. Other crop protection challenges (% of households) categorised by natural region

	Natural region		
	I	IIa	IIb
Challenge	n=10	n=10	n=10
1.Insect pest: i)Bean stem maggot	30	0	70
ii)Bruchids	80	70	90
2.Weeds	90	30	40

n=number of respondents in sample

Other crop protection challenges faced by farmers are insect pests such as bean stem maggot and aphids and storage insect pests like bruchids. Weeds are another culprit causing yield losses as farmers lack enough labour to control them in time (Table 4.2).

The common bean accessions used in the study are shown in plate 4.1

Moza

Honzo sweet (long)

Honzo sweet(s&r)

Pfumisai

Black speckled

Samanga sweet(s&r)

Red speckled

Giant

Plate 4.1: The common bean accessions used in the study

The characteristics of the landraces grown in Mutasa are given in Table 4.3. Only one class, the kidney bean is grown in Mutasa. Sole crops (plate 4.2a) have slightly better yields compared to intercrops (plate 4.2b)

Plate 4.2a) Sole crop of beans **Plate 4.2b)** Intercrop of maize and beans

Table 4.3. Characteristics of bean landraces collected from Mutasa district

				Estimated Yield(t/ha)		**Threshing method**	**Storage method**
Landrace	**Class**	**Growth habit**	**Maturity group**	**Sole crop**	**Intercrop**	Beating with sticks (%of farmers)	Polythene bags (%of farmers)
Giant	Kidney	bush	medium	0.06	0.04	100	100
Samanga (s&r)	Kidney	bush	medium	0.05	0.03	20	20
Honzo (long)	Kidney	vine	medium	0.06	n/a	90	100
Honzo (s&r)	Kidney	vine	medium	0.06	n/a	80	100
Pfumusai	kidney	bush	short	0.05	0.04	20	20
Black speckled	kidney	vine	medium	n/a	0.05	90	100
Red speckled	kidney	vine	medium	n/a	0.05	70	100
Moza	Kidney	bush	short	n/a	0.05	10	100

Yield estimates derived from converted data on average bags harvested by farmers.

The farmers grew any landrace of choice. The landrace with the available seed was planted without considering maturity group and/or growth habit.

Farmers stored their bean crop in polythene bags. The majority of farmers thresh the crop on open ground using sticks before packing and storing it in polythene bags (Table 4.3).

4.2 Incidence of fungal pathogens on bean samples

All the samples tested were infected by field fungal pathogens but at different levels. In all the eight samples pathogenic and saprophytic fungi were found namely; *Fusarium moniliforme* (100%), *Botryodiplodia theobromae* (12.5%), and *Collectotrichum lindemuthianum* (12.5%). Samples from natural region I had *Fusarium moniliforme*, but no *Collectotrichum lindemuthianum* and *Botryodiplodia theobromae.* Samples from natural region IIa had *Fusarium moniliforme* only while those from region IIb had *F. moniliforme*, *Collectotrichum lindemuthianum* and *Botrodiplodia theobromae* (Table 4.4).

Table 4.4. Infection of bean samples by field fungal pathogens.

Natural	Accession	*Fusarium*	*Collectotrichum*	*Botryodiplodia*

region		*moniliforme* (%)	*lindemuthianum* (%)	*theobromae* (%)
I(Samanga area	Pfumisai	0 (1.08[a])	0 (1.00[a])	0 (1.00[a])
	Giant	21.3 (4.73[b])	0 (1.00[a])	0 (1.00[a])
	Sweet bean (s&r)	0 (1.08[a])	0 (1.00[a])	0 (1.00[a])
	Sweet bean(long)	0.39(1.18[a])	0 (1.00[a])	0 (1.00[a])
IIa(Honzo area)	Sweet bean (s&r)	1.13(1.46[a])	0 (1.00[a])	0 (1.00[a])
	Black speckled	23 (4.90[b])	0 (1.00[a])	0 (1.00[a])
IIb(Tsonzo)	Moza	80.9 (9.05[c])	0.5(1.23[b])	0 (1.00[a])
	Red speckled	71.42(8.51[c])	0 (1.00[a])	4.1(2.263[b])
	Significance of F	***	***	***
	$LSD_{(0.05)}$	0.948	0.1262	0.2701
	CV (%)	46.6	24.1	45.8

Data separated using transformed values in brackets using square root of X+1 transformation. Means with same letters in the same column are not significantly different ($p<0.05$). ***= denote significance at <.001.

Plate 4.3: *Botryodiplodia theobromae* infection on Red speckled sweet bean

4.2.2 Infection of samples by storage contaminants

The infection of samples by storage pathogens was *Aspergillus flavus* (75%), *Cladosporium* sp. (100%), *Aspergillus niger* (50%) and *Rhizopus* sp. (75%). *Aspergillus flavus* had the highest infection rate in natural region I while *Cladosporium* sp. had highest infection of samples in natural region IIa. Samples from region I were infected by *A. flavus*, *Cladosprium* sp. and *Rhizopus* sp. All samples from natural region IIb were infected by *A. flavus*, *Cladosprium* sp. and *Rhizopus* sp. and *A. niger* (Table 4.5).

Table 4.5. Infection of bean samples by storage contaminants

Natural region	Accession	*Aspergillus flavaus* (%)	*Cladosporium* sp. (%)	*Aspergillus niger* (%)	*Rhizopus* sp. (%)
Ia(Samanga area	Pfumisai	4.2 (2.28^{b})	88.3 (9.45^{d})	0 (1.00^{a})	1.85(1.69^{a})
	Giant	86.9 (9.38^{d})	0.3 (1.12^{a})	0.12(1.12^{a})	15.4 (4.05^{c})
IIa(Honzo area)	Sweet bean (s&r)	25.3 (5.13^{c})	98 (9.95^{de})	0.15(1.15^{a})	1.22(1.15^{a})
	Sweet bean (long)	0 (1.0^{a})	100 (10.1^{e})	0 (1.00^{a})	0.69(1.30^{a})
	Sweet bean (s&r)	0.9 (1.38^{a})	100 (10.0^{de})	0 (1.00^{a})	0 (1.00^{a})
IIb(Tsonzo)	Black speckled	0 (1.0^{a})	0.4 (1.18^{a})	0 (1.00^{a})	0 (1.00^{a})
	Moza	3.3 (2.07^{a})	8.1 (3.02^{c})	0.37(1.54^{b})	3 (2.00^{b})
	Red speckled	0.5 (1.23^{a})	4.1 (2.26^{b})	0.82(1.35^{b})	0.44(1.20^{a})
	Significance of F	***	***	***	***
	$LSD_{(0.05)}$	0.6615	0.606	0.2911	0.861
	CV (%)	44.3	20.2	50.0	101.2

Data separated using transformed values in brackets using square root of X+1 transformation. Means with the same letters in the same column are not significantly different ($p<0.05$). ***=denote significance at <.001. s &r =small and round.

4.3. Incidence of bacterial pathogens on bean seed samples

Xanthomonas axonopodis pv. *phaseoli* was the only seed-borne bacteria found associated with the bean seed samples (plate 4.4). The bacterial colonies were identified on two landraces only; Honzo sweet (s&r) bean from region IIa and Moza from region IIb (Table 4.6). The percentage infection of the samples by *X. axonopodis* was 28.6%. No landraces from natural region I had the bacterium. The presence of the bacterium was scored using visual scores before analysis.

Table 4.6. Means for *Xanthomonas axonopodis* pv *phaseoli* colonies on samples

Landrace	Liquid assay(colonies)	Field Assessment score
Pfumisai	0 (1^{a})	5.00
Honzo sweet(s&r)	$5.25x10^{2}$(23.99^{c})	4.33
Honzo sweet(long)	0 (1^{a})	6.33
Giant	0 (1^{a})	4.33
Red speckled sweet	0 (1^{a})	7.67
Black speckled sweet	0 (1^{a})	6.33
Moza	$0.5x10^{1}$ (1.25^{b})	5.00
Significance of F	***	N.S
$LSD_{(0.05)}$	0.7065	-
CV (%)	6.9	26

Data separated using transformed values in brackets using square root of X+1 transformation. LSD was used to separate transformed values. Figures with same letter are not significantly different ($p<0.05$).

***=denote significance at <.001 and N.S = not significant. s&r=small and round.

There were significant differences in the incidence of *Xathomonas axonopodis* between the landraces. There were no significant differences in the tolerance of the landraces to bacterial blight. All the landraces are susceptible to bacterial blight.

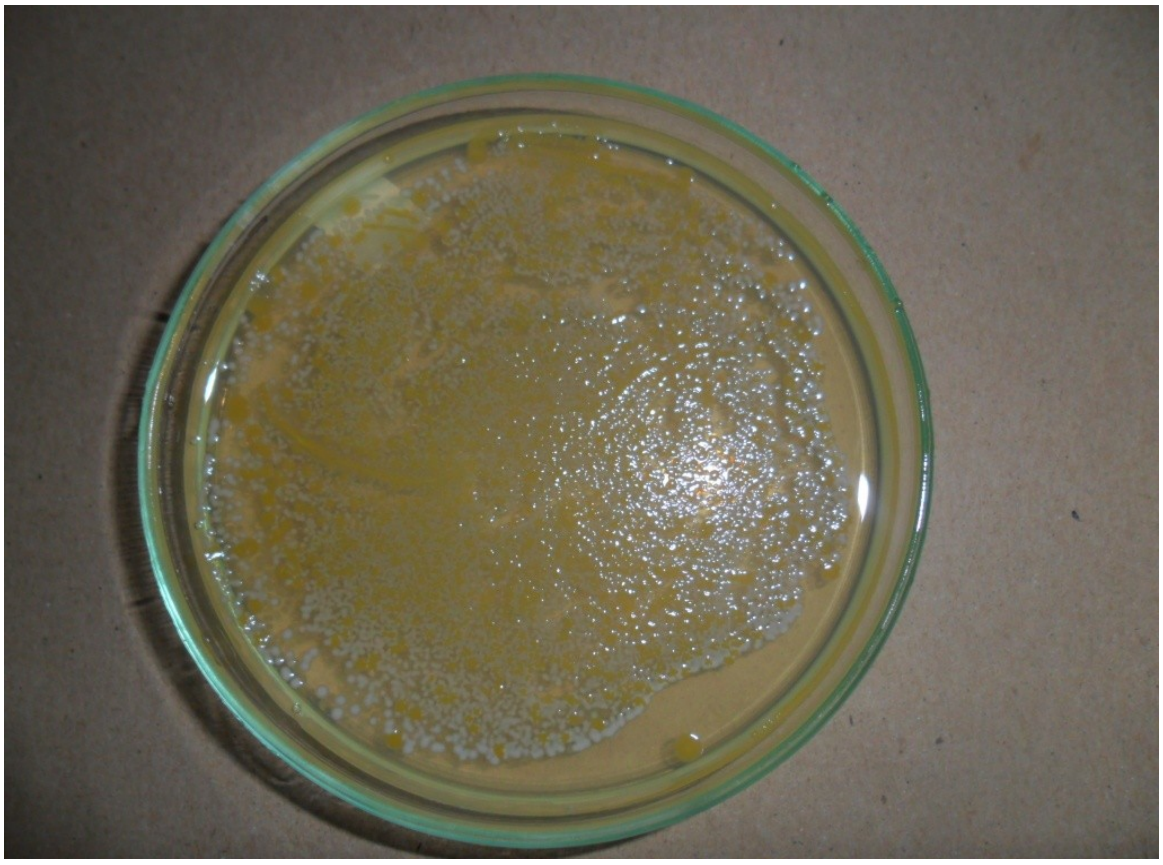

Plate 4.4: *Xanthomonas axonopodis* pv. *phaseoli* colonies isolated from Honzo sweet bean (s&r)

4.3.1 Growing- on test

No typical symptoms of bacterial disease infection were noted on all the landraces grown in the greenhouse after two and half weeks of seed planting.

4.4 ISTA Germination test

Standard normal germination of seeds varied across the landraces (Table 4.7). Giant and Pfumisai had the lowest normal germination rate while Red speckled sweet, Black speckled sweet, Honzo sweet (long) and Honzo sweet (s&r) had the highest. There were no significant differences ($p>0.05$) in normal germination rate between Red speckled, Black speckled, Honzo sweet (long) and Honzo sweet (s&r) seeds. The germination rate of Giant and Pfumisai seeds was not significantly different ($p>0.05$).

Table 4.7. Means for normal and abnormal seedlings, Fresh and dead seeds.

Location	Accession	Normal (%)	Abnormal (%)	Fresh (%)	Dead (%)
I(Samanga area)	Giant	21.27(4.72^{ab})	8.48 (3.08^{a})	54.8 (7.47)	5.9 (2.6^{b})
IIb)(Tonzo area)	Pfumisai	5.2 (2.49^{a})	19.8 (4.57^{b})	12.3 (3.65^{ab})	5.7 (1.9^{b})
	Moza	29.69 (5.54^{b})	6.89 (2.81^{a})	23.5 (4.95^{b})	27.5(5.3^{c})
	Red speckled sweet	79.82 (8.99^{c})	2.27 (1.81^{a})	8 (6.97^{c})	2 (4.9^{c})
IIa(Honzo area)	Black speckled sweet	86.6 (9.36^{c})	11.329(3.51^{b})	0.71(1.31^{a})	0 (1^{a})
	Honzo sweet (s&r)	67.89 (8.30^{c})	9.82 (3.29^{b})	5.7 (2.59^{ab})	0.7 (1.3^{a})
	Honzo sweet (long)	79.6 (8.98^{c})	9.62 (3.26^{b})	7.8 (2.96^{ab})	1.6 (1.6^{ab})
	Significance of F	***	N.S	***	***
	$LSD_{(0.05)}$	2.276	-	1.978	1.473
	CV (%)	22.4	34.4	31.5	37.5

Data separated using transformed values in brackets using square root of X+1 transformation. Means with the same letters in the same column are not significantly different at (p=0.05). ***= denote significance at <.001. N.S= not significant.

The infection of Giant by *Aspergillus flavus*, a storage contaminant was responsible for the low normal germination rate. Low germination percentage on Moza was due to infection by *Collectotrichum lindemuthianum*, *Fusarium moniliforme* and *Cladosporium* sp.

4.5 Tolerance of landraces to Angular leaf spot (*Phaeoisariopsis griseola*)

There were no significant differences in their tolerance to ALS (plate 4.5) using the field method between the landraces at p=0.05. The landraces were all susceptible to Angular leaf spot in all the methods used. The landraces can succumb to ALS given the same quantity of inoculum at the same crop growth (Table 4.8). There were

significant differences in infection levels for the spraying method and the detached leaf method even though all landraces were susceptible to the disease.

There were no significant differences in the field assessment as all landraces were susceptible to the disease.

Table 4.8. Average ALS disease scores for Spraying, detached leaf and field Assessment methods.

Landrace	Spraying method	Detached method	Field plot assessment
Black speckled sweet	2.33[a]	9.00[d]	5.67
Giant	5.00[c]	3.00[a]	6.33
Honzo sweet(long)	3.67[b]	7.67[c]	5.67
Honzo sweet(s&r)	3.00[ab]	5.67[b]	6.33
Moza	3.00[ab]	3.00[a]	4.33
Pfumisai	9.00[d]	8.33[cd]	6.33
Red speckled sweet	9.00[d]	5.00[b]	5.00
Significance of F	***	***	N.S
$LSD_{(0.05)}$	1.081	1.324	-
CV (%)	12.3	12.7	23.1

Data separated using transformed values using square root of X+1 transformation. Means with the same letters in the same column are not significantly different at (p=0.05). ***= denote significance at <.001. N.S = not significant.

4.5.1 Farmer field disease assessment

The three landraces were significantly different ($p<0.05$) in their tolerance to Angular leaf spot under farmers' field conditions but they are all susceptible to ALS (Table 4.9).

Table 4.9. Means for farmer bean field disease score –Angular leaf spot

Landrace	Average score
Black speckled sweet	6.67[b]
Giant	9.0[c]
Red speckled sweet	5[a]
Significance of F	***
$LSD_{(0.05)}$	0.666
CV (%)	4.8

Means with the same letters in the same column are not significantly different at (p=0.05). ***= denote significance at <.001.

Plate 4.5: Angular Leaf Spot leaf lesions on Pfumisai landrace in farmer field Assessment

CHAPTER 5

DISCUSSION

The study investigated the incidence of seed-borne fungal and bacterial pathogens on bean seed collected from Samanga area (natural region I), Honzo area (natural region IIa) and Tsonzo area (natural region IIb). The occurrence and level of infestation of the various field fungal pathogens varied in the three ecological zones. Tsonzo area recorded the highest average frequency of *Fusarium moniliforme*. Infection by *Botryodiplodia theobromae* and *Collectotrichum lindemuthiunum* was recorded in Tsonzo area only due to favourable environmental conditions such dry spell and temperature respectively. The rainfall amount (Appendix 1) and pattern in Tsonzo area seem to be promoting the survival and multiplication of *Botryodiplodia theobromae* and *Collectotrichum lindemuthiunum.* The two fungi seem to be favoured by extended periods of cool weather. The prevalence of dry spells in natural region II seems to be making the climate more conducive for the fungi (FAO, 2006).

Aspergillus flavus, Cladosporium sp., *Aspergillus niger and Rhizopus* sp. were the storage contaminants found on bean samples. Honzo area showed the highest frequency of *Cladosporium* sp. while *Aspergillus flavus* had the highest incidence in Samanga area. *Xanthomonas axonopodis* was found in natural region IIa (Honzo area) and IIb (Tsonzo area). The two areas receive 500-1020mm rainfall that enhanced the bacterium to develop and multiply. Also the distribution of rain in a

particular season provides necessary conducive conditions for the survival of the bacterium.

Aspergillus flavus is a thermo-tolerant fungus and can survive in temperatures that other disease pathogens would not.

The optimum temperature for its growth is 37^0C. The Samanga area offers the favourable temperatures and humid conditions for the fungus and thus its high infestation levels on samples collected from this area.

Post-harvest rot develops during harvest, storage, and/or transit. *A. flavus* infection can occur while bean plants are still in the field (pre-harvest), but often show no symptoms until post-harvest storage and/or transport.

The fungus' growth occurs at around 14% moisture content of the legume seed (Agrios, 2005). Dry beans are normally harvested at moisture content higher than 14% and this may have promoted infection by *A. flavus*. This is very particular with Samanga area which receives 970-1150mm that provided favourable conditions especially during the time of harvesting summer bean crops.

5.1 Infection of samples by field fungal pathogens

The occurrence of *Collectotrichum lindemuthium* in Tsonzo area might be due to high altitude since the disease is usually associated with high altitude and cool/temperate regions (Hagedorn and Inglis, 1986). The fungus was recorded in region IIb because it is prevalent in regions with cool to moderate temperatures and high relative humidity (Buruchara *et al.*, 2010).

Collectotrichum lindemuthianum and *Botyrodiplodia theobromae* were the only seed-borne fungi found on Moza and Red speckled sweet bean respectively which are grown in natural region IIb. The fungus *Collectotrichum lindemuthianum* might have come either from Mozambigue from where the seed was sourced or from retained seed. The pathogen is seed-borne and also farmers used untreated retained seed (Whingwiri *et al.*, 1992) which enhanced its transmission to new crops (Buruchara *et al*., 2010).

Botryodiplodia theobromae occurrence was limited to natural region IIb. The fungus seems to favour high altitude areas with cool weather and less periods of persistent wet weather.

The fungus is more prevalent in areas that experience drought/dry spells as reported by Schwartz and Pastor-Corrales, (1989) and such dry spells are common in Tsonzo area (IIb) and thus the presence of the fungus.

Fusarium moniliforme is a widely distributed pathogen causing seed rots. The fungus was more prevalent in agro-ecological region IIb although it was found in all the three natural regions. All the eight samples of bean seed examined were infected by *Fusarium moniliforme*. This could be because the fungi can thrive well in tropical and sub-tropical regions and its wide distribution was responsible for its presence in all the three ecological regions. Its highest incidence was in region IIb as the area provides very favourable conditions for its multiplication. Moza and Red speckled bean seeds had their germination reduced due to high infestation by *Fusarium*

moniliforme. Storage contaminants reduced germination rate more than field pathogens particularly *A. flavus* and *Cladosporium* sp.

5.2 Infection of samples by storage contaminants

The high frequency of *A. flavus* in Samanga area may be attributed to the fact that the area provides humid and warm conditions necessary for its multiplication. The lowest *A. flavus* infection was in natural region IIb. This may be due to low temperatures and dry spells that were unfavourable for fungal sporulation and multiplication. The bean crop might have been infected during pre-harvest period (Agrios, 2005).

Cladosporium sp. had highest frequency in agro-ecological region I and IIa. The two regions provide extended periods of high relative humidity promoting fungal sporulation and multiplication.

The seeds infected by *Cladosporium* sp. might have been exposed to inadequate moisture and temperatures during storage and this promoted infection of the beans by the fungus. This promoted growth of poisonous substances by the fungi (Costa and Scussel, 2002) which result in loss of seed quality and viability. High infestation of Honzo sweet (long) and Honzo sweet (s&r) by *Cladosporium* sp. did not cause a big decline in germination in the short term since the seed was still fresh (less than a season old) but over-storage of the infected seed might have some negative repurcations on seed germination ability.

5.3 Infection of samples by bacterial pathogens

The African Scholarly Science (2014) reported that *Xanthomonas axonopodis* was the only seed-borne bacterium found on all the seven samples examined.

It was recorded on Honzo sweet bean (s&r) and Moza in Honzo area and Tsonzo area respectively at variable infection levels. The bacterium was not noted in natural region I but region II as also reported by Giga (1989). Farmers used retained seed: seed-borne *X. axonopodis* is common in retained common bean seed lots (African Scholarly Science Trust, 2014). The high incidence of *X. axonopodis* in Honzo may also be because the crop was grown under overhead irrigation (early winter and early summer planting). The use of overhead irrigation resulted in dispersal of the bacterium to unaffected plants as also reported by Osdaghi *et al*. (2010). The use of overhead irrigation as reported by Helene and Legard (1991) and Wohleb and du Tolt (2011), increased secondary spread of the pathogen as compared to furrow or drip irrigation. The movement of irrigation equipment especially sprinklers at change times when the crop is wet also enhanced bacterial spread to unaffected parts of the field (Kaiser and Vakili, 1978; Lindemann and Upper, 1985).

5.4 Germination Percentage

Standard normal germination of seeds varied across the agro-ecological regions but there were no significant differences among landraces produced under intercrops and sole crops.

The poor germination rate of Moza, Pfumisai and Giant seeds may be as a result of poor storage conditions and incidence of seed-borne fungi such as *Collectotrichum lindemuthianum*) and other storage fungus e.g. *Aspergillus flavus* (African Scholarly

Science Trust, 2014). Most farmers threshed their crop on open dirty sacks using sticks and this may result in contamination of the beans by *A. flavus*.

The threshing action also increased the proportion of damaged seeds; *Apergillus flavus* enters the seeds through the damaged parts. Seed decay after harvest i.e. in storage (Agrios, 2005) increased and hence poor germination of highly infected landraces for instance Giant and Pfumisai. *Aspergillus flavus* seeds decayed prior to start of germination. The infection by *Aspergillus flavus* may also be as a result of the contaminated polythene bags that were used to store the beans.

Cladosporium sp. and *Fusarium* sp. can contaminate and cause decay of the seed during pre-harvest, harvesting and post harvest and in storage. Bean is harvested at physiological maturity when moisture content is still high and this promotes the growth of the fungus as reported by Agrios (2005). The fungus reduced germination by discolouring seeds or killing ovules or embryos or caused shrivelling of the seeds. Poor germination of Giant, and Pfumisai might have been caused by *Cladosporium* sp. and *Fusarium* sp. infection. *Fusarium* pathogens entered the seed through damaged tissue in the field and infection developed in storage (Agrios, 2005). *Fusarium* sp. may be responsible for reduced germination of Moza and Red speckled sweet bean.

5.5 Tolerance of landraces to Angular leaf spot and Bacterial blight.

The methods used to assess the landraces to ALS showed that all the landraces were susceptible to the disease. The laboratory methods are not appropriate methods of assessing tolerance since the inoculum may be too concentrated to the extent that all

samples show susceptibility. The field method is the most reliable method. Under field experimentation, all the landraces showed moderate tolerance to the inoculum.

This might be due to the fact that the inoculum load was low since the crop was grown after the main period of disease infection. The site may also have played a significant role in inoculum quantity and multiplication-continuous dry weather reduced fungal growth. In the farmer bean field assessment done in Samanga area, Giant had a high infestation level of ALS because the area provided moist and warm conditions required by the fungus to development.

All the landraces were susceptible to bacterial blight under field conditions in the experiment done at Africa university farm. The area provided enough inoculum for the disease to occur. The use overhead irrigation might have also necessitated disease development and spread.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.0 Conclusion

Three seed-borne fungi; *Collectotrichum lindemuthianum, Phaeoisariopsis gresiola* and *Botryodiplodia theobromae* and one seed-borne bacterium, *Xanthomonas axonopodis* were detected on bean seed samples from Mutasa district. The transmission of seed-borne fungi and bacteria to bean seedlings is very low; instead the pathogens kill and/or decay the seed and reduce germination ability. Dry bean is grown in all farming ecosystems in Mutasa at different times of the year. No bean landraces are tolerant to Angular leaf spot in Mutasa. The occurrence of seed-borne bacteria particularly *Xanthomonas axonopodis* is influenced by natural region. The occurrence of seed-borne fungal diseases is influenced by agro-ecological region in Mutasa. No common bean landraces are tolerant to bacterial blight in Mutasa District.

6.1 Recommendations for farmers

1. The exchange of seed among farmers should be confined to a ward to avoid introduction of seed-borne epiphytic populations into new agro-ecological regions.

2. Farmers should test the germination percentage of retained seed and carry-over certified seed before planting to ascertain optimum plant population and good crop stand.

4. Resistant varieties when available should be used instead of farmer- saved seed to reduce the carryover of seed-borne diseases to next generations.

5. Diversification in the classes of beans with disease resistant classes (for instance Canning type) can be used to reduce disease epidemics by farmers in Mutasa District.

6. Where possible farmers should replace overhead irrigation with furrow/flood irrigation to reduce incidence of *Xathomonas axonopodis*.

6.2 Recommendations for future research

1. Work should be done on adaptability of newly released bean varieties to diversify the classes of beans grown in Mutasa District.

2. Further work needs to be done on evaluating the tolerance of varieties to the major fungal and bacterial diseases prevalent in Mutasa.

3. Further work needs to be done on evaluation of affordable seed treatment methods that can be adopted by farmers.

REFERENCES

African Scholarly Science Trust., 2014. Assessment of common bean (*Phaseolus vulgaris* L.) seed quality produced under different cropping systems by smallholder farmers in eastern Ethiopia. *African journal of food nutrition and development*, **14:** 8568-8584.

Agrios G. N., 2005. Plant Pathology 5th Edition. Elsevier Academic Press: 922 pp.

Akhavan A., Bahar M., Saeidi G. and Lak M.R., 2009. Factors affecting epiphytic population of *Xanthomonas axonopodis* pv. *phaseoli* in epidemiology of bean common blight. *Journal of Science and Technology in Agriculture and Natural Resour*ces, **13**:265–277.

Allen D.J. and Edje O.T., 1990. Common bean in African farming systems: In Smithson J.B. (Ed) Proceedings of the Ninth SUA/CRSP and second SADCC/CIAT Bean Research Workshop, held at Sokonie University of Agriculture, Morogoro, Tanzania 17-22 September, 1990.CIAT Africa workshop series No.12.

Allen D.J., Ampofo J. K.O. and Wortman C.S., 1996. Pests, diseases and nutritional disorders of the common bean in Africa: a field guide. International Center for Tropical Agriculture, Cali, Colimbia.

Amannuel G. S., Kiihne R.F., Tanner D.G. and Vlek P.L.G., 2000. Biological nitrogen fixation in faba bean (*Vicia faba* L.) in the Ethiopian highlands as affected by P fertilization and inoculation. *Biology* and *Fertility of Soils*, **32**:353-359.

Andrews J. H. and Harris R.F., 2000. The ecology and biogeography of microorganisms on plant surface. *Annual Review of Phytopathology*, **38**:145–180.

Arnaud-Santana E. and Pena-Matos E., 1991. Longevity of *Xanthomonas campestris* pv. *phaseoli* in naturally infested dry bean (*Phaseolus vulgaris* L.) debris. *Plant Disease*, **75**:952–953.

Arulbalachandran D. and Mullainathan L., 2009. Changes on protein and methionine content of black gram (*Vigna mungo* (L.) Hepper) induced by gamma rays and EMS. *American- European Journal of Scientific Research*, **4** (2):68-72.

Baird R. E. and Brock J.H., 1999. First Report of *Macrophomina phaseolina* on Cotton (*Gossypium hirsutum*) in Georgia. *Plant Disease*, **83** (5):487-487.

Bassanezi R.B., Amorin L., Bergamin Filho A., Hau B. and Berger R.D., 2001. Acounting for photosynthetic efficiency of bean leaves with Rust, Angular leaf spot and Anthracnose to assess crop damage. *Plant Pathology*, **50**:443-452.

Beattie G.A., and Lindow S.E .1995. The secret life of foliar bacterial pathogens on leaves. *Annual Review of Phytopathology*, **33**:145–172.

Brentrup F., Küsters J., Kuhlmann H. and Lammel J., 2001. Application of the Life Cycle Assessment methodology to agricultural production: an example of sugar

beet production with different forms of nitrogen fertilisers. *European Journal of Agronomy*, **14** (3): 221- 233.

Buruchara R., 2007. Background information on Common Beans (*Phaseolus vulgaris* L.) in Biotechnology, Breeding and Seed Systems for African Crops. http://www.africancrops.net/rockefeller/crops/beans/index.htm.

Buruchara R., Mukankasi C. and Ampofo K., 2010. Handbook on Bean Diseases and Pest Identification and Management, handbook four. CIAT. Uganda: pp24-27

Capoor S.P., Rao D.G. and Sawant D.M., 1986. Seed transmission of French bean mosaic virus. *Indian Phytopathology*, **39**:343– 345

Central High Plains Dry Bean and Beet Group., 2004. Dry bean and Pest Management.2nd Ed. USA: p13.

CIAT., 2004 Enhancing farmers' access to seed of improved bean varieties in Rwanda,
CIAT, pp. 2.

Costa L.L.F. and Scussel V.M., 2002. Toxigenic fungi in beans (*Phaseolus vulgaris* L.) classes black and color cultivated in the state of Santa Catarina, Brazil. *Brazilian Journal of Microbiology*, **33**:138-144

Dawson W.A.J.M. and Bateman G.L., 2001. Fungal communities on roots of wheat and barley and effects of seed treatments containing fluquinconazole applied to control take-all. *Plant Pathology*, **50**: 75-82.

FAO., 2006. Fertilizer use by crop in Zimbabwe. Rome, Italy. www.fao.org.docrep

FAO., 2011. Statistical database of the Food and Agriculture Organisation of the United Nations, Rome, Italy. http://faostat.fao.org

Finisia C., 2003. Relationship between Common bacterial blight and bean yield loss in pure stand and bean intercropping systems. *International journal of pest management*, **49***:*177-185.

Fourie D., 2002. Distribution and severity of bacterial diseases on dry beans *(Phaseolus vulgaris* L.) in South Africa. *Journal of Phytopathology*, **150** (4–5):220–226.

Frison E.A., Bos L, Hamilton R.I., Mathur S.B. and Taylor J.D editors., 1990. (eds) FAO/IBPGR Technical Guidelines for the Safe Movement of Legume Germplasm. Food and Agriculture Organization of the United Nations, Rome/International Board for Plant Genetic Resources, Rome.

Giga D.P., 1989. Constraints to Bean production in Zimbabwe. In: Proceedings: First Meeting of the Pan African Working Group on bean Entomology, Nairobi, Kenya, 6-9 August 1989. CIAT African Workshop Series **11**: pp21-25.

Gilbertson R.L. and Hagedorn D.J., 1990. Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. *Plant Disease*, 7**4**:322–327.

Gilbertson R.L. and Maxwell D.P., 1992. Common bacterial blight of bean. In: Chaube HS, Kumar J, Mukhopadhyay AN, Singh US (eds) Plant diseases of international importance, Vol II Prentice Hall, Englewood Cliffs, New Jersey: pp18–39.

Goodwin P.H. and Sopher C.R., 1994. Brown pigmentation of *Xanthomonas campestris* pv. *phaseoli* associated with homogentisic acid. *Canadian Journal of Microbiology*, **40**:28–34

Graham P.H. and Vance C.P., 2003. Legumes: Importance and Constraints to Greater Use. *Plant Physiology*, **131**: 872–877.

Gulya T.J., Krupinsky J., Draper M. and Charlet L.D., 2002. First Report of Charcoal Rot (*Macrophomina phaseolina*) on Sunflower in North and South Dakota. *Plant Disease*, **86** (8):923-923.

Hagedorn D.J. and Inglis D.A., 1986. Handbook of bean diseases.Madison.Winsconsin:p6-7.

Harveson R.M., 2009. Common bacterial blight of dry beans in Nebraska. http:// ianrpubs.unl.edu/epublic/live/g1956/build/g1956.pdf.

Helene D.R. and Legard D.E., 1991. Fact sheet on bacterial diseases of beans. Cooperative Extension. New York States. Geneva: pp 729, 50.

Hirano S.S., Upper C., 1983. Ecology and epidemiology of foliar bacterial plant pathogens. *Annual Review of Phytopathology*, **21**:243–269.

International Seed Testing Association (ISTA)., 1999. International Rules for seed testing – Rules 1999. *Seed Science and Technology*., **27**, Supplement rules. p333.

International Seed Testing Association (ISTA)., 2007. International Rules for Seed Testing. Proceedings of International Seed Testing Association. ISTA, 8303 Bassersdorf, Switzerland: p1-44.

Islam S.M.M., Masum M. M. I. and Fakir M. G. A., 2009. Prevalence of seed-borne fungi in sorghum of different locations of Bangladesh. *Scientific Research and Essay*, **4**(3):175-179.

.**Jesus W.C.Jr (de)., Vale F.X.R., Coelho R.R., Hau B., Zambolin L., Costa L.C. and Berghamin Filho A., 2001.** Effects of Angular Leaf Spot and Rust on Yield Loss of *Phaseolus Vulgaris*. *Phytopathology*, **91**:1045-1053.

Kaiser W.J. and Vakili N.G., 1978. Insect transmission of pathogenic *Xanthomonads* to bean and cowpea in Puerto Rico. *Phytopathology*, **68**:1057–1036.

Kambewa S.P.,1997. The bean subsector in Malawi. Historical developments, current status and policy issues. A Master of Science thesis, Department of Agricultural Economics, Michigan State University. USA.

Karadimos D.A., Karaoglanidis G.S. and Klonari K., 2002. First Report of Charcoal Rot of Sugar Beet Caused by *Macrophomina phaseolina* in Greece. *Plant Disease,* **86** (9):1051.

Karavina C., Mandumbu R., Parwada C. and Zivenge E., 2011. Epiphytic Survival of *Xanthomonas axonopodis* pv. *phaseoli* (E. F SM). *Journal of Animal and Plant Science*, **9**(2): 1161- 1168.

Karavina C., Tigere T.A. and Chihiya J., 2008. The contribution of soil and crop debris inocula to the outbreak of bacterial common blight in field beans (*Phaseolus vulgaris* L.) under Zimbabwean conditions. *Journal of Sustainable Development in Africa*, **10** (3): 221–233.

Khangura R. and Aberra M., 2009. First Report of Charcoal Rot on Canola Caused by *Macrophomina phaseolina* in Western Australia. *Plant Disease*, **93** (6):666,3-667.

Koike S.T., 2008. Crown Rot of Strawberry Caused by *Macrophomina phaseolina* in California. *Plant Disease*, **92** (8):1253.

Kutywayo V., 2000. Identification and survival of bean (*Phaseolus vulgaris* L.) pathogens, and the effect of fungicide seed dressing and cultural practices on the incidence of the diseases they cause, under smallholder conditions in Zimbabwe. MPhil thesis, University of Zimbabwe. Department of Crop Science: 157pp.

Lak M.R., Shamsbakhsh M. and Bahar M., 2002. Identification of the bacterial agent of bean leaf and pod blight in Markazi province. *Journal of Science and technology in Agriculture and natural Resources*, **6**:231–243.

Lee K.W., Lee B.C., Park H.C. and Lee Y.S., 1990. Occurrence of green mottle mosaic virus disease of watermelon in Korea. *Korean Journal of Plant Pathology*, **6**:250–255.

Legesse D., Kumussa G., Assefa T., Taha M., Gohena J., Alemaw T., Mohhamed Y. and Terefe H., 2006. Production and Marketing of white pea beans in the Rift valley, Ethiopia. A subsector analysis. National Bean Research Program of the Ethiopia Institute of Agricultural Research.

Leterme P. and Munoz C., 2002. Factors influencing pulse consumption in Latin America. *British journal of Nutrition 88.* Supplement, **3**: s251-s255.

Lindemann J. and Upper C.D .1985. Aerial dispersal of epiphytic bacteria over bean plants. *Applied Environmental Microbiology*, **50**(5): 1229–1232.

Mahuku G.S., Jara C., Cuasquer J.B. and Castellanos G. 2002. Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. *Plant Pathology*, **51**: 594-604.

Mortensen C.N., 2000. Seed health testing for bacterial pathogens. Danish Government Institute of Seed Pathology for Developing Countries (DGISP).Thorvaldsensvej 57, DK-1871 Frederiksberg. Copenhagen.

Msikita W., James B., Wilkinson H.T., Juba J.H., 1999. First Report of *Macrophomina phaseolina* Causing Pre-harvest Cassava Root Rot in Benin and Nigeria. *Plant Disease*, **82** (12):1402.

Ndegwa A.M., Muchui M.N., Wachiuri S.M. and Kimamira, J.N., 2006. Evaluation of snap bean varieties for adaptability and pod quality. In: Proceedings of the 10th KARI Biennial Conference. KARI HQs, Nairobi, Kenya. 13th-17th Nov. 2006.

Nietsche S., Borém A., Carvalho G.A., Paula Junior T.J., Ferreira C.F., Barros E.G. and Moreira M.A., 2001. Genetic diversity of *Phaeoisariopsis griseola* in the state of Minas Gerais, Brazil. *Euphytica*, **177**:77-84.

Opio A.F., Allen D.J. and Teri J.M., 1996. Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. *Plant Pathology*, **45**: 1126–1133.

Opio A.F., Teri J.M. and Allen D.J., 1994. Survival of *Xanthomonas campestris* pv. *phaseoli* in Uganda. African Crop Science Conference Proceedings Volume **1**: 225-259.

Osdaghi E., Shams-bakhsh M., Alizadeh A. and Lak M.R., 2010. Study on common bean seed lots for contamination with *Xanthomonas axonopodis* pv. *phaseoli* by BIO-PCR technique. *Journal of Agricultural Technology*, **6**(3):503–513.

Pastor-Corrales M.A. and Tu J.C., 1989. Anthracnose. 2nd. Ed. In: Schwartz H.F, Pastor-Corrales MA, editors. Bean production problems in the tropics. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia: pp77-104.

Pastor-Corrales M.A., Jara C. and Singh S.P., 1998. Pathogenic variation in, sources of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean. *Euphytica*, **103**:161-171.

Pernezny K. and Jones J.B., 2002. Common bacterial blight of snap bean in Florida. **Pratt R.G., McLaughlin M.R., Pederson G.A. and Rowe D.E., 1998.** Pathogenicity of *Macrophomina phaseolina* to mature plant tissues of alfalfa and white clover. *Plant Disease*, **82**:1033-1038.

Saettler A.W., 1989a. Common bacterial blight. In: Schwartz HF, Pastor-Corrales MA (eds) Bean production problems in the tropics. Centro Internacional de Agricultura Tropical, Cali, Colombia: pp261–283.

Saettler A.W., 1994. Angular leaf spot. In: Compendium of bean diseases. R. Hall (Ed.), APS Press, Minnesota: pp 15-16.

Saettler A.W., Schaad N.M. and Roth D.A., 1995. Detection of bacteria in seed and other planting material. APS Press, Minnesota: p121.

Schaad N.W., Vidaver A.K., Lacy G.H., Rudolph K. and Jones J.B., 2000. Evaluation of proposed amended names of several *Pseudomonads* and *Xanthomonads* and recommendations. *Phytopathology*, **90**:208–213.

Schwartz H.F. and Pastor-Corrales M.A., 1989. Bean production problems in the tropics. CIAT. Colombia: p235-236.

Schwartz H.F., 1989. Additional fungal pathogens. 2nd.Ed. In: Schwartz H.F., Pastor-Corrales M.A, editors. Bean production problems in the tropics. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia: p231-259.

Shetty H.S., 1992. Different types of damages in seeds caused by seed borne fungi. In: Mathur, S.B. and Jorgensen, J. (eds). Seed pathology. Proceedings of CTA Seminar held at Copenhagen, Denmark, on 20- 25 June 1988, Technical Centre for Agricultural And Rural Cooperation, Danish Government Institute of Seed pathology for developing countries: pp53-62.

Spilsbury J., Jagwe J.J. and Wanda K., 2004. Evaluating the Marketing Opportunities for beans and its products in the principle beans growing countries of ASARECA, draft regional report compiled by International Institute of Tropical Agriculture and Food net.

Tinivella F., Hirata L.M., Celan M.A., Wright S.A.I., Amein T., Schmitt A., Koch E., Wolf J.M., Groot S.P.C., Stephan D., Garibaldi A., and Gullino M.L.,

2009. Control of seed-borne pathogens on legumes by microbial and other alternative seed treatments. *European Journal of Plant Pathology*, **123**:139–151.

US Dry Bean Council., 2011. Bibliography - Beans and cardiovascular disease research (Online). Available from: http://www.usdrybeans.com/ nutrition/benefits/ (Accessed 5 December 2011).

Vauterin L., Rademaker J.L.W. and Swings J., 2000. Synopsis on the taxonomy of the genus *Xanthomonas*. *Phytopathology*, **90**:677–682.

Weber R. B., Hrynczuk B., Runowska H. and Kita B., 2001. Influence of the mode of tillage on diseases of culm base in some winter wheat varieties, oats, and spring wheat. *Journal of Phytopathology*, **149**: 185-188.

Whingwiri E.E., Mashingaidze K. and Rukuni M., 1992. Small-scale Agriculture in Zimbabwe, Field crop production. Rockwood publishers. Harare. Zimbabwe:p150-154.

Wilson M., Hirano S.S., Lindow S.E., 1999. Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf. *Applied Environmental Microbiology*, **65** (4):1435–1443.

Wohleb C.H. and duTolt J., 2011. Common bacterial and Halo blight: Two bacterial diseases of phytosanitary significance for bean crops, Washington State University fact sheet No.FSO38E.http://pubs.wsu.edu.

Wortmann C.S., Kirby R.A., Eledu C.A. and Allen D.J., 1998. Atlas of common bean production in Africa, CIAT publication No.297.

Yang Y., Kim K. and Anderson. E. J., 1997. Seed transmission of cucumber mosaic virus in spinach. *Phytopathology*, 87: 924–931.

Zadoks J.C. and Schein R.D., 1979. Epidemiology and plant diseases management. Oxford University Press, New York, USA: p427.

Zamani Z., Bahar M., Jacques M.A., Lak M.R. and Akhavan A., 2011. Genetic diversity of the common bacterial blight pathogen of bean, *Xanthomonas axonopodis* pv. *phaseoli*, in Iran revealed by rep-PCR and PCR–RFLP analyses. *World Journal of Microbiology* and *Biotechnology*, **27**:2371–2378.

APPENDICES

Appendix 1: Annual rainfall (mm) for the three agro-ecological regions for the past three years.

Natural region	Altitude(m.a.s.L	2011/12	2012/13	2013/14
I(Samanga area)	1302.5	1150	1005	970
IIa(Honzo area)	1050	1021	964	787
IIb(Tsonzo area)	1415	682.1	508.1	953.6

Appendix 2: Survey questionnaire

District……
Ward…… Area……
Nat. Region……..

Village……. Altitude (m)……

Rainfall (mm/year)…….

Name of Interviewing officer……..

Date of interview…….

Farming sector…….

Farmer's Name……

1.Which bean varieties (landraces) are commonly grown by the farmer?(Rank according to quantities)

i……

ii……

iii……..

iv……

v…….

vi……

2. Where does the farmer get his/her seed?

i. Retained/Yemudura[ ] ii. Retail shops (Farm and City etc)[ ]

iii. Agric. Shows [ ] iv. Farmer exchanges [ ]
v.Other……..

3.When does the farmer normally grow the crop?

i.Summer[ ] ii.Early winter(February-March)[ ]

iii. Early summer (Aug-Sept)[ ]

4.0 What are the most problematic diseases in the area?

i……
ii…….
iii……...

iv…….
v……..

4.1. What control measures are being employed to reduce disease load?

a. Cultural methods

i. Crop rotation (>2years. [ ] ii. Destruction of crop residues [ ]

iii. Use of certified seed [ ] iv. Seed treatment [ ]

4.2. If crop rotation is practised, which crop(s) is rotated with the bean?

i. Maize [ ] ii. Yams [ ] iii. Cowpeas [ ] iv. Sweet potato [ ]

v. Wheat [ ]

4.3. If the farmer treats the seed before planting, which method is being used?

a. Hot water [ ] b. chemicals [ ] c. Other (specify) ……

5.0 What is the method of crop production being used by the farmer?

i. Dry land [ ] ii) Irrigated [ ]

5.1. If the crop is irrigated, what is the irrigation system in use?

i. Flood [ ] ii. Overhead/Sprinkler [ ] iii. Drip/trickle [ ]

6. What cropping system does the farmer use?

i. Intercropped with maize [ ] ii. Grown as a sole crop [ ]

iii. Mixed cropping with maize [ ]

7.What is the major crops grown by the farmer?(Rank them according to hectares)

i……
ii……

iii……..
iv……..

v……..

Appendix 3: Disease score scale for Angular leaf spot and Bacterial blight on infected leaves.

Score	Description of the score
1=	No visible symptoms of the disease.
3=	less than 2% of leaf area affected by the lesions.
5=	5% of leaf area affected by the lesions.
7=	10% of leaf area affected by the lesions
9=	More than 25% of leaf area affected by the lesions.

Appendix 4: Anova Tables

1. *Colectotrichum lindemuthianum*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	7	1.38862	0.19837	3.22	0.003
Residual	232	14.28300	0.06156		
Total	239	15.67163			

2. *Fusarium moniliforme*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	2358.773	336.968	97.05	<.001
Residual	232	805.525	3.472		
Total	239	3164.298			

3. *Botryodiplodia theobromae*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	7	41.8953	5.9850	21.23	<.001
Residual	232	65.3897	0.2819		
Total	239	107.2850			

4. *Aspergillus niger*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	7	8.4067	1.2010	3.67	<.001
Residual	232	75.9473	0.3274		
Total	239	84.3540			

5. *Cladosporium* sp.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	7	1810.093	258.585	152.92	<.001
Residual	232	392.307	1.691		
Total	239	2202.400			

6. *Rhizopus* sp.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	7	219.312	31.330	10.93	<.001
Residual	232	665.213	2.867		
Total	239	884.525			

7. *Aspergillus flavus*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	7	1810.093	258.585	152.92	<.001
Residual	232	392.307	1.691		
Total	239	2202.400			

8. *Xanthomonas axonopodis*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	6	903.44767	150.57461	1686.84	<.001
Residual	7	0.62485	0.08926		
Total	13	904.07252			

9. ISTA Germination test

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	6	170.815	28.469	11.88	<.001
Residual	21	50.309	2.396		
Total	27	221.125			

10. Angular leaf spot tolerance

a) Detached leaf method

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	6	108.9524	18.1587	31.78	<.001
Residual	14	8.0000	0.5714		
Total	20	116.9524			

b) Spraying method

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
landrace	6	146.6667	24.4444	64.17	<.001
Residual	14	5.3333	0.3810		
Total	20	152.0000			

c) Field method

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	6	10.667	1.778	1.04	0.442
Residual	14	24.000	1.714		
Total	20	34.667			

11. Tolerance to bacterial blight-Field assessment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	6	27.810	4.635	2.21	0.104
Residual	14	29.333	2.095		
Total	20	57.143			