SCREENING OF SELECTED BAMBARA NUT (*Vigna subterranea* (L.) Verdc) LANDRACES FOR TOLERANCE TO *FUSARIUM* WILT AND ITS MANAGEMENT USING FARMYARD MANURE IN BUSIA COUNTY, WESTERN KENYA

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the award of Master of Science degree in Plant Pathology of Egerton University

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been previously presented in this University or any other institution for the award of any degree.

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DEDICATION

This thesis is dedicated to my mother Margaret Nanjekho

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ABSTRACT

Bambara nut (*Vigna subterranea* (L.) Verdc.) is an indigenous legume crop in Kenya. *Fusarium oxysporum* f.sp *voandzeia* is a destructive fungal pathogen affecting Bambara nut in Kenya. An experiment was carried out in the green house and field to determine the incidence and severity of *Fusarium* wilt on local landraces and evaluate its management using goat farm yard manure (FYM). The field experiment was carried out in Busia County, where four villages (Bufisi, Bukati, Madola and Butunyi) were used to determine *Fusarium* wilt distribution in farmers' fields. A completely randomized design (CRD) experiment was conducted in the greenhouse at Egerton University to determine disease incidence and severity. The landraces used included black, red, maroon, maroon speckled, brown light eyed and brown dark eyed. The *Fusarium* wilt management experiment laid out in a completely randomized bock design (CRBD) was conducted in the greenhouse and in the field in Busia County. The black and red landraces of bambara nut were used in the study. The data were subjected to analysis of variance using statistical analysis system (SAS) software version 9 and treatment means separated using least significant different test (LSD). *Fusarium* wilt incidence ranged from 14.63 to 43.56% and varied with village. Bufisi, Butunyi and Bukati had the highest disease incidences while Madola had the lowest disease incidence. Incidence and severity of *Fusarium* wilt were significantly different and varied with landrace. There was no tolerant landrace. The maroon speckled and brown dark eyed landraces had the highest disease incidence of 80.5% and 80.0%. The brown light eyed, maroon, black and red landraces had incidences of 79.5%, 78.9%, 78.6% and 76.2% respectively. The maroon and brown dark eyed landraces had the highest disease severity of 45.5% and 44.3% respectively. The red, maroon speckled, black and brown light eyed landraces had severities of 43.5%, 43.1%, 42.8% and 42.6% respectively. The area under disease progress curve (AUDPC) varied with landrace i.e. maroon and brown dark eyed landraces had the highest while the brown light eyed landrace had the least AUDPC. In the greenhouse, FYM reduced the disease incidence and severity by 10.2% and 9.5% for the black landrace compared to 1.9% and 12.8% for the red landrace. In the field, FYM reduced disease incidence and severity by 9.1% and 6.9% for the black landrace compared to 10.4% and 10.4% for the red landrace. FYM had the lowest AUDPC irrespective of the landrace. The study confirmed the virulence of the pathogen on Bambara nut and the ability to manage the disease using FYM for improved yield performances.

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ABBREVIATIONS AND ACRONYMS

AUDPC	Area Under Disease Progress Curve
CRD	Completely Randomized Design
CRBD	Completely Randomized Block Design
DSI	Disease Severity Index
FYM	Farm Yard Manure
GIS	Geographic Information System
ILRI	International Livestock Research Institute
IFAD	International Fund for Agricultural Development
KALRO	Kenya Agricultural Livestock and Research Organization
LSD	Least Significant Difference
PDA	Potato Dextrose Agar
PDI	Percentage Disease Incidence
PROTA	Plant Resources of Tropical Africa
SAS	Statistical Analysis System
SFM	Soil Fertility Management

CHAPTER ONE

INTRODUCTION

1.1 Background information

Agriculture in Africa is highly underdeveloped with huge tracts of land remaining unutilized. The continent occupies 25% of the world's arable land generating only 10% of the global agriculture output (Jayaram *et al.,* 2010). In Kenya only about 20% of the landmass is classified as medium to high potential for agriculture. The high population estimated at 38.9 million people, has caused a big food deficit with approximately 46% of the population being categorized as food poor (World Bank, 2013). In order to achieve Sustainable Development Goal Number two (ending all forms of hunger and malnutrition by 2030), food production per unit area has to be increased to satisfy the growing population. Western Kenya generally classified as medium to high potential agricultural land (Jaetzold *et al.,* 2006) is also one of the areas with the highest population density of about 500-1200 persons/km^2 (Amadalo *et al.,* 2003), and hence food deficit. Bambara nut, *Vigna subterranea* (L.) Verdc. is one of the indigenous legume crops grown in sub Saharan Africa. The crop ranks third among grain legumes after ground nuts (*Arachis hypogaea* L.) and cowpeas (*Vigna unguiculata* L.) in most parts of Africa (Mkandawire, 2007).

The crop is highly nutritious (containing 49-63.5% carbohydrates, 15-25% protein, 4.5-7.4 lipids and 5.2-6.4% fibre), drought tolerant and able to produce higher yield than other legumes such as groundnuts and common beans grown under the same conditions (Masindeni, 2006). Bambara nut is used as a major component of food products such as legume milk, weaning food, beans pudding and bread baking (Poulter and Caygill, 2006). In addition, because of the high nutrition level, the crop contributes to food and nutrition security as well as improved dietary diversity and health of rural communities. Bambara is used in the low input agricultural production systems because of its ability to fix atmospheric nitrogen into the soil with the aid of *Bradyrhizobium* bacteria (Mwale *et al.,* 2007).

Furthermore, Bambara nut consumers have reported several medicinal values, such as using leaf sap to treat infected wounds and epilepsy, roots acting as aphrodisiac while pounded seeds treat eye cataract (Directorate of Plant Production, 2011). The crop is also a cheap but superior source of vitamin B (Basu *et al.,* 2007). Genotypes containing relatively higher levels of soluble fibres and tannins are thought to reduce incidences of heart disease, colon cancer and diarrhoea (Directorate of Plant Production, 2011).

A combined solution of Bambara nut leaf sap together with that of *Lantana trifolia* L. is used as an acaricide as well as a bio-cide (Bamshaiye *et al*., 2011). In Kenya, Bambara nut is mainly grown at the Coast, Eastern, Western and Nyanza regions (Wasula *et al.,* 2012). The production of this crop in Kenya has been low approximately 64kg/ha compared to a potential of 850kg/ha (Wasula, 2014). The ongoing changes in climate favour Bambara nut production because it can withstand harsh and fluctuating weather conditions and therefore will be important in resolving malnutrition, poverty and food security problems (Naylor *et al.,* 2004). Although previously regarded as a neglected underutilized species (NUS) in Kenya, the demand for Bambara nut is increasing because of its nutritional benefits and adaptation to the local conditions. In Central, West and South Africa, efforts to improve Bambara nut production have mainly targeted selection, characterization and nutritional analysis of landraces (Abu-Salam and Abou-Arab, 2011). In Kenya Odongo *et al*. (2015) genetically characterized the available landraces.

Biotic constraints are known to limit the full exploitation of the yield potential of crops. Several fungal diseases have been reported to attack Bambara nut (Brink and Belay, 2006). Soilborne fungal diseases including *Fusarium* wilt are a major challenge to legume production in Kenya (Medvecky *et al.,* 2007). *Fusarium oxysporum* the causative agent of *Fusarium* wilt is a common soilborne pathogen and saprophyte that feeds on dead and decaying organic matter. It survives in soil debris as mycelia and is commonly recovered from cultivated soil as chlamydospores (Maina *et al.,* 2015). *Fusarium oxysporum* may be found in many places and environments, but development of the disease is favoured mainly by high temperatures and warm moist soils. The optimum temperature for growth on artificial media is between 25-30°C and the optimum soil temperature for root infection is 30°C.

Infection through the seed can however occur at temperatures as low as 14°C (Pande *et al*., 2007). *Fusarium oxysporum* attacks Bambara nut at all stages of development. The propagules gain entry into the plant through cut seed surfaces, damaged roots and stem tissues of young and stressed plants, infected seeds and through wounds caused by insects (Leslie *et al.,* 2006). *Fusarium oxysporum* infestation on Bambara nut causes yield losses ranging from trace to total crop failure especially when adverse environmental conditions persist after planting through flowering (Cook, 1978).

Recommended strategies for control of *Fusarium* wilt include use of plant host resistance, crop rotation and selected fungicides such as carbendazin and prochloraz (Ajiloqba and Babalola, 2013). As an adaptation strategy, farmers grow the crop mainly during the short rain season when conditions for fungal sporulation and survival are low. Management of *Fusarium* wilt using resistant varieties has been achieved in many legumes such as chickpea (Chaudhry *et al*., 2006). Selection of Bambara nut genotypes for wilt tolerance has rarely been achieved. Use of synthetic fungicides for wilt management is too costly for most smallholder farmers. In addition, large scale use of chemical treatment contributes to environmental contamination and exposes farmers to health hazards related to handling chemical pesticides (Schwartz *et al.*, 2007). Occurrence of fungicide resistance in pathogens and breakdown of host resistance by pathogen populations are some of the reasons undermining efforts to develop and adopt new disease control measures (McDonald and Linde, 2002; Castan˜o *et al.*, 2013).

Cultural practices that can be used to manage the disease include crop rotation, field sanitation, proper crop spacing, improved water drainage, and soil nutrient availability through incorporation of organic and inorganic amendments among others (Chandel and Deepika, 2010). Farmyard manure is recommended for use in agricultural systems because of its benefits that include improving soil fertility and soil health. Aspects of soil health include reduction of disease incidences caused by a number of soil borne pathogens such as *Fusarium* and *Pythium* species (Diab *et al.*, 2003; Noble and Coventry, 2005). The study therefore aimed at testing available local landraces for wilt tolerance and enhancing Bambara nut production in Busia County Western Kenya through management of *Fusarium* wilt using farmyard manure application.

1.2 Statement of the problem

Food insecurity is a major problem to most people especially those living in the rural areas in Kenya. The increasing population and declining land area per capita implies that the problem of food deficit will continue. Bambara nut has the potential of improving human nutrition and ecosystem health. The demand for the crop is increasing because of the emerging medicinal and nutritional uses coupled with the current climate change. Efforts to improve Bambara nut production in Kenya have mainly targeted collection and cataloguing of the available land races. Biotic constraints however limit full exploitation of the yield potential in Bambara nuts. *Fusarium* wilt, a soil borne fungal disease contributes to immense yield losses in many legumes including Bambara nut. Use of pesticides in the control of *Fusarium* wilt is not feasible in small scale farming systems because they are expensive and contribute to environmental degradation. Crop tolerance and use of organic fertilizers are generally known to contribute to improved crop protection. Limited work has however been done to evaluate *Fusarium* wilt tolerance level among different landraces and the management of the disease using FYM at farm level.

1.3 Objectives

1.3.1 General objective

To contribute to sustainable Bambara nut production in Western Kenya through screening of selected landraces for tolerance to *Fusarium* wilt and its management using farmyard manure.

1.3.2 Specific objectives

1. To determine the distribution of *Fusarium* wilt in selected Bambara nut farmers' fields in Busia County Western Kenya.
2. To determine the incidence and severity of *Fusarium* wilt on selected Bambara nut land races.
3. To determine the effect of farmyard manure on the incidence and severity of *Fusarium* wilt on selected susceptible Bambara nut landraces.

1.4 Hypotheses

1. There is no significant difference in the distribution of *Fusarium* wilt in selected Bambara nut fields in Busia County Western Kenya.
2. There is no significant difference in the incidence and severity of *Fusarium* wilt on selected Bambara nut landraces.
3. Farmyard manure does not significantly affect the incidence and severity of *Fusarium* wilt of Bambara nut.

1.5 Justification

Bambara nut is one of the indigenous crops that is highly nutritious and has been used as a major component in various food products improving human health. The crop also improves soil fertility by fixing atmospheric nitrogen with the aid of *Bradyrhizobium* bacteria. Bambara nut is thus an important crop for reducing malnutrition, poverty and food insecurity problems in rural areas of Kenya. As one of the indigenous crops, Bambara nut production has remained low with its potential value being underexploited placing it in danger of genetic erosion and disappearance. This further restricts its development options. Its genetic erosion can have severe consequences on nutritional status and food security of the resource poor farmers and consumers. Biotic constraints are among the factors that contribute to low yields of this crop. Losses resulting from *Fusarium* wilt have been quantified on other legumes and management options exploited but not in Bambara nut.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bambara nut production and use

Bambara nut (*Vigna subterranea* (L.) Verdc) is an indigenous crop grown in most parts of sub Saharan Africa (Brink and Belay, 2006; Okwonko and Opara, 2010). A global mapping activity initiated by FAO in 2001 to identify areas suitable for Bambara nut production revealed a huge production potential for the crop in the warmer climate zones of the earth (Azam-Ali *et al*, 2001). Africa has an annual production of about 330,000 metric tons of which Bukina Faso, Chad, Corte d' Ivore, Ghana, Mali, Niger and Nigeria contributes 45-50% (PROTA, 2006). In Kenya, Bambara nut is grown by smallholder farmers at the Coast, Eastern, Western and Nyanza regions (Wasula *et al.,* 2012). Bambara nut has the potential to improve nutrition and boost food availability because it is highly nutritious i.e. the seed contains about 49-63.5% carbohydrates, 15-25% protein, 4.5-7.4% lipids and 5.2-6.4% fiber (Abdulsalami and Sheriff, 2010; Okwonko and Opara, 2010; Bamishaiye *et al.,* 2011; Mazahib *et al.,* 2013). The crop is thus a major component of food products such as legume milk, weaning food, beans pudding and bread baking because of its nutrient richness (Poulter and Caygill, 2006). The seed is preferred for use in balancing nutrient deficiencies in sorghum and maize diets because of its high carbohydrate, protein and fat content as well as presence of vitamin B (Masindeni, 2006; Basu *et al.,* 2007).

Consumers of bambara nuts have also reported medicinal values. For example, the leaf sap is used to treat infected wounds and epilepsy, roots act as aphrodisiac while pounded seeds treat eye cataract (Basu *et al.,* 2007). Genotypes containing relatively high levels of soluble fibres and tannins are thought to reduce incidences of heart disease, colon cancer and diarrhoea (Directorate of Plant Production, 2011). In Western Kenya, a combined solution of bambara nut leaf sap and *Lantana trifolia* L. is used as an acaricide as well as a vegetable insecticide (Bamshaiye *et al*., 2011). Bambara nut is a drought tolerant crop with ability to withstand high temperatures and therefore fit for marginal soils where other leguminous crops cannot grow. It is also of great use in low input agricultural production systems because of its ability to fix atmospheric nitrogen into the soil with the aid of *Bradyrhizobium* bacteria (Masindeni, 2006; Mwale *et al.,* 2007).

Thus bambara nut has potential to contribute to sustainable food production and food security among smallholder farmers.

2.2 *Fusarium* wilt of bambara nut (*F. oxysporum* fsp. *voandzeia*)

2.2.1 Biology and distribution of *Fusarium* genus

Fusarium is a genus of filamentous fungi classified in the Order Hypocreales of the class Ascomycetes. It has over 50 species with cosmopolitan distribution in the air, soil and in association with many other plants (Leslie *et al.,* 2006). Most of the *Fusarium* species are plant-pathogenic, causing diseases in several agriculturally important crops. Some *Fusarium* species produce mycotoxins whose biological activity can be detrimental to plants, and are also associated with cancer and other diseases in humans and domesticated animals (Lukasz *et al.,* 2013). The effect of *Fusarium* species on crops in the field results into contamination of cereal grains and other plant-based foods (Moretti, 2009). Plant infections by *Fusarium* occurs at all developmental stages, from germinating seeds to mature vegetative tissues, depending on the host plant. Out of 101 most economically important plants, 81 have at least one plant associated with *Fusarium* disease (Moretti, 2009). Studies on *Fusarium* prevalence in Kenyan soils indicate that it is a highly distributed and diverse genus (Maina *et al.,* 2009) causing a variety of plant diseases, that include vascular wilts, cankers, rots of seed, fruit, root and stem, and blights.

F*usarium* chlamydospores, conidia and hyphae are distributed widely in cultivated soil and soil debris (Maina *et al.,* 2015). The propagules gain entry into the plant through cut surfaces of seeds, damaged roots and stem tissues of young and stressed plants. Infection through wounds caused by insects can also act as entry points (Leslie *et al.,* 2006). *Fusarium* species are able to survive in the soil for long periods of time as chlamydospores (Vakalounakis and Chalkias, 2004). These are resting spores produced in the soil during periods of unfavourable conditions (Leslie *et al.,* 2006). Many *Fusarium* species also exist as harmless saprophytes in the soil while others establish long-term associations with crop plants as endophytes (Vakalounakis and Chalkias, 2004)

2.2.2 Lifecycle of *Fusarium oxysporum* fsp. *voandzeia)*

Fusarium oxysporum Schl. is a ubiquitous soil-borne fungus that includes pathogenic and non-pathogenic members. The pathogenic members are best known for causing *Fusarium* wilt diseases of many economically important agricultural crops (Maina *et al.,* 2015). Bambara nut is affected by a special form of the fungus known as *F. oxysporum* f.sp. *voandzeia.* In Kenya, *Fusarium* wilt of bambara nuts has been reported in almost all the bambara nut growing regions (Cook, 1978; Masindeni, 2006). The species produces three types of asexual spores: microconidia, macroconidia and chlamydospores. The microconidia are the most abundant spores, crescent in shape and are produced on aerial mycelia. Macroconidia, have three to five cells with gradually pointed or curved edges. Chlamydospores are survival spores produced either singly or in pairs and can be clustered or in short chains that are either intercalary or terminally placed (Agrios, 2005). *Fusarium oxysporum* survives in the soil debris as a mycelium and is most commonly recovered from the soil as chlamydospores (Maina *et al.,* 2015). *Fusarium oxysporum* may be found in many places and environments but development of the disease is favoured by high temperatures and warm moist soils.

The optimum temperature for root infection and growth on artificial media is between 25-30°C, while infection through the seed is as low as 14°C (Pande *et al*., 2007). The pathogen usually infects the host by means of mycelia or by germinating spores penetrating the plant's root tips, root wounds, or lateral roots. The mycelium then advances intracellularly through the root cortex and into the xylem, where it remains exclusively producing microconidia (asexual spores). The microconidia enter into the sap stream and are transported upward. The spores and mycelia eventually clog the vascular vessels, preventing the uptake and translocation of water and nutrients, leading to wilt and plant death. The fungus invades all tissues, sporulates, and continues to infect neighbouring plants (Summerell *et al*., 2010; Maina *et al.*, 2015). Wilt and plant death is thus a result of a combination of pathogen activities that include accumulation of fungal mycelium in the xylem and/or toxin production, host defence responses, including production of gels, gums and tyloses, and vessel crushing by proliferation of adjacent parenchyma cells (Groenewald, 2006).

Plants may appear stunted and yellowed exhibiting premature leaf drop and poor pod fill. Initial underground symptoms appear as reddish-brown streaks (lesions) on the hypocotyl and primary roots, two to three weeks after emergence (Zemouli-Benfreha *et al*., 2014). Diseased areas of the plant enlarge with age and gradually turn brown. Symptoms may extend up the main root and hypocotyl to the soil surface with longitudinal cracks developing in older lesions. Severely infected primary and secondary roots are normally killed, but persist on plants as dried remnants (Leslie and Summerell, 2006; Umar *et al.*, 2013).

2.3 Management of *Fusarium* wilt

Diseases are major contributors to yield losses in crop production. Appropriate management strategies that are sustainable and safe to the environment and consumers are therefore necessary. The main goal of plant disease management is to reduce the economic damage caused by plant diseases on crops. The principles of disease management often aim at reducing the disease pressure to levels too low to cause economic injury (Maloy, 2005). The general principles of disease management include avoidance, exclusion, eradication, protection, therapy and immunization all of which can be achieved using different methods (William, 2012). *Fusarium* wilt management can therefore be achieved through various chemical, biological, cultural and use of host plant resistance methods.

2.3.1 Chemical control

Synthetic pesticides have been used to manage pests and diseases for a long time (Unsworth, 2010). Application of fungicides alters the structure and function of soil microbial communities by lysis of membranes and interference with the biosynthesis of amino acids and proteins and inhibition of spore germination, germ tube elongation and mycelium growth (Madhuri and Rangaswamy, 2003). Some fungicides lead to low growth rate, abnormality and destruction of the process of cell division (Yang *et al.*, 2011). Thus different fungicides and soil fumigants can be used to control soil borne plant pathogens. Amini and Sidovich (2010) found Prochloraz, bromuconazole, benomyl and carbendazim fungicides, to be efficient in controlling *Fusarium* wilt of tomatoes. These fungicides kill *Fusarium* by damaging the cell membranes and interfering with key metabolic processes such as respiration.

Rajput *et al.* (2006) noted carbendazim, thiophanate methyl and dithane to be effective in reducing mycelial growth of *Fusarium* wilt in cotton. Mao *et al*. (2014) found combined application of chloropicrin and dimethyl disulfide fumigants to have a positive synergistic activity against the pathogen. A mixture of metamidoxime and copper oxychloride has been found to be effective against *F. oxysporum* f. sp. *lycopersici in vitro*. Thiram and Topsin-M are also effective in reducing populations of *F. oxysporum* f. sp. *lycopersici* (Amini and Sidovich, 2010).

In as much as these chemicals offer significant reduction in *Fusarium* wilt incidences of various crops their toxicological effects outweigh their benefits in agriculture (Das *et al.,* 2010). For instance, a group of benzimidazole carbamates (benomyl and carbendazim) which are systemic in action have been reported to have carcinogenic effects on mammals after a long term exposure (Amini and Sidovich, 2010). Most small scale farmers are also poor and cannot afford the chemicals and their use on large scale could contribute to environmental pollution (Schwartz *et al.,* 2007). Occurrence of fungicide resistance in pathogens and breakdown of host resistance by pathogen populations are some of the reasons underlying efforts to develop new disease control measures (McDonald and Linde, 2002; Castan˜o *et al.*, 2013).

2.3.2 Biological control

Biological control is a component of an integrated pest management strategy that aims at reducing or eradicating pathogen populations by use of natural enemies such as microbial antagonists (Pal and Gardener, 2006). Microorganisms protect the plant from fungal attacks by production of antifungal metabolites and competition with the pathogen for nutrients or niche exclusion, parasitism, lysis of the pathogen or induction of plant resistance mechanisms (Leelavathi *et al*., 2014). Fungal antagonists especially *Trichoderma* species have proved to be potential biocontrol agents of *Fusarium* wilt of potatoes (Akrami *et al.,* 2011; Ommati *et al*., 2013). *Fusarium* wilt of lentils has also been successfully controlled using *Bacillus pumilus, Pseudomonas alcaligens* and *Rhizobium* species (Bapat and Shah, 2000; Akhtar *et al.,* 2010). Many yeast species have been used in the biocontrol of fungal pathogens by producing cell wall degrading enzymes and antifungal diffusible metabolites (Yonggang *et al*., 2013).

Use of botanicals in integrated disease management systems is an effective biocontrol strategy and of great relevance in the organic farming systems (Gurjar *et al.*, 2012), because it is eco-friendly. Extracts isolated from several plants have been reported to have biological effects such as antioxidant activities against *Fusarium* wilt (Riaz *et al.*, 2008). Neela *et al*. (2014) also noted the antifungal activity of ethanol extracts of *Piper betel, Lowsonia inermis, Psidium guajava, Carica papaya and Moringa oleifera* plants against *Fusarium* wilt in tomatoes. Time of application of biological control agents should however coincide with the period of host susceptibility. The extracts should persist as long as the plant remains susceptible for them to be effective in controlling the pathogen. Insufficient survival of the antagonists may lead to inadequate or partial control of the pathogen (Satish *et al.,* 2009; Rongai *et al.,* 2012). Introduction of a food base such as compost, which supports the activity of antagonists but does not stimulate the activity of the pathogen, also improves the efficiency of biocontrol agents (Otsyula *et al*., 1998).

2.3.3 Use of host plant resistance

Host plant resistance is the use of in- built mechanism of the host plant to resist various activities of the pathogen (Staskawcz, 2001). The host plant may possess genes that enable it to avoid, tolerate or recover from pathogen attacks under conditions that may cause more injury to other plants within the same species (Thakur, 2007). The genetic makeup of the plant can also be improved to either resist or tolerate the pathogen effects. Specific genes for tolerance obtained from wild relatives or other crops can be introduced into the plant (Hajjar and Hodgkin, 2007). Effective screening for disease resistance requires accurate simulation of natural environmental conditions where plants are exposed to the inoculum. Optimum inoculation and incubation conditions should be established so that susceptible and resistant genotypes can be easily differentiated (Infantino *et al*., 2006; Swarupa *et al.,* 2014).

Currently there are no bambara nut varieties that have been selected for resistance to specific soil borne fungal disease. In chickpea, resistance to *Fusarium* wilt is governed by major resistance genes (Sharma *et al*., 2005). Odeny *et al*. (2009) reported presence of *Fusarium* wilt resistance genes in some varieties of African and Indian pigeon peas. Chaudhary *et al.* (2008) similarly reported presence of *Fusarium* wilt resistance genes in several varieties of lentils.

Evaluation and selection of superior genotypes using various scientific techniques for utilization in breeding for *Fusarium* wilt resistance in bambara nuts is important.

2.3.4 Cultural management

Cultural management involves management of the crop in the field using farm practices such as field sanitation, crop rotation, use of clean and certified seed, proper spacing, good choice of planting site and date and proper use of fertilizers for optimum growth and yield of crops (Katan, 2010). For example, bambara nuts require little rains (500-600mm annually) for optimal production hence the best planting time is during the short rain season when pathogen sporulation levels are low. Proper spacing also leads to optimal microclimate that reduces pathogen sporulation. Planting bambara nuts on raised beds has been shown to be effective in reducing incidences of wilts in the field (Directorate of Plant Production, 2011). The use of fertilizers has proven not only to be of nutritional importance but also able to suppress soil borne diseases. This is because the ability of the plant to express its genetic potential for disease resistance can be improved by mineral nutrition (Dordas, 2008).

i) Organic fertilizers

Organic fertilizers are soil amendments obtained from vegetable and animal matter (Linquist *et al.,* 2007). Introduction of synthetic inorganic fertilizers and fungicides has led to reduced organic input, decreased soil organic matter, declining soil fertility and an increase in the population of soil borne pathogens in the agro-ecosystems (Bailey and Lazarovits, 2003). There is however a renewed interest in the application of organic matter such as farmyard manure to the soil for the control of soil borne pathogens. This is due to an increasing concern about adverse effects of soil fumigants and fungicides on the environment and the need for healthy agro-ecosystem (Lazarovits, 2001). The use of farmyard manure has been proposed for conventional and biological systems of agriculture to improve soil structure and fertility as well as decrease disease incidence caused by soil borne pathogens (Noble and Coventry, 2005). Organic amendments can be effective in controlling diseases caused by some soil borne fungal pathogens.

The amendments alter physical, chemical and biological properties of the soil that directly or indirectly affect pathogen survival and crop infection (Bunemann *et al*., 2006). Farmyard manure serves as a source of energy for microorganisms accelerating their microbial activity and overall biomass. Some of these microbes are antagonists that deter pathogens and therefore serve as prey as well as niche and nutrient competitors (Noble and Coventry, 2005; Bonilla *et al.,* 2012). Microbe-microbe interactions such as competition, antibiosis, parasitism and predation are often the underlying mechanisms of disease control in organic systems. Application of nitrogen-rich manure amendments may reduce soil-borne diseases by releasing allelochemicals generated during microbial decomposition (Suarez-Estrella *et al.,* 2007). High concentrations of volatile fatty acids in manure may also inhibit plant pathogens (Pérez-Piqueres *et al*., 2006; Shen *et al.,* 2015). Other potential disease-suppressing mechanisms include altered environmental conditions in the root zone, such as pH, electrical conductivity, porosity, water-holding capacity and nutrient concentrations, which directly or indirectly affect plant health (Millner *et al*., 2004; Mathur *et al.,* 2006).

Some of the pathogens that are controlled by organic fertilizers include, *Fusarium* spp, *Phytophthora* spp, *Pythium* spp, *Rhizoctonia solani* Kuhn, *Scleroctinia* spp and *Sclerotium* spp (Diab *et al.,* 2003). The general effect of organic manure for control of some pathogens is known, but conflicting results have been reported because of the variation in pathogen species such as the level at which the mechanism is switched on in different hosts. This is critical especially under field conditions where the soil conditions vary (Termorshuizen *et al.,* 2007; Bonilla *et al.,* 2012). Despite its wide use as a source of nutrient by small scale farmers, farmyard manure has not been exploited for its potential as a soil borne disease management strategy. Effectiveness of farmyard manure against plant diseases caused by a broad range of pathogens, including bacteria, fungi and nematode species, has been demonstrated in numerous studies and on different crops (Noble and Coventry 2005; Bonanomi *et al*., 2010; Avile´s *et al*., 2011). Suppressive levels have been found to vary depending on the kind of manure used and the crop (Borrero *et al.,* 2006; Castan˜o *et al*., 2011).

ii)Use of Inorganic fertilizers

Inorganic fertilizers are chemically produced and contain main mineral based nutrients (nitrogen, phosphorus and potassium) manufactured for immediate application on the crop (Ibrahim *et al.,* 2013). Unlike organic fertilizers they do not need to undergo decomposition to supply nutrients to the plant. Excessive and inappropriate application of inorganic fertilizers however has negative effects to the environment and consumers (Morris *et al.,* 2007). Some inorganic fertilizers increase crop infestation by soil pathogens through supply of nutrients (Nakhro and Dkhar, 2010). Inorganic fertilizers especially nitrogen, phosphorus and potassium based fertilizers not only serve to maintain or improve crop yield, but their application also directly or indirectly induces changes in soil chemical, physical and biological properties (Ibrahim *et al.,* 2013). Some of the inorganic fertilizers lower the soil pH especially the acid forming nitrogen fertilizers (Ibrahim *et al.,* 2011). Others lead to increase in disease tolerance by facilitating the development of thicker cuticle and cell walls while others lead to formation of more schlerenchyma tissues causing difficulties in the penetration of pathogens into the plant tissues (Zhong and Cai, 2007).

Application of chemical fertilizers has been shown to have a direct effect on the composition of the soil microbial community in plant monoculture and fallow soils. For example, toxicity of ammonia ion released during degradation of urea exerts an adverse effect on soil borne pathogens (Irshad *et al.,* 2006). Frutan and urea fertilizers have been effective in the control of *Macrophomina phaseolina* infection in mung bean. Inorganic fertilizer composed of nitrogen, phosphorus and potassium has been reported to suppress *Rhizoctonia solani* Kuhn (Zhong and Cai, 2007). Available information about the long-term influence of inorganic fertilizers on soil microbial biomass and microbial diversity is however inconsistent. Integration of inorganic fertilizer with organic manure will not only sustain the crop production but also be effective in improving soil health and enhancing the nutrient use efficiency (Verma *et al*., 2005; Avile´s *et al*., 2011).

2.3.5 Integrated Disease Management

Integrated disease management is one of the ecology based method which aims at minimizing damages caused by diseases through combining the use of all available disease management measures in a sequence or simultaneously by actions taken before and after planting the crop (Jimenez-Dias and Jimenez-Gasco, 2011). Integrated disease management is one of the effective disease control approach which combats environmental degradation and increases agricultural productivity especially in developing countries (Waiganjo *et al.,* 2006). Such integrated practices include use of high residue tillage implements, crop rotation and organic matter additions. The practise of including green manure as well as cover crops in rotational manner is a good approach of sponsoring soil fertility, suppressing weeds, providing a break in the pest cycles and ensuring diversity in the sources of organic matter (Jeff, 2009). Some of the integrated management strategies for *Fusarium* wilt include crop rotation using non similar crops such as cauliflower and cabbage, use of natural antagonists such as *Bacillus* species, embracing farm hygiene and use of fungicides such as prochloraz (Ajilogba *et al.*, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site description

Busia County, Western Kenya, lies between latitudes 0°30’ and 0°45’ N and longitudes 33º55’ and 34°25’ E, at an altitude of 1200 m above sea level. It receives a mean annual rainfall of between 900 to 1800 mm and mean annual minimum and maximum temperature of 17 and 30°C, respectively. The county lies within the Lower Midland 1 and 2 agro ecological zones and has predominantly humic *acrisol* soils (Jaetzold *et al.*, 2006). The study was carried out during the short rainy season between September and December 2015 when most farmers grow Bambara nut.

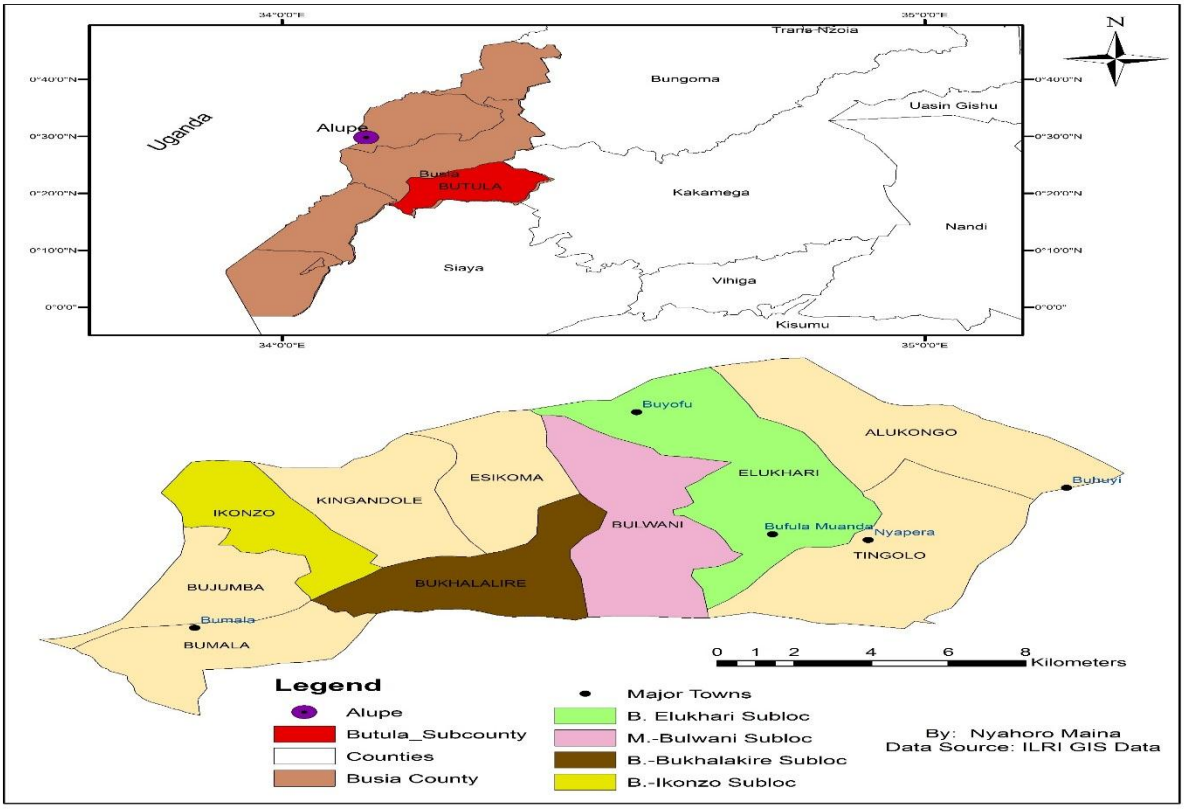

Figure 1: Map of Western Kenya showing study sites

Source: ILRI GIS Data

3.2 Sampling for wilt incidence in farms

Purposive sampling was used to select four villages (Madola, Bukati, Butunyi and Bufisi) in Butula sub-county, Busia County where Bambara nut production predominantly occurs. A total of 20 farms (having between 1/8-1/4 acres under the crop) were used to determine wilt incidence. In each farm disease incidence (proportion of diseased plants in a population) was visually assessed in quadrats (1m x 1m), as shown below;

$$\text{Wilt incidence} = \frac{\text{No. of plants wilted}}{\text{Total number of plants in the quadrat}} \times 100$$

The wilt incidence percentages generated from each farm were transformed using logarithms before data analysis. The mean incidences were then rated as described by Traperos-Casas (1983). Where;

0% -zero

0.1 to 0.5%- low

1 to 20% - moderately high

20.1 to 50% - high

> 50% - very hig

3.3 Laboratory experiment

Fusarium oxysporum isolation and identification

The root samples of diseased Bambara nuts were collected, placed in press sealed plastic bags (75 mm x 100 mm), labelled, kept in a cool box and transported to the Department of Biological Sciences laboratory, Egerton University for pathogen isolation and identification. The protocol described by Kristin and James (2000) was used for fungal isolation. Root tissues of plants showing symptoms were washed in running tap water, cut into 1cm portions, surface sterilized in 1.5% sodium hypochlorite for one minute, double rinsed in sterile distilled water and then blot dried between sterile paper towels.

The growth medium- Czapekdox agar (Sodium nitrate 3g, Potassium chloride 0.5g, Di-potassium hydrogen phosphate 1 g, Sucrose 30g, Magnesium sulphate 1g, Ferrous sulphate 0.01 g, Agar 15g and water 1 litre) a selective media for *Fusarium* growth was autoclaved at 121°C for 20 minutes allowed to cool down to touch temperatures (40- 45°C) before adding 5ml/litre of streptomycin sulphate to eliminate bacteria contaminants. Glass petri dishes (Pyrex®) sterilized in the oven at 160°C for 45 minutes were used to grow the isolates. The sterile plates containing the samples were sealed using parafilm and incubated at 25- 26ºC followed by examination of colony formation within 2-14 days. The *Fusarium* wilt fungus was purified by repeated sub-culturing on PDA media and identified with the help of relevant literature (Leslie and Summerell, 2006). The photographic images of mycelia growth on PDA plates were taken using Nikon Coolpix camera S2900-20.1 MP. The microscopic identification was achieved using cello tape impression technique, where fungal spores picked by an adhesive tape were mounted on microscope slides smeared with cotton blue stain and observed under a camera mounted LED light compound microscope observed at a magnification of X400 (Tiwari *et al.*, 2009).

3.4 Greenhouse experiment

3.4.1 Pathogenicity test

The *in-vivo* assay was carried out in the greenhouse at Egerton University using the method described by Ros *et al.* (2005). Pure sub-cultures were prepared from cultures that had been isolated from Bambara nut infected roots and an inoculum made from these cultures. Sterilized PDA plates amended with 5ml/litre of streptomycin sulphate were inoculated with three mycelia plugs of *Fusarium oxysporum* from actively growing regions of the mycelia growth and incubated for seven days at 25-26ºC.The arising spores were scrapped off from the petri dishes surfaces by adding sterile distilled water so as to obtain a suspension. The suspension was filtered through one layer of Mira cloth and the concentration adjusted using a haemocytometer to 10^6 conidia/ml.

Seeds of the black and red landraces of Bambara nut were surface sterilized in 1% sodium hypochlorite for three minutes, rinsed in three changes of sterile distilled water and air dried in the laminar flow.

Three seeds per landrace were then planted in pots (18 cm long and 19 cm wide) containing sterilized sandy- loam soil (2.5 kg per pot) (autoclaved at 120°C for one hour on three consecutive days) with adequate watering. Fourteen days after emergence, 10 ml of the 10^6 conidia/ml of *Fusarium oxysporum* spore suspension was applied over the base of the hypocotyl of the seedlings using a 10 ml syringe. Development of pathogenicity signs and symptoms was observed on a weekly basis and records taken. Re- isolation was then carried out as described in section 3.3 to confirm isolates identity 12 weeks after planting.

3.4.2 Incidence and severity of *Fusarium* wilt

(i) Inoculum preparation and seedlings inoculation

A pure sub culture was made from the cultures that had been isolated from bambara nut infected roots and an inoculum prepared from these cultures using the protocol described in section 3.4.1. The greenhouse experiment was laid out as a Completely Randomized Design replicated three times. The most cultivated Bambara nut landraces in Busia county (black, red, maroon, maroon speckled, brown light eyed and brown dark eyed) were used in the study. Two seeds per landrace were germinated in pots (18 cm long and 19 cm wide) containing sterilized sandy- loam soil (2.5 kg per pot) (autoclaved at 120°C for one hour on three consecutive days) with adequate watering. At three-leave stage, the seedlings were inoculated with 10 ml of the 10^6 conidia/ml of *F. oxysporum* spore suspension at the base of the hypocotyl using a 10 ml syringe as described by Kristin and James (2000). Post inoculation symptom monitoring was commenced and disease symptoms recorded.

ii). Disease assessment

Disease incidence (DI) at 45 days after inoculation was determined using the equation proposed by Cooke (2006) for a period of 60 days.

DI= (Number of infected plants /total number of plants assessed) x 100

Fusarium wilt symptom rating was done at an interval of 10 day using a scale of 0-5 based on leaf yellowing grading by Abdou *et al.* (2001). Where;

0= Healthy

1= One leaf yellowing

2= More than one leaf yellowing

3= One wilted leaf

4= More than one leaf wilted

5= Completely dead plants

Disease severity index (DSI) was then determined using the formula described by Liu *et al.* (1995).

$DSI = \Sigma d / (d\ max \times n) \times 100$ where;

d -the disease rating of each plant

d max -the maximum disease rating

n- The total number of plants/samples examined in each replicate.

The area under disease progress curve (AUDPC) was calculated using the formula suggested by Pandy *et al.* (1989).

$AUDPC = D\ [1/2\ (Y1+Yk) + (Y2+Y3+\ldots\ldots +Yk-1)]$

Where;

D= Time interval

Y1= First disease severity

Yk= Last disease severity

Y2, Y3……Yk-1= Intermediate disease severities

3.4.3 Management of *Fusarium* wilt

Sandy-loam soil was first sterilized by autoclaving at 120°C for one hour on three consecutive days. About 2.5 kg of soil was filled in pots (18 cm long and 19 cm wide) and inoculated with the pathogen using the method described by Prasad and Weigler (1976). *Fusarium* wilt inoculum used had been prepared as described in section 3.4.1. Five ml of distilled water containing 1.0×10^6 spore / ml of *F. oxysporum* was drenched into the soil using a 10ml syringe and left for one week to allow inoculum build up. The pots were arranged in a 2x2 factorial experiment laid out in a Completely Randomized Design replicated three times. Farm yard manure (at the rate of 5tons/ha) was then added to one set of the pots while another set was left as negative control.

Seeds of black and red landraces were planted in the pots (two seeds per pot) with adequate watering. Disease incidence and severity was determined using the formula described in section 3.4.2. This was done 30 days after germination at an interval of 10 days for a period of 40 days just before the onset of maturity stage so as not to confuse adult senescence with disease wilting.

3.5 Field experiment

This was performed under natural infestation of *Fusarium* wilt at Kenya Agricultural and Livestock Research Organization, Alupe station, Busia County. Alupe lies at 0˚ 28´ N and 34˚ 07´E, and at an altitude of 1189 m above sea level. It receives a mean annual rainfall of between 1100- 1450 mm, a mean annual minimum and maximum temperature of 17 and 30°C respectively. The station is situated in agro-ecological zone of lower midland 3 and has dark red (*Orthic ferrasols*) soils (Jaetzold *et al.,* 2006). The field was ploughed and levelled using a tractor before the experimental set up. The 2x2 factorial experiment was laid out as a Completely Randomized Block Design replicated three times with each plot measuring about (4x2) m^2 .

Seeds of the black and red Bambara nut landraces were used in the experiment. Sowing was done at a spacing of 20 cm within rows and 45 cm between the rows. The seeds were planted at a depth of 2 cm with the application of FYM (at a rate of 5 tons/ha) treatment and zero treatments (where no manure was applied). Weeding and other cultural management practices were carried out manually. Disease incidence and severity were determined using the formula described in section 3.4.2. This was done 30 days after germination at an interval of 10 days for a period of 40 days just before the onset of maturity stage so as not to confuse adult senescence with disease wilting.

3.6 Data analysis

All the data collected were transformed using logarithms to achieve normality as well as reduce variability before being subjected to analysis of variance using SAS version 9 and the means were separated using the least significant difference

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Distribution of *Fusarium* wilt in farmers' fields

4.1.1 *Fusarium* wilt distribution in the farms

The *Fusarium* wilt incidence varied with village (Figure 2), and ranged from 14.63% to 43.56%. Bufisi, Butunyi and Bukati villages had the highest disease incidence while Madola had the lowest disease incidence. Variation in disease incidence among villages could be attributed to differences in chemical, physical and biological properties of the soil. These soil properties are highly influenced by the farming practices adapted by farmers which varied among the villages. For instance, the soil pH and biological properties could be altered depending on fertilizer use by farmers. Soils also harbour natural bacteria such as *Pseudomonas* species (Shen *et al.,* 2015) that are antagonists to *F. oxysporum* which may be killed due to wrong farm practices such as misuse of inorganic fertilizer (over application) leading to increased *F. oxysporum* spores in the soil hence the high wilt incidences. Mehmood *et al.* (2013) similarly noted differences in incidence of *Fusarium* wilt which they attributed to variations in soil properties and rainfall amounts. Landa *et al*. (2006) and Navas-Cortes *et al*. (1998) similarly reported variation in intensity of *Fusarium* wilt in farmers' fields which they attributed to differences in cultivar, amount of inoculum in the soil, virulence of the pathogen and environmental humidity and temperature.

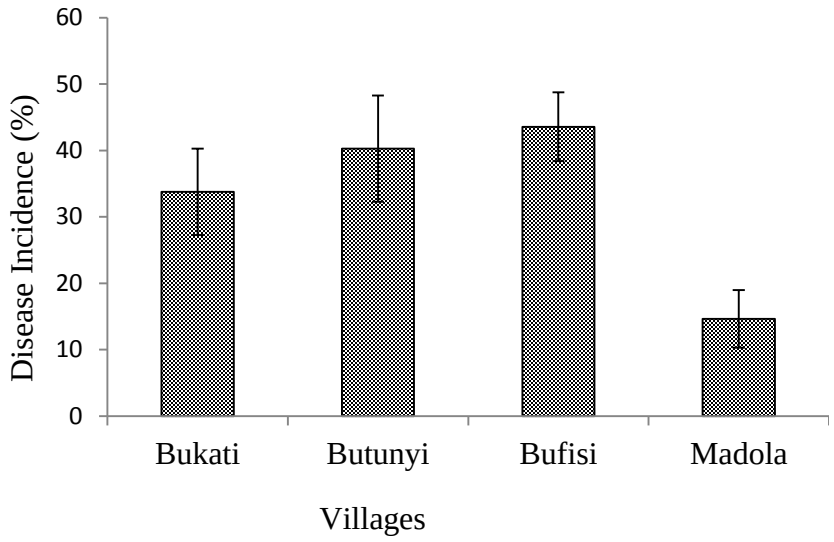

Figure 2: Distribution of *Fusarium* wilt incidence in farmers' fields in the year 2015

The *Fusarium* wilt incidence was generally high probably because of the high rainfall and air temperature that were favourable for pathogen sporulation. The short rains of 2015 were heavier (787 mm) compared to the year 2014 (655 mm) (http://me.awhere.com). These conditions may have enhanced fungal sporulation and hence the high disease incidence. Similarly, Misra and Pandey (2000) reported an increase in *Fusarium* wilt on guava seedlings during the heavy rains. Repeated cultivation of Bambara nut on soils/areas that had previously been used for legume production could also have contributed to the high wilt incidences in the farms. This is because most legumes serve as alternate hosts to *F. oxysporum* thus favouring inoculum build up in the soil (Gimenez-Dias and Jimenez-Gasco, 2011).

4.1.2 *Fusarium oxysporum* isolation and identification

Colony and spore characteristics

Fusarium oxysporum was isolated from all the root samples of bambara nut collected from farmers' fields. This indicates an abundance of the pathogen in the bambara nut farmers' fields. Wakelin *et al.* (2008) similarly reported abundance of *Fusarium* species in cultivated soils where they were mostly associated with plant roots. The mycelia were white cottony to pink with the aerial part becoming tinged with purple colouration (Plate 1). The reverse was non-descript pale to yellow (Plate 2). Microconidia were abundant, born on phialides with straight, curved or ellipsoidal shapes.

The macroconidia were sparse, found accumulating along the hyphae or scattered, often 3-5 septate and pointed at both ends more ellipsoidal than microconidia (Plate 3). Chlamydospores which developed after seven days were smooth and sometimes rough walled, abundant and formed terminally or intercalary (Plate 4). The colony characteristics were consistent with the descriptions by Kleczewski and Egel (2011).

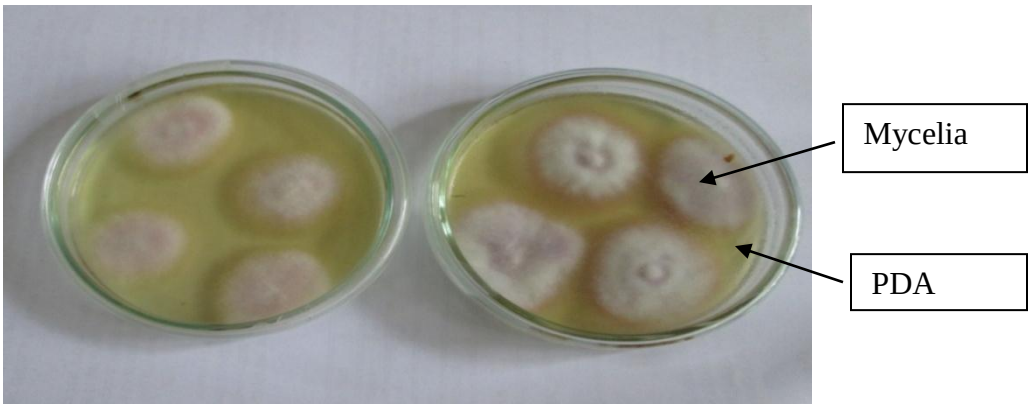

Plate 1: Growth of *Fusarium oxysporum* on potato dextrose agar plates -aerial view

Source: Author

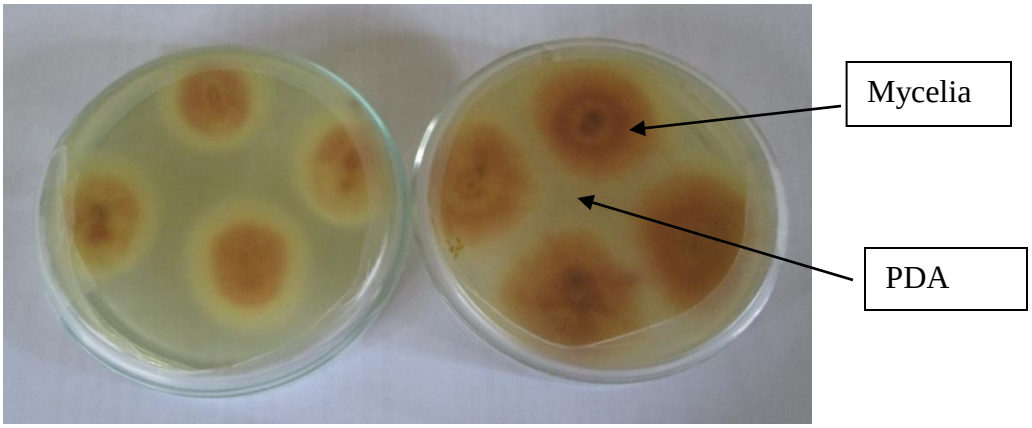

Plate 2: Growth of *Fusarium oxysporum* on potato dextrose agar plates -reverse view

Source: Author

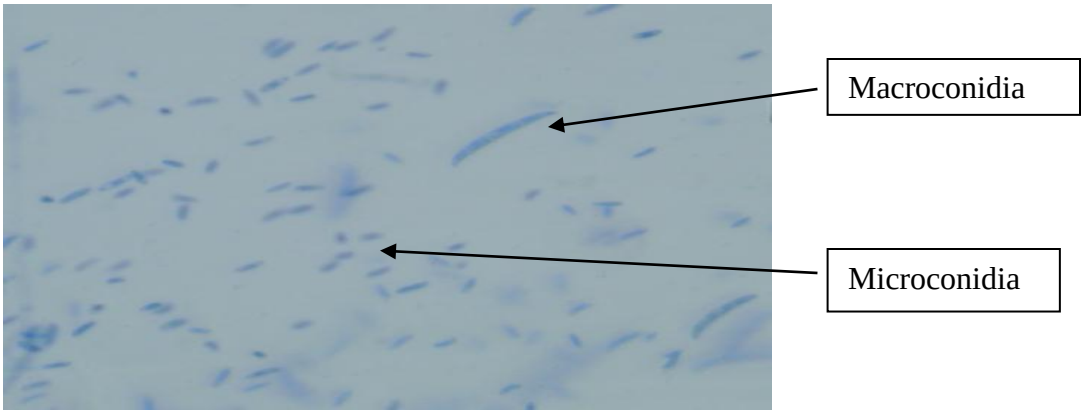

Plate 3: *Fusarium oxysporum* conidia (x40

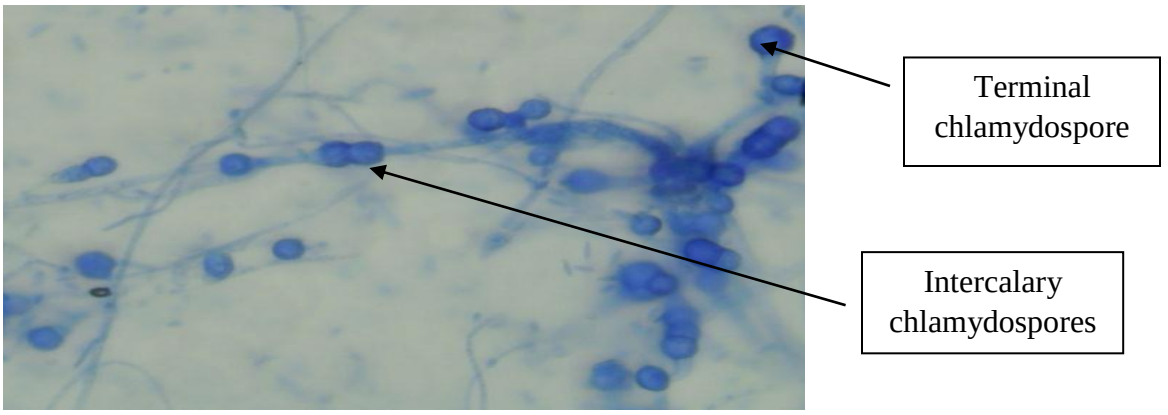

Plate 4: *Fusarium oxysporum* chlamydospores (x400)

4.1.3 Pathogenicity of *Fusarium* wilt on Bambara nuts

Fusarium wilt symptoms occurred 45 days after inoculation. The first indication of the disease was yellowing of leaves that intensified with time, before the entire crop eventually wilted (Plate 5). Destructive sampling showed brown vascular discolourations. The isolate was virulent with the two landraces (Black and Red) being susceptible to the pathogen. *Fusarium oxysporum* identical to the parent isolate was re-isolated from the diseased root tissues.

Plate 5: Pathogenicity symptoms of *Fusarium* wilt on the black and red landraces eight weeks after inoculation

Source: Author

Pathogenicity was indicated by presence of the disease on the landraces since the symptoms expressed in the greenhouse were similar to those observed in farmers' fields. Ebbels and Billington (1972) in Tanzania similarly reported presence of *Fusarium* wilt on different bambara nut landraces. Occurrence of symptoms varied with landraces but ranged from 14 to 50 days after inoculation. Early symptoms were observed in the maroon and maroon speckled landraces while late symptom expression was seen in the brown landrace. The variation may be attributed to the differences in genetic makeup of landraces and the specific pathogen races involved. Rodriquez-Molina *et al*. (1995) also noted differences in the time of *Fusarium* wilt symptom expression among different genotypes of tomato. The characteristic symptoms which included yellowing of leaves, wilting of the plant and brown vascular discolouration throughout the trial period were consistent with those observed earlier by Sharma (2011).

4.2 Incidence and severity of *Fusarium* wilt

The incidence and severity of *Fusarium* wilt was high and varied significantly ($P \leq 0.05$) with landrace and time after inoculation (Tables 1 and 2). The disease severity for the landraces varied with time. However, at 75 days after inoculation, all the landraces had similar (approximately 43%) disease severity. The time coincided with flowering and pod initiation and set of the crop. The inoculum levels at this stage were also expected to be high due to rapid sporulation by the pathogen within the vascular system.

Chaudhry *et al.* (2006) similarly observed higher *Fusarium* wilt intensity at flowering and podding stages of chickpea development.

Table 1: Mean *Fusarium* wilt incidence (%) on Bambara nut landraces over time in the greenhouse

	Type of landrace					
Time (days) after inoculation	Black	Red	Maroon	Maroon speckled	Brown dark eyed	Brown light eyed
45	46.67±3.33*	43.33±3.33	48.52±4.55	56.67±3.33	53.33±3.33	46.67±3.33
55	56.67±6.67	53.33±8.82	58.89±4.84	56.67±3.33	56.67±6.67	53.33±3.33
65	66.67±3.33	63.33±3.33	68.89±1.11	66.67±3.33	63.33±3.33	76.67±3.33
75	83.33±3.33	83.33±3.33	86.30±3.16	86.67±3.33	90±0	86.67±3.33
85	96.67±3.33	90±0	90±5.77	96.67±3.33	93.33±3.33	96.67±3.33
95	100±0	100±0	100±0	100±0	100±0	100±0
105	100±0	100±0	100±0	100±0	100±0	100±0

*Values shown are the mean disease incidence (%) ± SE of the landraces.

Table 2: Mean *Fusarium* wilt severity (%) on Bambara nut landraces over time in the greenhouse

	Type of landrace					
Time (days) after inoculation	Black	Red	Maroon	Maroon speckled	Brown dark eyed	Brown light eyed
45	19.33±0.67*	15.33±0.67	18.67±0.67	18±0	20±2	18±1.15
55	22±1.15	18.67±1.76	22.15±1.29	22±1.15	22.67±1.76	20.67±1.76
65	25.33±1.33	26±1.15	27.63±0.85	26.67±1.33	25.33±1.33	30.67±1.76
75	41.33±1.76	43.33±1.76	44.96±2.06	44.67±1.33	44±1.15	42.67±0.67
85	54.67±1.33	51.33±0.67	50.44±1.56	50±1.15	52±2	56.67±2.40
95	63.33±1.76	67.33±1.76	72.59±2.84	62.67±1.76	59.33±2.40	64±2.31
105	73.33±2.40	82.67±1.76	82.30±3.72	77.33±1.33	74.67±1.33	77.33±1.76

*Values shown are the mean disease severity (%) ± SE of the landraces.

Irrespective of landrace, disease incidence and severity increased with time. At about 95 days after inoculation, the landraces had attained 100% incidence. Generally, the maroon speckled and brown dark eyed landraces had the highest disease incidences while the red landrace had the lowest disease incidence (Table 3). The maroon and brown dark eyed landraces had the highest disease severities with the brown light eyed landrace having the lowest disease severity. Disease incidence and severity among the landraces were statistically different at $p \leq 0.05$.

Table 3: Mean *Fusarium* wilt incidence (%) and severity (%) among the bambara nut landraces

Landrace	Incidence (%)	Severity (%)
Black	78.6^{ab}*	42.8^{bc}
Red	76.2^{b}	43.5^{bc}
Maroon	78.9^{ab}	45.5^{a}
Maroon speckled	80.5^{a}	43.1^{bc}
Brown light eyed	79.5^{ab}	42.6^{c}
Brown dark eyed	80.0^{a}	44.3^{ab}
LSD ($p \leq 0.05$)	3.62	1.67

* Means followed by the same letter in each column are not significantly ($p \leq 0.05$) different from each other

The high disease incidence and severity on the selected landraces is an indication of the virulence of the pathogen on the crop. The results are consistent with Cook (1978) who reported presence of *Fusarium* wilt on Bambara nut in Kenya. The symptoms were noted starting from the vegetative phase through flowering with varying degrees among the landraces. This is probably because the pathogen is soil borne (Zhao *et al.*, 2014) hence infects the plant starting from seedling phase. Zemouli-Benfreha *et al.* (2014) similarly reported presence of *Fusarium* wilt symptoms at all stages of chickpea development.

None of the tested landraces were tolerant or resistant to the disease. The variation in disease incidence and severity among the landraces could be attributed to differences in genetic makeup among other factors. For example, van den Berg *et al*. (2007) noted low *Fusarium* wilt incidence on Cavendish banana cultivar which they attributed to cell wall strengthening genes in the roots. Aslam *et al.* (2013) similarly noted influence of genetic makeup of different chickpea cultivars on the *Fusarium* wilt severity.

The differences in symptom expression and hence variance in wilt incidence and severity could be due to variation in *F. oxysporum* f.sp. *voandzeia* races. Several studies have indicated the influence of different races of *Fusarium oxysporum* on incidence and severity of the wilt disease in various crops (Haware and Nene, 1982; Jimenez-Diaz *et al.*, 1993; Ben-Yephet *et al*., 1996; Wang *et al*., 1999; Navas-Cortes *et al.*, 2000; Abd-Elsalam *et al.*, 2004). Since the landraces were exposed to the same intensity of inoculum (10^6 conidia/ml), the variation in symptom expression and hence disease incidence and severity observed could be attributed to differences in genetic makeup of the landraces.

Wang *et al.* (1999) similarly noted that the highly susceptible cultivars of cotton required less spore concentration compared to the more tolerant cultivars for symptom expression. The area under disease progress curve (AUDPC) for severity was generated for all the landraces and it varied with landrace (Figure 3). The AUDPC was directly related to disease severity i.e. the higher the severity the higher the AUDPC. The maroon and brown dark eyed landraces had the highest area under disease progress curves i.e. 2730 and 2673 % disease days respectively followed by the red (2604), maroon speckled (2584) and black (2578) landraces respectively. The brown light eyed landrace had the least area under disease progress curve of 2555 % disease days. The AUDPC indicates disease progress over time on specific landrace and provides an insight on the appropriate time to apply management interventions (Simko and Piepho, 2011). Bani *et al.* (2012) also noted positive correlation between AUDPC and *Fusarium* wilt severity on field peas.

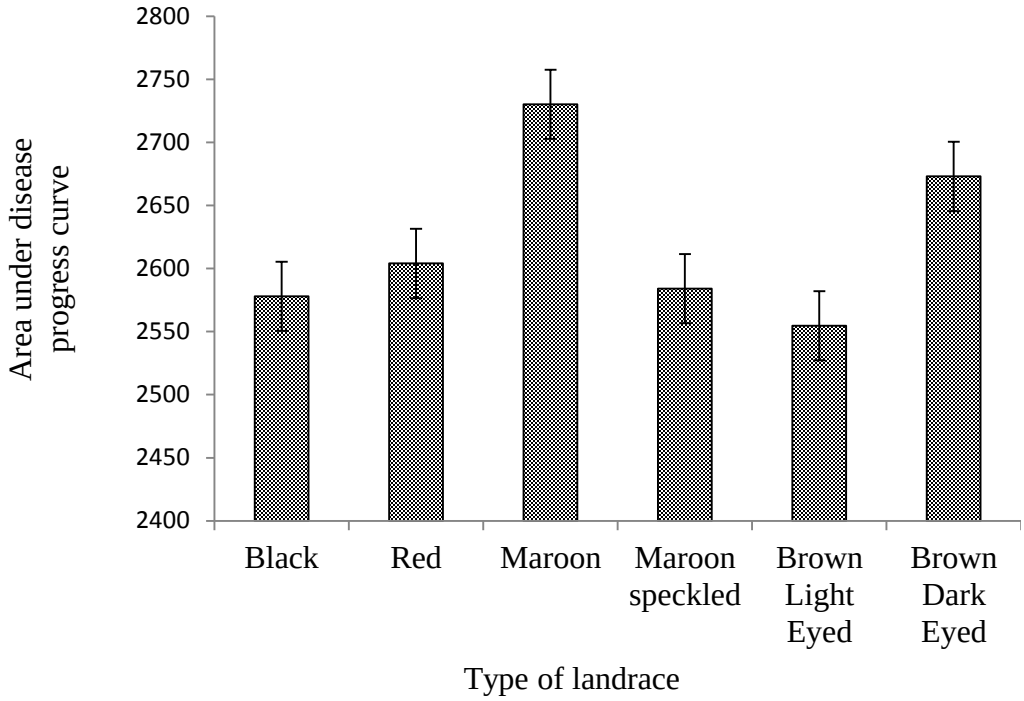

Figure 3: Area under disease progress curve of *Fusarium* wilt on different Bambara nut landraces

4.3 Effect of farm yard manure (FYM) on incidence and severity of *Fusarium* wilt

4.3.1 Greenhouse experiment

Disease incidence and severity in the greenhouse was high and varied with landrace, soil management practice and time (days) after germination (Figures 4, 5, 6 and 7). The difference was more explicit in the black landrace. This could be attributed to either genetic difference among the landraces or seed source since they were purchased from the local market. Application of farm yard manure (FYM) resulted in lower disease incidence and severity irrespective of the landrace. *Fusarium* wilt suppressive effect by FYM could be attributed to several factors such as increased soil pH and microbial antagonist effect (Bonilla *et al.*, 2012). Gangopadhyay and Gopal (2010) and Umar *et al*. (2013) similarly noted a significant reduction in *Fusarium* wilt incidence of cumin and tomatoes when FYM was incorporated in the soil. The reduction was attributed to increased antagonistic microbial community in the soil.

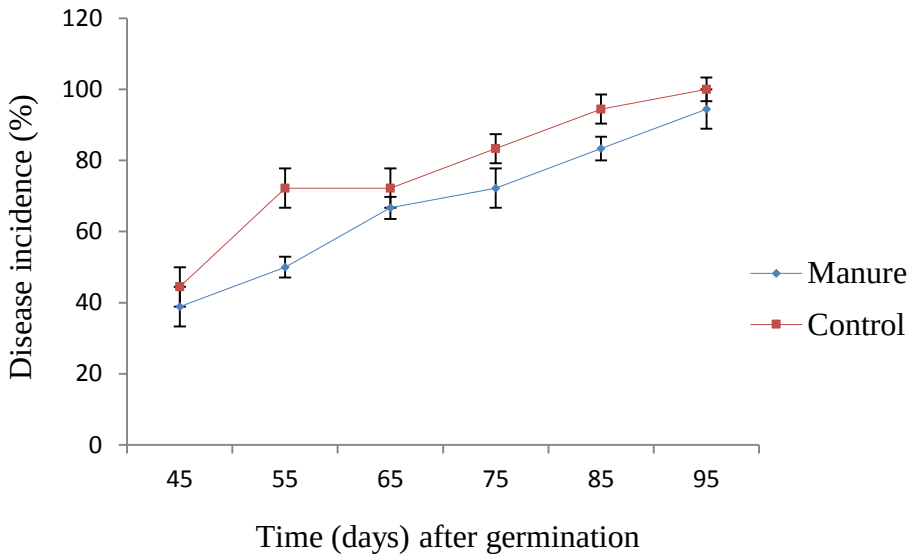

Figure 4: Effect of farm yard manure on *Fusarium* wilt incidence of the black Bambara nut landrace over time in the greenhouse

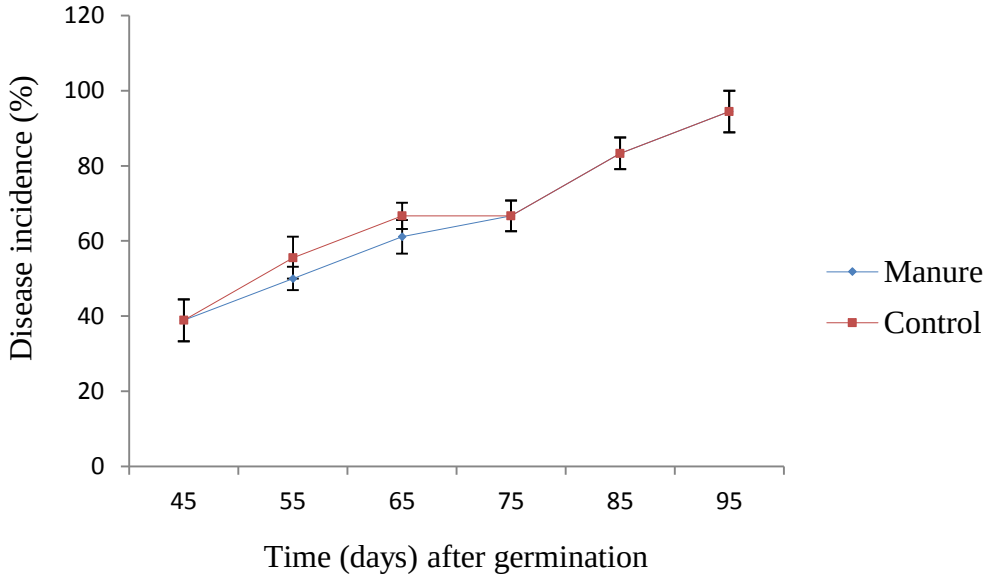

Figure 5: Effect of farm yard manure on *Fusarium* wilt incidence of the red Bambara nut landrace over time in the greenhouse

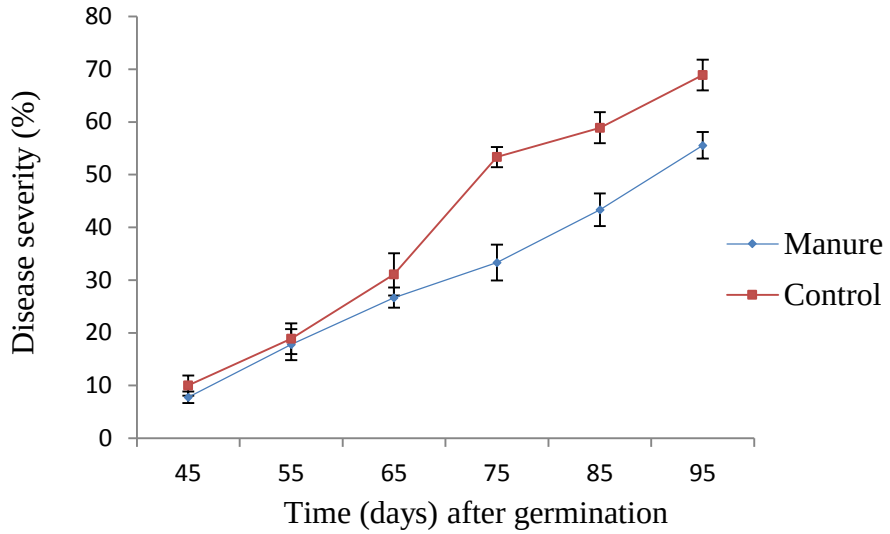

Figure 6: Effect of farm yard manure on *Fusarium* wilt severity on the black Bambara nut landrace over time in the greenhouse

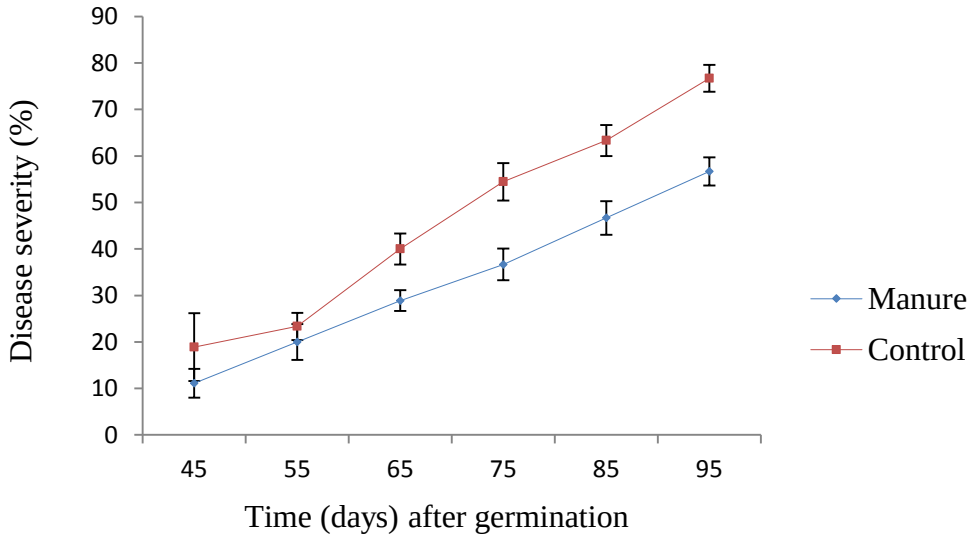

Figure 7: Effect of Farm yard manure on *Fusarium* wilt severity on the red Bambara nut landrace over time in the greenhouse

Significant differences in disease severity were observed 65 days after germination on the black landrace compared to 55 days on the red landrace (Figures 6 and 7). The difference may be attributed to variation in growth behaviour of the landraces. The black landrace matures faster compared to the red landrace implying the possibility of reducing intense pathogen infestation unlike the slow maturity of the red landrace (Abu and Buah, 2011).

Farm yard manure application had lower disease curves compared to the control. Area under disease progress curve (AUDPC) was determined for each landrace and treatment (Figure 8). The control treatment had the highest AUDPC i.e. 2006 and 2289 % disease days on the black and red landraces respectively while manure treatment recorded an AUDPC of 1527 and 1672 % disease days on black and red landraces respectively.

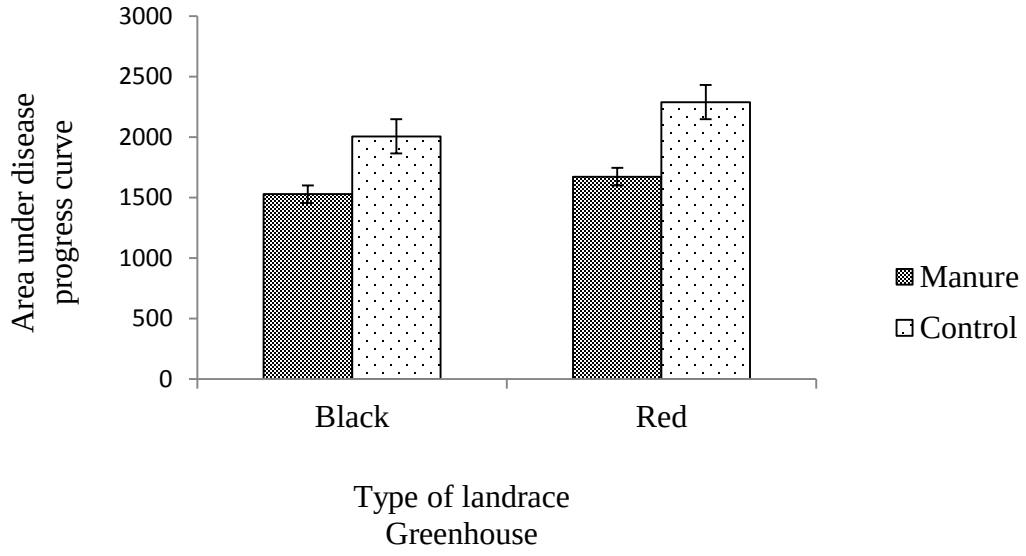

Figure 8: The effect of Bambara nut landrace and farm yard manure on area under disease progress curve in the greenhouse

4.3.2 Field experiment

The disease incidence and severity was high and varied with landrace (Figure 9, 10, 11 and 12). The trend was similar with the greenhouse experiment. However, unlike in the greenhouse, the increase in disease incidence in the field was generally low. This could be due to differences in the biotic and abiotic conditions such as temperature, humidity and microbial antagonists in the field. The FYM treatment had lower disease incidence and severity for both landraces compared to the control. This is as a result of the disease suppressive effect of FYM (Bonilla *et al.,* 2012). Haruna *et al*. (2012) also reported low *Fusarium* wilt incidence and severity on FYM treated tomato plants which they attributed to induced natural defence mechanisms as a result of nutrient supply to the plant. Escuadra and Amemiya (2008) reported the suppressive effect of organic amendments on *Fusarium* wilt of spinach, which they attributed to the nutrient competition between the antagonists and *Fusarium oxysporum*. Melero-Vara *et al*. (2011) also noted a reduction in disease severity of *Fusarium* wilt of carnation as a result of manure application. They attributed the reduction effect to increased soil pH as a result of ammonia increase in the soil and antagonism effect from microbial community.

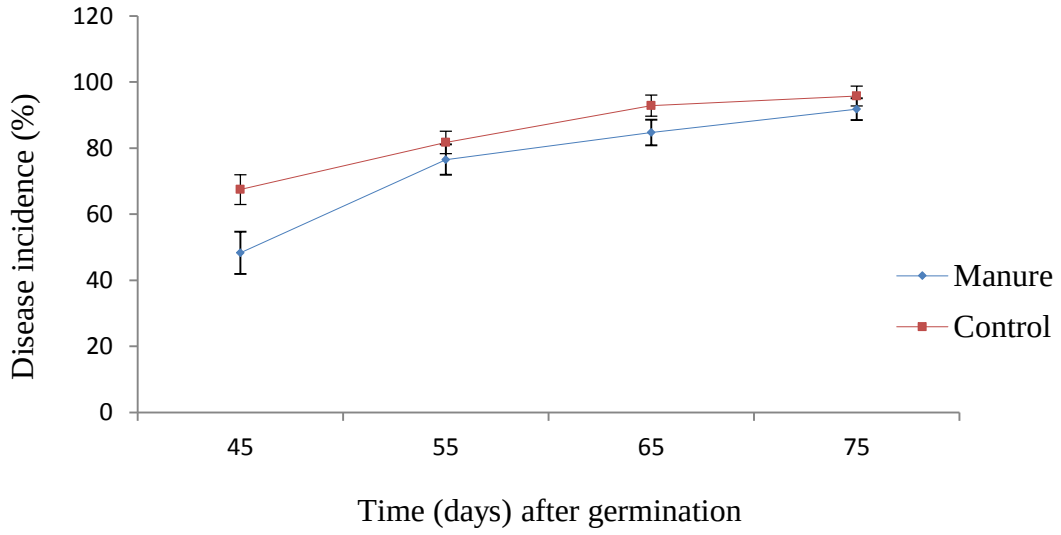

Figure 9: Effect of farm yard manure on *Fusarium* wilt incidence on the black Bambara nut landrace over time in the field

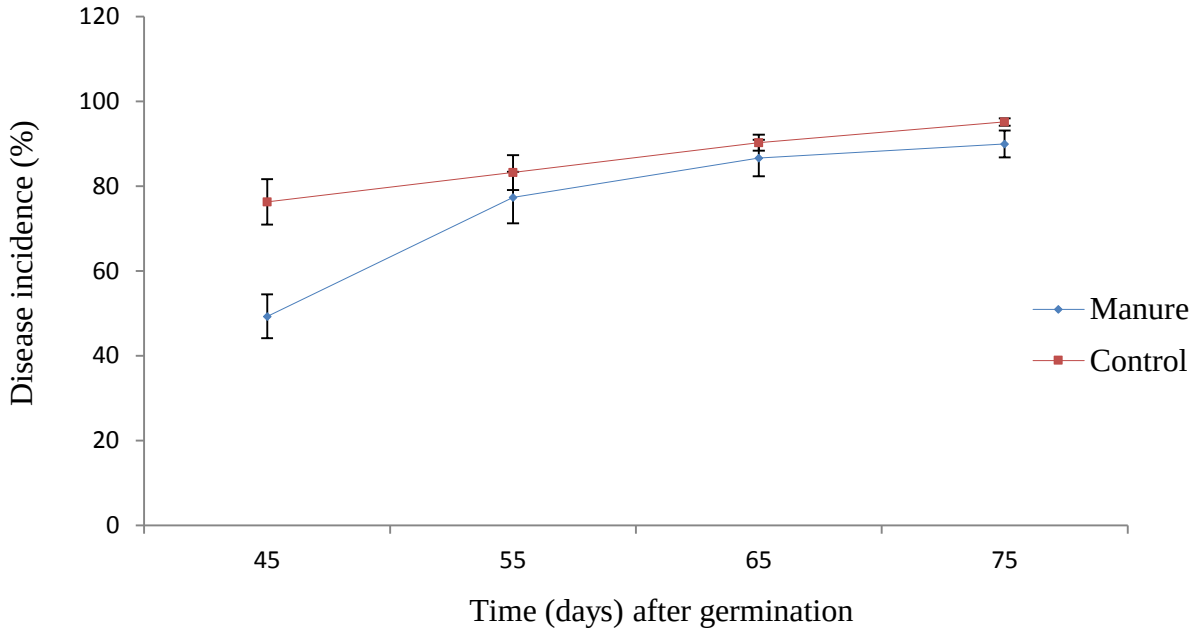

Figure 10: Effect of farm yard manure on *Fusarium* wilt incidence on the red Bambara nut landrace over time in the field

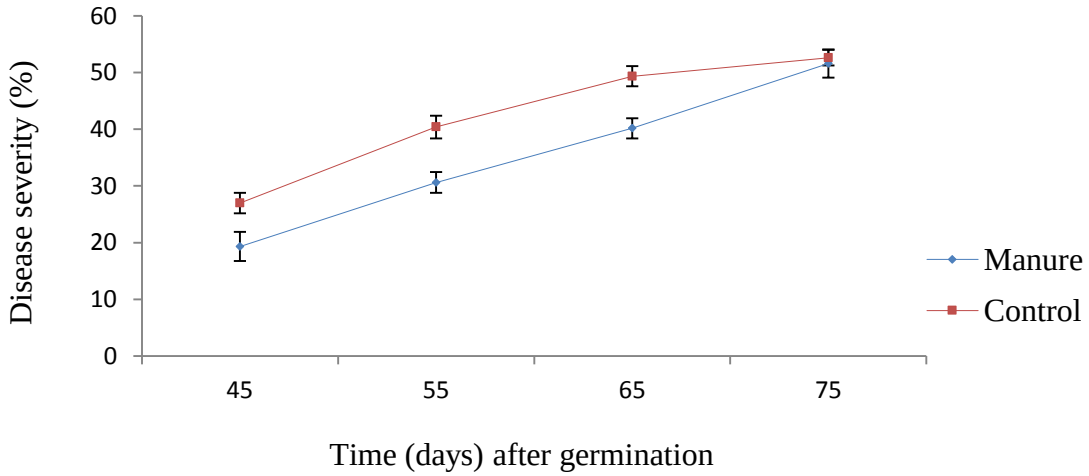

Figure 11: Effect of farm yard manure on *Fusarium* wilt severity on the black Bambara nut landrace over time in the field

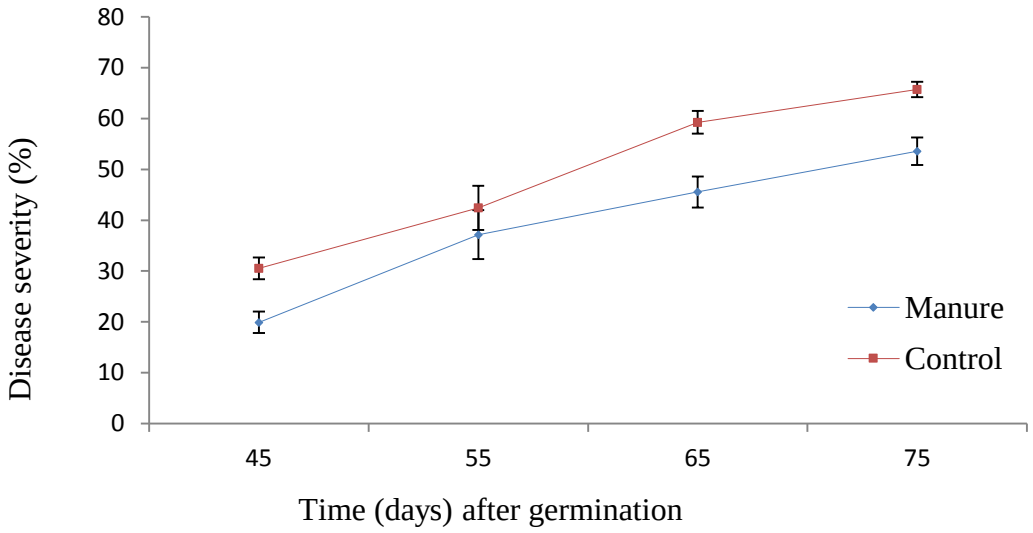

Figure 12: Effect of farm yard manure on *Fusarium* wilt severity on the red Bambara nut landrace over time in the field

Area under disease progress curve (AUDPC) was determined for each landrace and treatment (Figure 13). For both landraces, FYM treatment resulted in lower disease curves than the controls. The control treatment had the highest AUDPC i.e. 1295 and 1500 % disease days on the black and red landraces respectively while manure recorded an AUDPC of 1061 and 1195 % disease days on black and red landraces respectively.

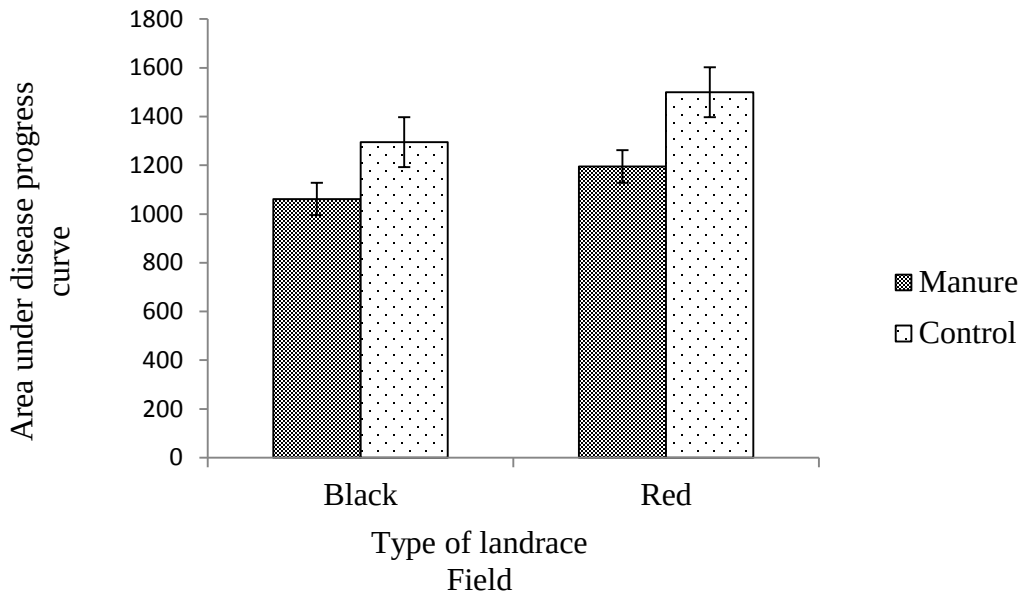

Figure 13: The effect of Bambara nut landrace and farm yard manure on area under disease progress curve in the field

Organic amendments have often been incorporated in the soil to basically promote growth and yield of crops and soil health (Ghorbani *et al.*, 2008). Current focus is on the use of organic amendments to improve soil health and manage plant diseases. Several research scientists noted effectiveness of FYM in suppression of soil borne diseases (Noble and Coventry, 2005; Bunemann *et al*., 2006; Bonilla *et al.*, 2012). Lazarovits (2001) and Lazarovits *et al.* (2001, 2005, 2008) also noted the suppressive effect of FYM on *Fusarium* wilt. They attributed the FYM suppressive effects to the increase in the soil pH. Most of the underlying mechanisms of disease control in organic systems include competition, antibiosis, parasitism and predation among the microbial community in the soil (Pérez-Piqueres *et al*., 2006). Soil borne diseases are reduced by the application of nitrogen-rich manure amendments through the release of allelochemicals that are generated during microbial decomposition (Suarez-Estrella *et al.*, 2007). Plant pathogens may also be inhibited by high concentrations of volatile fatty acids in manure (Shen *et al.*, 2015). Application of FYM also alters environmental conditions in the root zone, such as porosity, water-holding capacity, pH, nutrient concentration and electrical conductivity, which may directly or indirectly affect plant health and pathogen activity (Millner *et al*., 2004; Mathur *et al.*, 2006).

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Fusarium wilt contributes to great yield losses in bambara nut. *Fusarium oxysporum* was abundant in the farmers' fields of Busia County, Western Kenya. The wilt incidences however varied with villages. The variation was mainly attributed to differences in biological, physical and chemical properties of the soil, farm practices and seed used. The isolation of *F. oxysporum* from all the root samples collected is an indication of the abundance of the pathogen in the Bambara nut farmers' fields. Positive pathogenicity test was also a clear indication of the *F. oxysporum* virulence on Bambara nut.

None of the local landraces tested was resistant to the disease; they however had differences in the wilt incidence and severity. The variation could be attributed to differences in the genetic makeup of the landraces, presence of different *F. oxysporum* races and to some extent the seed used which is locally sourced and therefore could have different inoculum levels.

Farm yard manure effect in the greenhouse was consistent with that in the field. The FYM treatment had lower disease incidence and severity on both landraces indicating that it has the ability to suppress *F. oxysporum* in the soil. The application of FYM has been reported to increase microbial community within the soil most of which are antagonistic to *F. oxysporum*. Farm yard manure supplies nutrients to the plant thus contributing to its proper growth and induced natural defence against diseases. Farm yard manure application increases soil pH to levels that are toxic to the pathogen which in return lowers disease incidences.

5.2 RECOMMENDATIONS

1. *Fusarium* wilt analysis at farm level should be scaled up and related to the biotic and abiotic conditions so that the disease 'hotspots' can be identified. There is need to assess the quality and health of seeds used by farmers' by determining the *F. oxysporum* inoculum levels in the seeds for appropriate management interventions.
2. More studies should be carried out on specific races of *F. oxysporum* f.sp *voandzeia* that exist using molecular tools so as to develop the wilt control strategies. There is also the need to assess other Bambara nut landraces and wild relatives for any resistant type that could be used for breeding purposes.
3. Farm yard manure should be adapted by farmers for the management of *Fusarium* wilt of Bambara nut at farm level. In addition, more research work needs to be carried out on alternative *Fusarium* wilt control strategies and the mechanisms involved in using them.

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7.0 APPENDICES

Appendix 1: Mean squares for percentage disease incidence (PDI) over time after inoculum application in the greenhouse at Egerton University

		Days after inoculation				
Source of variation	DF	45	55	65	75	85
Replicate	2	11.58	48.75	4.32	19.82	72.22
Landrace	5	72.33	14.32	73.21*	18.71*	32.22
Error	10	43.45	117.66	33.21	28.71	32.22
C V (%)		13.40	19.40	8.53	6.23	6.05

* Significance at ($P \leq 0.05$).

Appendix 2: Mean squares for disease severity index (DSI) over time after inoculum application in the greenhouse at Egerton University

		Days after inoculation						
Source of variation	DF	45	55	65	75	85	95	105
Replicate	2	2.89	0.53	2.44	16.02	15.73	29.31	31.78
Landrace	5	7.82	0.53	12.29	5.50	20.49	62.61*	44.40*
Error	10	3.42	8.08	5.82	5.18	6.30	11.24	11.30
C V (%)		10.15	13.31	8.95	5.23	4.78	5.17	4.31

* Significance at ($P \leq 0.05$).

Appendix 3: Mean squares for percentage disease incidence (PDI) over time after germination in the greenhouse at Egerton University

		Days after germination					
Source of variation	DF	45	55	65	75	85	95
Replicate	2	23.16	23.14	0.00	23.13	23.16	69.47
Landrace	1	23.16	208.33	92.57	370.07*	92.63	23.16
Treatment	1	23.16	578.80*	92.57	92.52	92.63	23.16
Error	7	102.55	76.05	52.90	33.04	33.08	62.86
C V (%)		25.14	15.31	10.91	7.96	6.68	8.27

* Significance at ($P \leq 0.05$).

Appendix 4: Mean squares for disease severity index (DSI) over time after germination in the greenhouse at Egerton University

		Days after germination					
Source of variation	DF	45	55	65	75	85	95
Replicate	2	95.29	58.33	8.33	25.92	25.92	14.83
Landrace	1	111.94	33.37	92.57	14.87	45.47	59.27
Treatment	1	74.95	14.81	181.35	1070.50***	778.60***	833.17***
Error	7	26.86	14.57	33.07	10.05	9.66	15.36
C V (%)		43.39	19.08	18.16	7.13	5.86	6.08

*** Significance at ($P \leq 0.001$).

Appendix 5: Mean squares for percentage disease incidence (PDI) over time after germination in the field at KALRO- Alupe

		Days after germination			
Source of variation	DF	45	55	65	75
Replicate	2	92.99	40.15	22.94	27.70
Landrace	1	73.95	4.17	0.27	4.11
Treatment	1	1601.06**	92.24	103.43	63.66**
Error	7	80.60	57.38	19.25	4.06
C V (%)		14.88	9.50	4.95	2.16

** Significance at ($P \leq 0.01$).

Appendix 6: Mean squares for disease severity index (DSI) over time after germination in the field at KALRO- Alupe

		Days after germination			
Source of variation	DF	45	55	65	75
Replicate	2	13.60	44.02	1.30	23.75
Landrace	1	13.04	55.73	176.03*	171.69*
Treatment	1	250.71**	169.95	392.85**	130.61*
Error	7	13.23	32.02	19.49	21.52
C V (%)		15.04	15.03	9.09	8.30

*,**Significanceat($P \leq 0.05$)and($P \leq 0.01$)respectively