A STUDY ON THE OCCURRENCE, DIVERSITY AND DISTRIBUTION OF *FUSARIUM*SPP. IN THE ARABLE SOILS IN ZAMBIA.

BY

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DEPARTMENT OF BIOLOGICAL SCIENCES

2012

DECLARATION

I, CHIWAMA LUKALI hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other university.

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Signature

……

Date

APPROVAL

THIS DISSERTATION BY CHIWAMA LUKALI IS APPROVED AS FULFILLING THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MYCOLOGY BY THE UNIVERSITY OF ZAMBIA.

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ABSTRACT

Fusarium species are ubiquitous soil inhabiting fungi. They have the ability to exist as saprophytes degrading lignin and complex carbohydrates associated with plant debris. Although the predominant role of these saprophytes is harmless, many species are pathogenic to plants, especially in agricultural settings.The occurrence, distribution, richness and diversity of *Fusarium* species were determined from selected arable soils around Lusaka District. The experimental soils were collected from 12 commercial farms of the Lusaka District situated in four sites*viz*Lusaka North, East, South and West. One hundred and five (105) isolates of *Fusarium* species were obtained and plated on a Selective *Fusarium* Agar mediumfollowing the conventional soil dilution plating method. The general objective of the study was to determine the occurrence, diversity, richness and distribution of *Fusarium* spp. in arable soils of the District. The specific objectives of the study wereto: i) evaluate the occurrence of *Fusarium* spp. ii) determine their species diversity and iii) establish their distributionin the study area.A total of eight species of *Fusarium* were identified on the basis of colony morphological characteristics; number and type of phialids; and shape, size and type of conidia.This study showed that *Fusarium oxysporum*(47.6%) and *F. solani*(30.5%) were the most dominant species with regard to the frequency of occurrence. The other six *Fusarium* species showed much reduced occurrence and ranged between 1.9% and 5.7%.There were significant differences($p=0.05$) in their occurrenceof among farms. There were also significant interactions between farms and sites with regard to species diversity and richness.The soils differed inphysical and chemical properties. The studyhas also demonstrated a clumped distribution of*Fusarium* spp. as shown by the variance/mean ratio analysesin the 8 of the 12 sampled farms. The study provides the baseline information on the occurrence, diversity and distribution of *Fusarium* spp. in certain agricultural soils of Zambia. These findings suggest that farmers should avoid cultivating *Fusarium*-susceptible crops in such soils.

DEDICATION

To my family and friends for their love, support and patience.

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LIST OF ACRONYMS

spp.	**Species**
mc	**Moisture content**
%	**Percent**
°C	**Degree Celcius**
m	**Meters**
cm	**Centimeters**
cm^3	**Cubic centimeters**
g	**Grams**
mg/kg	**Milligram/kilogram**
K	**Potassium**
S	**Sulphur**
N	**Nitrogen**
vs	**Versus**

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1. 0.CHAPTER ONE: INTRODUCTION

Fusarium is a large and diverse genus of filamentous fungi classified in the Order Hypocreales of the Phylum Ascomycetes. This form-genus was first described by Link (1809). Later, the genus *Fusarium* was defined by Wollenweber and Reinking (1935) based on morphological and cultural characteristics. However, *Fusarium* is a polyphyletic genus which includes over 50 species and has cosmopolitan distribution in the air, soil and in association with many plants (Alexopoulos *et al*, 1996; Leslie *et al*., 2006).

The occurrence of *Fusarium* spp. conidia in the air is common (Fernando *et al*., 2000). Conidia of *Fusarium* species are often introduced and dispersed in the atmosphere from the soil by wind currents or by rain (Ooka and Kommedahl, 1977;Jenkinson and Parry, 1994; Paul *et al*., 2004). Airborne spores of *Fusarium*are also involved in several types of respiratory conditions in humans. These include allergic rhinitis and asthma, which occur in individuals who are predisposed to a number of allergens(Alexopoulos*et al.*, 1996).

Apart from causing airborne respiratory infections, some species of *Fusarium* may also cause a range of opportunistic infections such as keratomycosis (Mselle, 1999), onychomycosis (Godoy *et al.,* 2004), pulmonary infections (Rolston, 2001; Gorman *et al.,* 2006) and endophthalmitis (Goldblum *et al.,* 2000) in immune-compromised humans.

Fusarium species also produce a wide array of mycotoxins.These are fumonisins(Lamprecht *et al.,* 1989; Lamprecht *et al.,*1994 and Katta *et al*., 1997), beauvericin, moniliformin, zearalenone(Thiel *et al.,* 1991**;**Abramson*et al*., 2002),

nivalenol, deoxynivalenol (Thiel *et al.,* 1982; Blaney and Dodman, 2002), fusaproliferin, and trichothecene in cereals (Bullerman and Tsai, 1994).These mycotoxins are responsiblefor allergies, growth defects and cancer in humans (Linnabary and Tarrier, 1988)and domestic animals(Jeschke *et al.,* 1987;Engelhardt *et al*., 1989; Broomhead *et al*., 2002).

The predominant interest in the genus *Fusarium* is their role as plant pathogens (Booth, 1971). *Fusarium*spp. cause a variety of serious plants diseases. These include vascular wilts, cankers, rots of seed, fruit, root and stem, and blights in a wide range of economically important crops. The affected crops are tomatoes (*Lycopersicon esculentum*)(Fletcher and Lord, 1985;), maize *(Zea mays*) (Bullerman and Tsai, 1994), wheat (*Triticum aestivum*) (Boshoff *et al.,* 1999), groundnuts (*Arachis hypogaea*)(Joffe 1973;Kranz and Pucci, 1963), pawpaw (*Carica papaya*), pineapples (*Ananas sativus*)(Bolkan *et al.*, 1979),cassava (*Manihot esculenta*), mangoes (*Mangifera indica*) (Britz *et al*., 2002), potatoes (*Solanum tuberosum*),cucumbers (*Cucumis sativus*), bananas (*Musa acuminata*) (Frisullo *et al.,* 1994) and cotton (*Gossypium arboretum*)(Davis *et al.,* 1996; Pitt *et al.,* 1994).This is due to the fact that *Fusarium*chlamydospores, conidia andhyphae are distributed widely in cultivated soil and soil debris (Bolkan *et al.,* 1979). These 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 point of entry, therefore, causing diseases to susceptible plants(Leslie *et al*., 2006).

Fusarium species are able to survive in the soil for long periods of time as chlamydospores (Haware *et al.,* 1996;Vakalounakis and Chalkias, 2004). Theseround, thick-walled, single-celled or chained spores are produced in abundance in dead tissues or pieces of colonised organic matter in the soil. Chlamydospores are resting spores of *Fusarium* spp. produced in the soil during periods of unfavourable conditions (Leslie *et al*., 2006). Many *Fusarium* species also exist as harmless saprobes in the soil while others establish long-term associations with crop plants as endophytes (Thomas and Buddenhagen, 1980; Bacon and Hinton, 1996).

Diversity is the number of different species in a particular area (i.e., species richness) weighted by some measure of abundance such as number of individual or biomass (Swingland, 2001). The Shannon diversity index (H') is an index that is commonly used to characterize species diversity in a community (Hurbert, 1971). High values of H' are representative of more diverse communities. A high diversity index of soil *Fusarium* species and other soil fungi is important for their involvement in soil structure formation; decomposition of organic matter; toxin removal;and the cycling of carbon, nitrogen, phosphorus, and sulphur (Garbeva *et al*., 2004). However, the presence of diverse species of *Fusarium* in agricultural soil may lead to disease infection of susceptible plants.

It is, therefore, apparent from the foregoing that cultivated soil is one of the sources of fusarial spores that may cause serious crop diseases leading to low yields. However, there is no information on the occurrence,distribution and diversity of *Fusarium* species in cultivated soils of Zambia. Therefore, the general objective of this study was to

determine the occurrence, diversity, richness and distribution of *Fusarium* spp. in selected arable soils.

1.1.Statement of the Problem

*Fusarium*is an important soil-inhabiting fungus known to cause diseases in many economic plants in Zambia (Naik and Burden,1981; Augus, 1964). However, information on theoccurrence, distribution and diversity in the arable soils has not been documented.Therefore, crop losses due to infections caused by soil *Fusarium* species cannot be ascertained.

1.2.Significance of the Study

Information obtained from this study will provide the baseline data on the occurrence, diversity and distribution of *Fusarium* spp. in agricultural soils. Thesefindings of the occurrence of *Fusarium* species in arable soils could be used to advise farmers to avoid the cultivation of *Fusarium*-susceptible crops in such soils.

1.3.Objectives

The objectives of this study were to:

1. evaluate the occurrence of *Fusarium* spp. in selectedsoils of the study area.
2. determine the species diversity in the selected sites of the study area.
3. establish the distribution of *Fusarium* spp. in the study area.

1.4.Research Hypotheses

The study tested the following hypotheses:

- *Fusarium* species occur widely in arable soils of Lusaka District,
- Species composition of *Fusarium*is diversein arable soils,
- Sufficient richness of *Fusarium* species existsin arable soils of Lusaka District.

1.5.Null Hypotheses

- *Fusarium* species do not occur widely in arable soils of Lusaka District,
- Species composition of *Fusarium*is not diversein arable soils of Lusaka District,
- Sufficient richness of *Fusarium* species does not existin arable soils of Lusaka District.

2.0. CHAPTER TWO: REVIEW OF LITERATURE

2.1. Importance of *Fusarium* in soil

The soil in general serves as a medium for the growth of plants and existence of microoganisms including *Fusarium. Fusarium* species in the arable soil exist as mycelium, microconidia, macroconidia and chlamydospores but most commonly as chlamydospores. The soil and plant debris generally serve as a reservoir for the survival of *Fusarium* propagules which may eventually cause disease in plants (Agrios, 1988;Leslie *et al.,* 1990).

Some *Fusarium* species in the soil are saprophytic, living on dead organic material and commonly causing its further decay and thereby reducing and degrading lignin (Sutherland*et al*., 1983; Rodriguez *et al.*, 1996)and other complex organic compounds(Went and DeJong, 1966;Christakopoulos *et al*., 1995) to basic nutritional elements essential for the growth of plants. However, under certain circumstances, these saprophytic *Fusarium* species may attack living plants and become parasitic(Booth, 1971; Hawksworth *et al.*, 1983; Agrios, 1988) resulting in low yields.

2.2. Occurrence and distribution of *Fusarium* speciesin soil

Fusarium species are ubiquitous and have been isolated from various soil types. They havebeen reported from tropical and temperate regions, from desert and alpine soils. The majority of *Fusarium* species are recoverednear the surface of cultivated soils. It has been shown that the occurrence and population diversity of *Fusarium* species in the soil are

influenced by climatic factors, mainly temperature and rainfall (Liddell and Burgess, 1985; Saremi and Burgess, 2000).

In Africa, the most prevalent *Fusarium* species recovered in soils planted to millet and sorghum as reported from Lesotho, Nigeria and Zimbabwe were *F. oxysporum, F. equiseti, F. solani, F. moniliforme, F. compactum, F. nygamai,* and*F. chlamydosporum*.Other *Fusarium* species isolated were *F. merismoides, F. polyphialidicum, F. graminearum, F. subglutinans, F. sambucinum, F. longipes, F. semitectum, F. lateritium* and a groupof cultures designated as population A which resembled *F. camptoceras. Fusarium equiseti* was the predominant species in the soil samples from Nigeria and Zimbabwe, while *F. oxysporum* was the predominant species recovered in soil from Lesotho (Nwanwa and Nelson, 1993). In Mahlanya, Swaziland, high populations of *Fusarium moniliforme* and *Fusarium thapsinum*were isolated from soil randomly sampled in maize and sorghum fields during the 1998/1999 cropping season. These populations were lowest before planting but increased with advancement in the growth stage of maize crop (Mansuetus *et al*., 2000).

In a study conducted in Transkei, South Africa by Jeschke *et al.,*(1990),*Fusarium* spp. were isolated from soil samples and debris obtained at different altitudes of 0, 250, 500, 800, 1100, and 1400 m. Nineteen species were isolated, of which *F. oxysporum, F. equiseti, F. semitectum F. pallidoroseum*, *F. nygamai* and *F. solani* were frequently isolated. *Fusarium oxysporum* was the predominant species and was isolated from all soil samples. Other species recovered were *F. chlamydosporum, F. merismoides, F. lateritium*

*(*syn. *Gibberella baccata)*, *F. culmorum, F. compactum, F. dlamini, F. poae, F. proliferatum, F. moniliforme*, *F. graminearum*, *F. sambucinum*, *F. napiforme* and 3 unknown populations. This study demonstrated that debris was a more important source of *Fusarium* species diversity than soil (Jeschke *et al.*, 1990).

Species distribution is the manner in which a biological taxon is spatially arranged.The pattern of distribution is not permanent for each speciesas they respond to the availability of resources in their environment(Houchmandzadeh, 2009). There are three basic types of population distribution within an area, uniform, random and clumped. Clumped distribution is the most common type of dispersion found in nature where the distance between neighboring individuals is minimized (Taylor *et al*., 1978; Houchmandzadeh, 2009). This type of distribution is found in environments that are characterized by patchy resources. Clumped distribution is the most common type of dispersion found in nature because fungi need certain resources to survive, and when these resources become rare fungi tend to "clump" together around these crucial resources.

Fusarium propagules populate all agricultural fields, and have been found to be unevenly distributed in soil (Trujillo and Snyder, 1963; Wearing and Burgess, 1977;Smith, 2007).These fusarial populations vary from one area of a field to another and this erratic distribution is dependent on the immediate environmental conditions such as soil moisture, temperature, competitive and antagonistic microflora and a conducive soil type (Smith, 2007). Less common than clumped distribution, uniform distribution is where species are evenly spaced. In this type of distribution, the distance between neighboring

individuals of a population is maximized. The need to maximize the space between individuals generally arises from competition for a resource such as nutrients or moisture (Houchmandzadeh 2009).

Random distribution is rare in nature as biotic factors, such as the interactions with neighboring individuals, and abiotic factors, (such as climate or soil conditions) generally cause organisms to be either clustered or spread apart. Random distribution usually occurs in habitats where environmental conditions and resources are consistent, and also based on the idea that every species has equal opportunity and access to resources (Austin 2007;Houchmandzadeh, 2009).

There are various ways to determine the distribution pattern of species. Variance/mean ratio (VMR) is one such method that used to characterize the distribution of species, events or objects in time or space as described by Clark and Evans (1954). If the distribution is random, the VMR is about 1.0. , larger values (VMR >1.0) correspond to existence of "clumps" - spatial or temporal clusters. Smaller values (VMR < 1.0) correspond to a more-uniform-than-random distribution (often named "even", "uniform")(Clark and Evans, 1954; Ormerod and Vaughan, 2005).

2.3. Spread, Survival and Factor affecting survival of *Fusarium* species in soil

Fusarium species can be spread through, stakes, water, soil, infected transplants andsoiled equipment, tools, shoes and clothing (Egel and Martyn, 2007). Long distance spread is through seed, transplants or infected soil (Ploetz, 1994). *Fusarium*species can alsobe disseminatedvia wind blowing dust particles. Studies have also shown that

pathogenic *Fusarium* spp. in the soil may be disseminated by earthworms (Toyota and Kimura,1994). Insects generally do not appear to spread *Fusarium* species (Agrios, 1988; Yamoah *et al.*, 2011) except for the fungus gnats (*Bradysia* spp.), which have been shown totransmit *F.oxysporum* spores to host plants (Gillespie and Manzies, 1993; Elmer, 2008).

The survival of *Fusarium* species in soil is mainly in the form of chlamydospores. Thesespores are thick-walled and contain conspicuous lipid food reserves (van Eck, 1978). They develop directly from hyphal cells, microconidia or macroconidia that become stranded in soil and where germination cannot occur immediately (Li*et al.*, 1998). Chlamydospores enable *Fusarium*spp. to remain dormant in the soil during periods of absence of a suitable host. In this state, chlamydospores can survive long periods of unfavourable conditions such as droughts and low temperatures. These surviving propagules can persist in the soil for between 13 months to 40 years (Haware *et al.,* 1996; Vakalounakis and Chalkias, 2004).

On the other hand, species of *Fusarium* that do not produce chlamydospores such as *Fusarium verticillioides, F. proliferatum and F. graminearum* survive in the soil by colonising host tissues. In the absence of host tissues, these species can survive saprophytically from season to season by colonizing any stubble or plant debris remaining in the field after harvest (Bolkan *et al.,* 1979; Golkari *et al*., 2008;Wakelin *et al*., 2008). Besides survival by chlamydospores, some species of *Fusarium* have been

shown to survive in the fields by colonizing weeds thereby acting as reservoirs of infection (Katan, 1971; Fassihiani, 2000).

Studies have shown that survival of *Fusarium* spp. generally decreases with increased soil moisture content. Optimum growth and survival of *Fusarium*in soil has been reported to be between 15% to 30% moisture content(Oritsejafor, 1986; Bolkan *et al.,* 1979; Means and Kremer, 2007). However, *Fusarium* species have been shown to survive in soils with moisture content as low as 2.6% (Mandeel and Abbas, 1994) and as high as 78% (Senthilkumar *et al.,* 2011).Soil organic matter content is also another factor that is important for the survival of *Fusarium* in the soil as it provides a source of carbon for the growth of the fungus in the soil (Agrios, 1988). Generally, *Fusarium* spp. survive better in soils with higher soil organic matter content (Agrios, 1988; Fawole and Olowonihi, 2005), however, studies have shown that soils with low organic matter content (0% organic matter) can still cause Fusarium wilts (Larkin and Fravel, 2002;Wakelin *et al.* 2008). This survival is likely due to the fungus ability to produce chlamydospores that can continue to exist in the soil for a long time in an unfavorable environment (Vakalounakis and Chalkias, 2004).

On the other hand, high levels of soil organic matter content have been reported to supportthe existence of *Fusarium*-antagonistic rhizobacteria(*Bacillus* and *Pseudomonas*) and fungi (non-pathogenic *Fusarium* spp. and *Trichoderma* spp.)(Mandeel and Baker, 1991;Larkin and Fravel, 2002). Besides soil moisture content and organic matter, soil reaction with pH values ranging between 5–7 are most favourable for growth and survival of*Fusarium oxysporum*(Oritsejafor, 1986;HargreavesandFox, 1977).

Temperature is also a major climatic factor responsible for controlling the distribution pattern of *Fusarium* species(Agrios, 1988).Warm weather favors pathogenic species of *Fusarium* and species of *Fusarium* have been shown to survive at temperatures ranging from 10 to 35^{o}C (Fravel *et al.*, 1996; Larkin and Fravel, 2002). High temperatures stress the host plants, thereby increasing its susceptibility to infection(Saremi and Burgess, 2000; Doohan *et al.,* 2003).Zambia experiences a unimodal rainfall. It is wet between December to March and dry the rest of the year. The dry season can be divided into cool-dry season between April to July and the hot dry season(August- November). These conditionsfavoring survival of *Fusarium* spp. are characteristic to soils of Zambia thus making the occurrence of fungus in arable soils inevitable.

Fusarium species, including the wilt -causing species have been reportedoccur, survive, and grow in soils of all types,but sandy soils provide conditions that are most favorable forgrowth and development (Amir and Alabouvette, 1993;Yang *et al.,* 2000;Weber *et al.,* 2006). *Fusarium* wilt tends to be mostsevere in sandy soils and generally less of a problem in heavierclay soils (Scher and Baker, 1980; Larkin *et al*., 1993;Amir and Alabouvette, 1993). Arable soils in Zambia are of a diversity of textures from heavy clays to loose sands.

2.4.Species Diversity and Richness

Species diversity is a measure of the number of species and the relative contribution of each of these species to the number of individuals in a community (Swingland, 2001). A diversity index is a mathematical measure of species diversity in a community.The Shannon diversity index (H') is one such index that is commonly used to characterize

species diversity (Hurbert, 1971). This index not only measures species richness (species count), but also takes into account the relative abundance of species, or evenness. Normal values of H' as depicted in natural systems ranges from 1.5 for systems with low species richness and eveness to 3.5 for systems with high species evenness and richness. In general, it is thought that more disturbed and less stable environments should have lower H' values. High values of H' are representative of more diverse communities. The criteria adopted for the interpreting the Shannon- Wiener's diversity (Sudarma and Suprapta 2011) are as follows: H'<1= low diversity; H' 1-3= fair diversity and H' >3= high diversity.

It is reported that soils with high species diversity have high species richness (Rosenzweig, 1995; Stirling and Wilsey, 2001). A high diversity index of soil *Fusarium* species and other soil fungi is important for their involvement in soil structure formation (McIntosh 1967; McNaughton 1977; Tilman 1996); decomposition of organic matter; toxin removal;and the cycling of carbon, nitrogen, phosphorus, and sulphur (Garbeva *et al*., 2004). However, presence of diverse species of *Fusarium* in agricultural soil may lead to disease development in susceptible plants.

2.5. Plant infection

The pathogenic species of *Fusarium* in arable soil survives between growing seasons in infected plant debris as mycelium, microconidia, macroconidia and chlamydospores but most commonly as chlamydospores. *Fusarium* propagules spread by water, contaminated farm equipment, and also through infected transplants. Usually, once an area becomes

infested with *Fusarium*, it remains so for a long time (Hawar*et al.*, 1996; Vakalounakis and Chalkias, 2004).

When healthy plants grow in contaminated soil, the germ tube of spores or the mycelium penetrate root tips directly, or enter the roots through wounds caused by or at the point of formation of lateral roots (Rodriguez-Galvez and Mendgen, 1995). The mycelium advances through the root cortex intercellularly and, when it reaches the xylem vessels, enters them through pits. The mycelium then exclusively remains in the vessels and travels through them, mostly upwards, through the stem to the crown of the plant. While in the vessels, the mycelium branches and produces microconidia, which are detached and carried upward in the sap stream. The microconidia germinate at the point where their upward movement is stopped, the mycelium penetrates the wall of the vessel, and more microconidia are produced in the next vessels penetrating them through the pits (Charest, *et al*., 1984; Mendgen *et al.,* 1996).

The processes of xylem vessel clogging by mycelium, spores, gels, gums, and tylosis and crushing of the vessels by proliferating adjacent parenchyma cells are responsible for the breakdown of the water economy of the infected plant. When the amount of wateravailable to the leaves is below the required minimum for their function, the stomata close and the leaves wilt. The leaves finally die and this results in death of the rest of the plant (Agrios, 1988). The fungus then extensively invades the parenchymatous tissues of the dead plant and reaches the surface of the dead tissues, where it sporulates profusely. The spores may be disseminated to new plants or areas by wind, water, and so

on (Beckman, 1964;Agrios, 1988).Occasionally, the fungus in the vascular system may reach grains of cereals such as kernels of maize, rice and wheat thereby causing seed-borne infections (Griffiths and Lim, 1967; Kini, 2002; Wulff *et al*., 2010).Pumpkin, peas and tomato areotherexamples of plants whose seeds can be infected systemically by *Fusarium* in the vascular system (Agarwal and Sinclair, 1997; Kasuyama, 2000).

It is, therefore, apparent from the foregoing that cultivated soil is one of the sources of fusarial spores that may cause serious crop diseases leading to low yields. However, there is no information on the occurrence,distribution and diversity of *Fusarium* species in cultivated soils of Zambia.

3.0.CHAPTER THREE: MATERIALS AND METHODS

3.1.Study Area

This study was carried out around Lusaka District in the southern part of the central plateau of Zambia. Lusaka experiences a tropical Savannah type of climate. The soil types of Lusaka include sandy, silt and clay. These soil types are variously and widely distributed across the district.

Lusaka District was chosen as the study area because of its proximity to the laboratory facilities at the University of Zambia. The area also offers a variety of farms on which diversity of crops are grown.

3.2. Sampled Farms

The study included soil samples collected from four (4) sitesaround Lusaka District. Thesewere Lusaka North(LN), Lusaka East (LE), Lusaka South (LS) and Lusaka West (LW). The farthest points sampled being Fringilla area in the North (Great North road); Chilanga area in the South (Kafue road); Silverest area in the East (Great East road); and Kacheta area (Mumbwa Road) in the West as shown in Fig 1. The sampled farms were Morningside farm (LNF1), GART Research Field1 (LNF2), GART Commercial field 2(LNF3) in the North of Lusaka, Anviona farm (LEF1), Silver-Rivers(LEF2), Sable Walkover farm (LEF3) in Lusaka East, Mahesh farm(LSF1),Mt Makulu (LSF2), Thandiwe farm(LSF3) in the South of Lusaka,Sunlight farm(LWF1), Kaypi farm(LWF2) and Okapi farm (LWF3) in the West of Lusaka (Table 1).

Table 1.Codes of sampled farms

Location	Farm	Code
Lusaka North	Morningside	LNF1
Lusaka North	GART Research Field1	LNF2
Lusaka North	GART Commercial field 2	LNF3
Lusaka East	Anviona	LEF1
Lusaka East	Silver-Rivers	LEF2
Lusaka East	Sable Walkover	LEF3
Lusaka South	Mahesh	LSF1
Lusaka South	Mt Makulu	LSF2
Lusaka South	Thandiwe	LSF3
Lusaka West	Sunlight	LWF1
Lusaka West	Kaypi	LWF2
Lusaka West	Okapi	LWF3

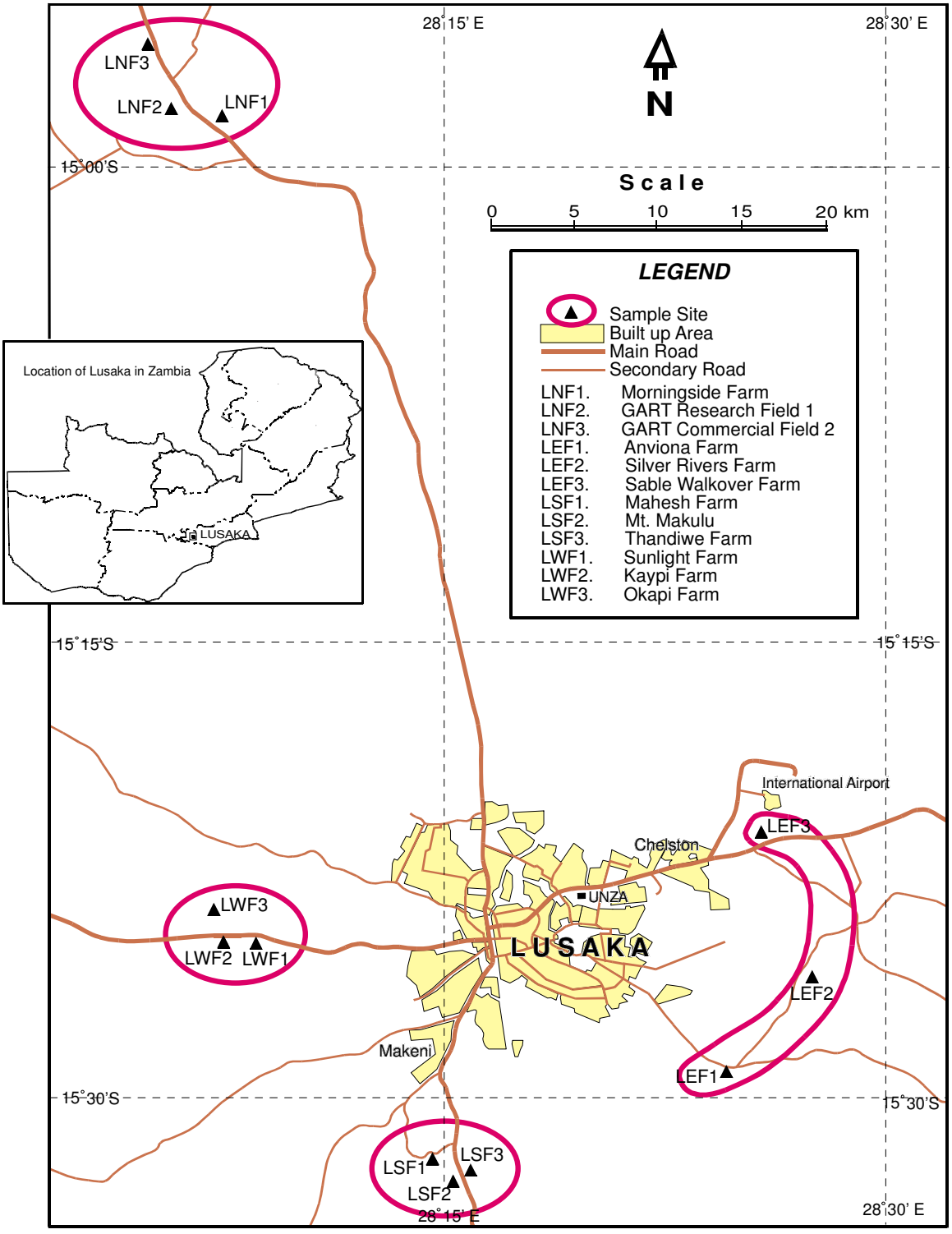
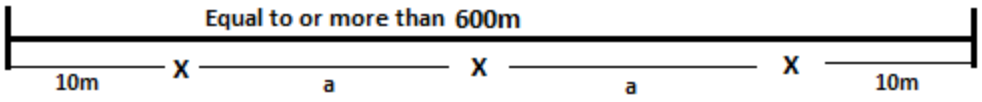


Figure 1:Location of study sites aroundLusaka District.

3.3.Sampling Design

Three (3) farms, approximately one kilometer apart, were randomly selected from each of the four sampling sites. Therefore, a total of twelve (12) farmswereincluded in the study. A transect along the longest line possible (length not less than 600m) was established in each study field in each field. Three (3) quadrats measuring 5m X 5m were established along the transect lines. The first quadrat was located ten metres (10m) from one end of the transect line, the second in the centre while the last quadrat was positioned ten metres (10m) from the other end of the transect line as shown in Figure 2. A total of 36 quadrats were established in the study area.



Key: a= equal distances, X=position of quadrats

Figure 2: Sampling design.

3.4. Sampling Procedure

Soil sampleswere collected within each established quadrats. Ten soil samples from a depth of 10cm were randomly collected per quadrat using anauger that had been sterilized by wiping with70% alcohol. Samples from each quadrat were bulked and mixed thoroughly (by hand shaking) in a clean and alcohol-swabbed bucket to make one composite soil sample.Hence,three subsamples were collected from each farm. The

subsamples were stored in labeled plastic bags in the laboratory at the University of Zambia until processing.

3.5. Data Collection Techniques and Tools

3.5.1. Determination of the occurrence, richness, diversity and distribution of *Fusarium*species in the study area.

In order to determine the occurrence, richness, diversity and distribution of *Fusarium* spp. in arable soils around Lusaka, fungal isolations were performed on soil samples collected from each quadrat following the Soil DilutionPour Platemethodaccording to Leslie *et al*. (1990). This method involves thorough mixing (by shaking) of a known mass of soil in sterile water and making serial dilutions from it.

Soil samples were air dried for 48 hours and then crushed to fine particles usingheat-sterilised mortar and pestle. One (1) g of crushedsoil sample was suspended aseptically into a beaker containing99cm^3 of sterile distilled water to make a 10^{-2} dilution. The soil suspension was then shaken for 5 minutes until all soil particles were evenly dispersed in the water. Following this dilution, 1cm^3 of the suspension (10^{-2} dilution) was withdrawn using a sterile pipette and transferred to another beaker containing 9 cm^3 of sterile distilled water to make the 10^{-3} dilution. Similarly, a 10^{-4}soil suspension was made. Four (4) replicate agar Petri plates of Selective Fusarium Agar (SFA) medium were inoculated with 1cm^3aliquots of each dilution. SFA was used for the isolation of *Fusarium* spp. (Leslie *et al*., 2006). The inoculated plates were incubated at room temperature (25$\pm$1^oC) until fungal colonies developed to sporulation stage.

Identified *Fusarium* colonies in mixed cultures, were then subcultured on fresh Potato Dextrose Agar (PDA) media to obtain pure isolates of *Fusarium* spp. The isolates were also incubated at room temperature for a period of six weeks to allow growth and production of chlamydospores.

Fusarium colonies appearing on culture plates were microscopically examined and identified to species levelfollowing established classification schemes of Booth (1971) and Leslie *et al.,* (2006). Images of the isolated *Fusarium* colonies were taken after staining with methyleneblue (Plates 1-8).The data collected from the above procedurewere summarized as the number of *Fusarium* spp. obtained per quadrat of a sampled farm.

3.5.2. Determination of soil physical and chemical characteristics

Soil samples collected were taken to the School of Agriculture, University of Zambia for analysis and for physical and chemical parameters. Soil reaction(pH $CaCl_2$), moisture content(oven drying method), organic matter (OM) (Walkley and Black method), Nitrogen(Kjeldahl Method), Phosphorus (Bray P1 Method), Potassium (K)(Ammonium acetate buffered at pH 7), Sulphur (S)(Morgan's Reagent), soil texture (Hydrometer method) tests were carried out on the sampled soils as follows (Schumacher *et al*., 1995);

Soil Reaction (pH – $CaCl_2$)

Ten grams soil was weighed into a 50cm^3 plastic container to which 25cm^3 of 0.01M $CaCl_2$ solution was added. The mixture was shaken for 30 minutes and pH read on pH meter (Radiometer Copenhagen PHM82) after the sediments had settled to the bottom.

Organic Matter – Walkley and Black Method

One gram sample was weighed and 10cm^3 Potassium dichromate added followed by 20cm^3 concentrated sulphuric acid. The mixture was then left to digest for 30minutes. Ten cubic centimetres ofconcentrated phosphoric acid was then added followed by 150cm^3distilled water. Ten drops diphenylamine indicator was then added before titrating with 1M ferrous sulphate solution.

Nitrogen–Kjeldahl Method

One gram of the sample digested with concentrated sulphuric acid for 1 hour in the presence of mixed catalyst ($CuSO_4$+KSO_4+Se). The digest was then distilled and the distillate collected in Boric acid indicator which turned from purple to green at the end point. The indicator solution was then titrated to the purple colour with 0.01N HCl solution and percent N calculated from the amount of acid consumed.

Plant Available Phosphorus –Bray P1 Method

Three grams of the soil was weighed and extracted with 21cm^3 Bray 1 Extraction solution. The mixture was shaken for 1 minute and filtered. Five cubic centimetres of filtrate were pipetted and mixed with 4cm^3 of ammonium molybdate, potassium antimony tartrate and dilute sulphuric acid mixture to develop a blue colour. The intensity

of the colour was read on a spectrophotometer (Milton Roy Spectronic601) to give concentration of P.

Potassium (K) –Ammonium acetate buffered at pH 7

Potassium was determined by atomic absorption spectrometer (AAS)[PerkinElmer Analyst 400] after being extracted with neutral ammonium acetate. Fifty cubic centimetres of ammonium acetate were added to 10g of soil and shaken for 30minutes. The mixture was then filtered and K determined from filtrate on AAS.

Sulphur (S) –Morgan's Reagent

Five grams of the sample soil was weighed and extracted with 25cm^3 Morgan's reagent (Sodium acetate dissolved in acetic acid). The mixture was shaken for 30 minutes and filtered. A portion of the filtrate was mixed with Barium Chloride to form a white precipitate whose turbidity was read on spectrophotometer (PerkinElmer Analyst 400) to give the concentration of S.

Soil Texture by Hydrometer method

Fifty grams of soil sample was dispersed with Calgon (sodium hexametaphosphate) for 5 minutes and transferred to a 1 litre measuring cylinder. The suspension was agitated for 1 minute using a metal plunger and the density of the suspension read after 40seconds to give the density of clay plus silt. The sample was left to stand for 2 hrs after which the density was again read to give the density of clay. The readings were then used to calculate the percentages of Sand, Clay and Silt. For the texture class, the USDA soil texture classification triangle was used to assign the soil its texture class.

Soil Moisture Content -Oven Drying Method

Ten grams (10g) of the soil sample was weighed and oven dried at 105^{o}C for 24hrs. After oven drying the soil was re-weighed and the difference in weight recorded as soil moisture content (mc).

3. 6. DATA ANALYSIS

3. 6. 1. Determination of the species richness and diversityof *Fusarium* species in the study area.

The*Fusarium*species diversity and richness (number of different species of *Fusarium*spp. encountered) wasobtained usingPC-ORD 5.0. computer software.PC-ORD is a Windows program for multivariate analysis of ecological data entered in spreadsheets. Data obtained was summarized in PCORD compact format, a text file consisting of pairs of species codes and abundance's. When using the compact format, two files were required: a species file (containing a list of species and codes), and the compact data file. Two types of compact data fileswere describednamely the quantitative lists of species present in each sample unit, and presence lists of species. The quantitative lists of species consist of species codes and abundance values whilethe presence lists of species consist of a list of species' code numbers. The data was then summarized with descriptive statistics (mean, standard deviation, sum), and diversity measures. The measures of calculateddiversity included species richness (S; the number of species in a sample unit) andH' = Shannon diversity- species diversity.

Shannon-Wiener's Diversity Index

$$H' = \sum_{i=1}^{s} - (P_i * \ln P_i)$$

where:

H' = the Shannon diversity index

P_i = fraction of the entire population madeup of species i

S = numbers of species encountered

$\sum$ = sum from species 1 to species S

The general criteria for interpreting the Shannon-Wiener's diversity (Ferianita-Fachrul *et al*., 2005) is as follows: H'<1= low diversity; H' 1-3= fair diversity and H'>3= high diversity.

The data obtained was subjected to the square root transformation by taking the square root of each observation.Analysis of Variance (ANOVA)was applied to the transformed data generated (occurrence, diversity and richness) to determine if there were significant differences among the twelve sampled farms around Lusaka District.

3. 6. 2. Determination of the distribution of *Fusarium* species in the study area

The distribution index ratio defined as thevariance/mean ratio(Southwood, 1978),was calculated for the *Fusarium* species datato determine the distribution of*Fusarium*speciesamong sampled farms and within sampled sites. Its formula consists of

dividing the variance (square of the standard deviation) by the mean. A distribution index ratio of less than 1 is indicative of a regular dispersion. If the ratio equals 1, it indicates that the population is randomly distributed, while a distribution index ratio greater than 1 indicates an aggregated dispersion pattern.

4.0.CHAPTER FOUR: RESULTS

4.1. General

This study consisted of analyzing soil samples from 12 farms around Lusaka District for occurrence of fungal species. The geographical locations of the farms, crops grown on each farm at the time of soil sampling and the previously grown crops are shown in Appendix 1. The major crop grown was wheat. Previously (during the 2009/10 cropping season), these farms had soybeans. At the time of sampling,LEF1 and LNF1 farms were growing vegetables as in the previous season. There were no crops grown in the fields at LSF2 but previously maizewas grown.

4.2. Soil Physical and Chemical Properties of the soils at the farms sampled

Soil fertility status was assessed from the analysis of nitrogen (N), phosphorus (P), potassium (K), organic matter content (OM), moisture content (MC) and soil texture. These physical and chemical properties of soils from the sampled farming units are shown in Table 2 and Table 3.

Table 2: Chemical properties of soils from selected farmsaround Lusaka District.

FARM	N%	P(mg/kg)	K(mg/kg)	S(mg/kg)	OM%	pH
LEF1	0.21	61.67	147.39	42.67	2.97	6.8
LEF2	0.16	45.19	88.48	54.61	1.85	6.7
LEF3	0.18	39.40	90.36	17.78	1.30	7.0
LNF1	0.15	15.09	87.47	24.34	2.30	7.9
LNF2	0.18	20.50	135.00	19.80	2.50	7.2
LNF3	0.19	19.47	81.88	22.61	1.85	6.9
LSF1	0.16	34.25	75.29	17.50	1.81	7.5
LSF2	0.19	8.44	155.30	35.11	2.87	6.9
LSF3	0.18	4.21	140.29	23.72	2.23	5.5
LWF1	0.19	14.29	112.61	27.45	2.64	6.8
LWF2	0.16	44.05	108.41	26.28	2.34	7.6
LWF3	0.21	46.27	92.68	29.94	2.85	7.4

Key:

N=Nitrogen, P=Phosphorus, K=Potassium, S=Sulphur, OM= Organic matter.

The highest values of plant available soil phosphorus (61.67 mg/kg) and potassium (147.39 mg/kg) were recorded from LEF1. The farm with the lowest amount of phosphorus (4.21 mg/kg) was LSF3, whereas the one with the lowest amount of potassium was LSF1 (75.29 mg/kg). Sulphur was highest in soils from LEF2 (54.61 mg/kg) while LSF1 showed the lowest sulphur content (17.5 mg/kg). Total soil nitrogen was highest in soils from LWF2 and LEF1 (0.21%) and low in soil from LNF1 (0.15%) in the North of Lusaka District.

Table 3: Physical properties of soils from selected farms around Lusaka District.

FARM	MC (%)	Sand (%)	Clay (%)	Silt (%)	TEXTURE
LEF1	19.6	47	21	32	Loam
LEF2	11.3	55	16	29	Sandy loam
LEF3	8.7	53	9	38	Sandy loam
LNF1	19.2	49	35	16	Sandy clay loam
LNF2	13.3	42	37	22	Clay loam
LNF3	14.3	43	33	24	Clay loam
LSF1	18.7	47	21	32	Loam
LSF2	13.4	44	31	25	Clay loam
LSF3	16.7	48	29	23	Sandy clay loam
LWF1	15.6	49	19	32	Loam
LWF2	8.1	48	27	25	Sandy clay loam
LWF3	9.6	39	31	30	Sandy clay loam

Key:

MC=Moisture content.

The measured pH ranged from 5.5-7.8, with the most alkaline soils (pH=7.86) being from LNF1 while the most acid (pH=5.48) was from LSF3 in the South of Lusaka. Significant differences (p=0.05) in pH among sampled farms andsignificant interactions between farms and sites were noted.

The soil moisture content of samples ranged from8.1% to 19.6%. Soil from LEF1 contained the highest moisture content (wetter) while LWF2 was drier (8.1%). Significant differences (p=0.05) in soil moisture content among farms were observed. Organic matter content was also observed to be high (~3%) in soil from LEF1 while soil from LEF3 measured the lowest organic matter content (1.3%).Significant differences (p=0.05) in soil organic matter content among sampled sites were noted.The textures of the soils were loam, sandy-loam, sandy-clay-loam and clay-loam.Sandy-clay-loam soils

were encountered at four of the twelve sites, loam and clay-loam soil texture were each identified at three farm sites while sandy-loam soil was found in two of the twelve farm sites (Table 3).

4.3. Occurrence of Fusarium species in arable soils of Lusaka District.

Thirty-seven (37)isolates of *Fusarium* species were recovered from sandy-clay-loam soils, 27 from loam soils, 23 isolates from clay-loam and 18 from sandy-loam soils (Fig 3).

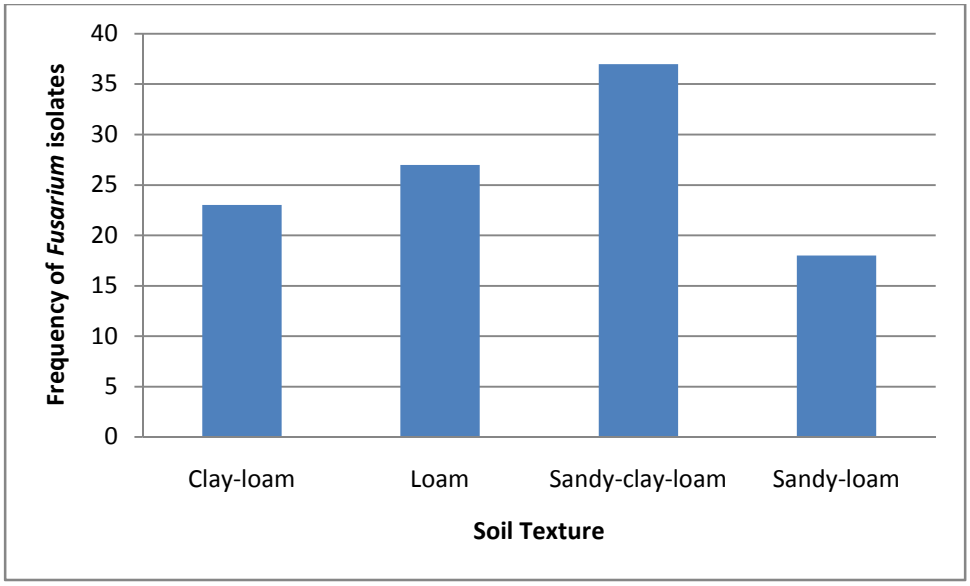


Figure 3: Occurrence of*Fusarium* isolates in different textured soil.

From the soil samples collected from the 12 farms, a total of 105 isolates of *Fusarium* species were recovered (Table 4). Out of these, eight (8) species of *Fusarium* were characterized and identified (Table 5).

Table 4: *Fusarium* species isolated from arable soils around Lusaka District

Fusarium species*/Farms	LEF1	LEF2	LEF3	LNF1	LNF2	LNF3	LSF1	LSF2	LSF3	LWF1	LWF2	LWF3	Tot	%
F. oxysporum	6	4	4	8	3	3	6	3	2	5	4	2	50	48
F. solani	1	2	4	4	1	0	2	5	3	2	6	2	32	31
F. verticillioides	1	0	0	2	0	1	0	0	0	1	1	0	6	5.7
F. nelsonii	0	0	2	1	0	1	1	0	0	0	0	0	5	4.8
F. proliferatum	0	1	0	0	0	0	0	1	0	0	1	0	3	2.9
F. badinda	1	0	0	0	1	0	0	2	0	0	0	0	4	3.8
F. crookwellense	0	0	1	0	0	1	0	1	0	0	0	0	3	2.9
F. denticulatum	1	0	0	0	0	0	0	0	0	0	0	1	2	1.9
	10	7	11	15	5	6	9	12	5	8	12	5	105	100

Key:
LEF1=Lusaka East farm 1(Anviona), LEF2= LusakaEast farm 2 (Silver Rivers), LEF3= LusakaEast farm 3 (Sable Walkover), LNF1=LusakaNorth farm 1 (Morningside), LNF2=LusakaNorth farm 2 (GART research field 1), LNF3=LusakaNorth farm 3 (GART commercial field 2), LSF1 =LusakaSouth farm 1 (Mahesh), LSF2= LusakaSouth farm 2 (Mt Makulu), LSF3=LusakaSouth farm 3 (Thandiwe), LWF1=LusakaWest farm 1(Sunlight),LWF2=LusakaWest farm 2 (Kaypi), LWF3= LusakaWest farm 3 (Okapi).

*Plates 1 to 8 in Appendix.

Fusarium oxysporum was isolated from all the 12 farms, followed by *F. solani* occurring in 11 farms, *F. verticillioides* in five farms, *F. nelsonii* in four, while *F. proliferatum, F. badinda* and *F. crookwellense* were isolated in three farms. *Fusarium denticulatum* was the least abundant having been isolated in two out of twelve farms (Table 4).*Fusarium oxysporum* also gave the highest number of isolates (50) identified followed by *F. solani*(32).

The Analysis of Variance for occurrence of *Fusarium* spp. revealed that the occurrence of the fungi at sites North, East, South and West of Lusaka were non-significant (Table 5) but there were significant differences (p=0.05) among farms and measuredinteractionsbetween farms and sites were also significant.The highest

occurrence of the isolates was at LNF1 while the lowest were at farms LNF2, LSF3 and LWF3 (Table 6).

The significant interactionsbetween site and farm were noted with regard to occurrence. Occurrence at farm 1 (LWF1) was lower than that at farm 2 (LWF2), 2.236 and 1.911, respectively at site 4 (West of Lusaka). At site 1 (North of Lusaka), LNF1 was greater than LNF2, and at site 3(South of Lusaka) the reverse happened(Table 6 and Figure 4).

Table 5. Analysis of Variance-Occurrence

Source of variation	d.f.	s.s.	m.s.	f (v.r.)	p (F pr.)
Site	3	0.04819	0.01606	0.65	0.610 (NS)
Farm	2	0.65161	0.3258	3.88	0.042***
Site x Farm	6	1.92459	0.32077	3.82	0.015***
Total	35	4.27607			

Table 6.Means ofoccurrence of *Fusarium*species at four sites and three farms used in the study.

	Farm 1(F1)	Farm 2(F2)	Farm 3(F3)	Means
Lusaka West (LW)	1.911	2.236	1.609	1.919
Lusaka North (LN)	2.444	1.609	1.715	1.923
Lusaka South (LS)	1.989	2.215	1.626	1.943
Lusaka East (LE)	2.079	1.794	2.157	2.010
Means	2.106	1.964	1.777	1.949
Site				
LSD	0.181			
CV%	4.600			
Farm				
LSD	0.251			
CV%	4.200			
For interactions (Site x Farm)				
LSD	0.432			
CV%	14.900			

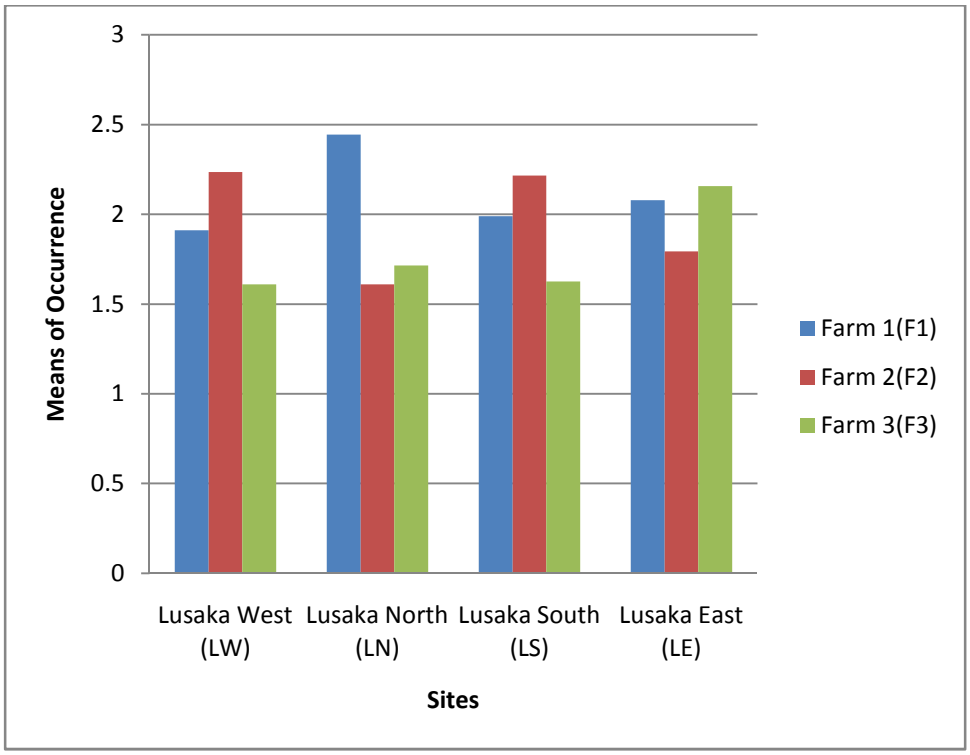


Figure 4. Occurrence of *Fusarium* species at different sites around Lusaka District

4.4. Diversity of *Fusarium* species in arable soils of Lusaka District.

The Analysis of Variance for species diversity of *Fusarium* spp. revealed that the diversity of the fungi at sites North, East, South and West of Lusaka were non-significant(Table 7), but the interactionsbetween farms and sitesfor this parameterwere significant (p=0.05).The highest *Fusarium* species diversity was at LSF2 and the lowest at farms LNF2, LSF3 and LWF3 (Table 8). A significant interaction wasmeasured with differential response with regard to diversity. Diversity at farm 1 (LWF1) was lower than that at farm 2 (LWF2), 1.194 and 1.369, respectively at site 4 (West of Lusaka). At site 1 (North of Lusaka), LNF1 measured a higher value than LNF2, and at site 3(South of Lusaka) the reverse happened(Table 8 and Figure 5).

Table 7. ANOVA-Diversity

Source of variation	d.f.	s.s.	m.s.	f (v.r.)	p (F pr.)
Site	3	0.03457	0.01152	0.57	0.652 (NS)
Farm	2	0.0718	0.0359	1.8	0.197 (NS)
Site x Farm	6	0.39746	0.06624	3.32	0.026***
Total	35	0.9462			

Table 7. Means of Species diversity of *Fusarium* species at the four sites and three farms used in the study.

	Farm 1(F1)	Farm 2(F2)	Farm 3(F3)	Means
Lusaka West (LW)	1.194	1.369	1.094	1.219
Lusaka North (LN)	1.391	1.094	1.194	1.226
Lusaka South (LS)	1.277	1.422	1.100	1.266
Lusaka East (LE)	1.326	1.183	1.375	1.295
Means	1.297	1.267	1.191	1.252
Site				
LSD	0.1633			
CV%	6.500			
Farm				
LSD	0.122			
CV%	0.800			
For interactions (Site x Farm)				
LSD	0.240			
CV%	11.300			

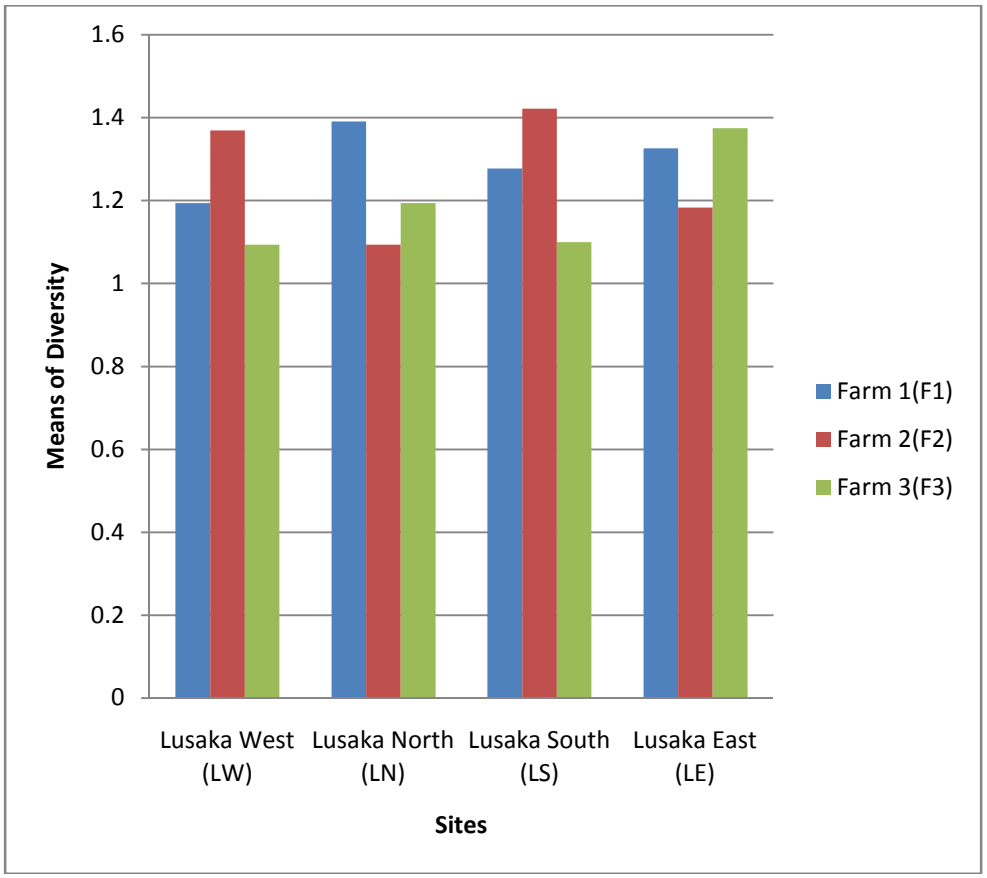


Figure 5. Species diversity of *Fusarium* species at different sites around Lusaka District.

4.5. Species richness of *Fusarium* species in arable soils of Lusaka District.

Analysis of Variance for species richness of *Fusarium* spp. showed that species richness was non-significantfor the sites and farms (Table 9), but there was a significant difference($p=0.05$) in the interaction between farms and sites.The highest species richness was recorded at LSF2 and the lowest at farms LNF2, LSF3 and LWF3 (Table 10).

The significant interaction was noted with differential response with regard to species richness. Richness at farm 1 (LWF1) was lower than that at farm 2 (LWF2), 1.626 and

1.911, respectively at site 4 (West of Lusaka). At site 1 (North of Lusaka), LNF1 was higherthan LNF2, and at site 3(South of Lusaka) the reverse happened(Table 10 and Figure 7).

Table 9. ANOVA -Richness

Source of variation	d.f.	s.s.	m.s.	f (v.r.)	p (F pr.)
Site	3	0.05234	0.01745	0.63	0.619 (NS)
Farm	2	0.14825	0.07412	2.34	0.128 (NS)
Site x Farm	6	0.93385	0.15564	4.92	0.005***
Total	35	1.8093			

Table 10. Means of Species richness of *Fusarium* species at the four sites and three farms used in the study.

	Farm 1(F1)	Farm 2(F2)	Farm 3(F3)	Means
Lusaka West (LW)	1.626	1.911	1.520	1.686
Lusaka North (LN)	1.989	1.520	1.626	1.712
Lusaka South (LS)	1.732	2.000	1.520	1.751
Lusaka East (LE)	1.821	1.626	1.911	1.786
Means	1.792	1.764	1.644	1.734
Site				
LSD	0.191			
CV%	5.500			
Farm				
LSD	0.154			
CV%	0.700			
For interactions (Site x Farm)				
LSD	0.295			
CV%	10.300			

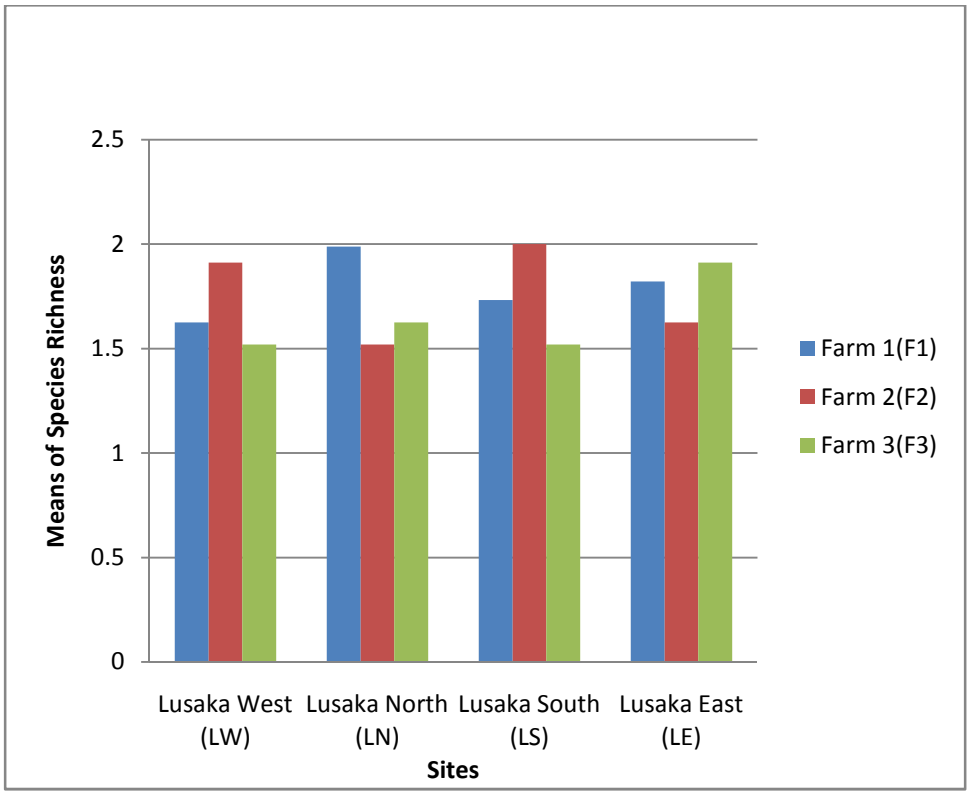


Figure 6.Species richness of *Fusarium* speciesat different sites on 12 farms around Lusaka District.

4.6. Distribution pattern of *Fusarium* species in arable soils on farms around Lusaka District.

Fusarium species isolated from soils from LSF2 were distributed randomly (variance/mean ratio=1) as shown in Table 8. Samples from LEF2 in the East, LNF3and LSF3 showed a regular distribution of *Fusarium* spp. (variance/mean ratio<1) and the other eight samples showed an aggregated distribution of *Fusarium* species (variance/mean ratio>1).

Table 11: The distribution of *Fusarium* species within sampled farms.

FARM	No. samples	Mean *Fusarium* spp.	Variance	Variance/Mean
LEF1	3	3.33	4.33	1.30
LEF2	3	2.33	0.33	0.14
LEF3	3	3.67	4.33	1.18
LNF1	3	5.00	12.00	2.40
LNF2	3	1.67	2.33	1.40
LNF3	3	2.00	1.00	0.50
LSF1	3	3.00	7.00	2.38
LSF2	3	4.00	4.00	1.00
LSF3	3	1.67	1.33	0.80
LWF1	3	2.67	6.33	2.38
LWF2	3	4.00	7.00	1.75
LWF3	3	1.67	2.33	1.40

Table 12: Distribution of*Fusarium* species across sampled sites.

Sites	No. samples	Mean *Fusarium* spp.	Variance	Variance/Mean
East (LE)	3	9.33	4.33	0.46
West (LW)	3	8.33	12.33	1.48
South (LS)	3	8.67	12.33	1.42
North (LN)	3	8.67	30.33	3.50

Fusarium species isolated from East of Lusaka (LE) were regularly distributed (variance/mean ratio<1) and the other three sampled areas indicated an aggregated distribution of *Fusarium* species (variance/mean ratio>1) as shown in Table 12.

5.0.CHAPTER FIVE: DISCUSSION

A total of 105 isolates of *Fusarium* species were obtained and characterized from soils collected from 12 farms around Lusaka District. The current study has shown that eight species of *Fusarium*are widely distributed in the arable soils namely *F. oxysporum, F. solani, F. verticillioides, F. nelsonii, F. proliferatum, F. badinda, F. crookwellense* and *F. denticulatum*.

Earlier studies of soil fusaria in Africa and Asia show variable numbers and species composition. Forty two isolates of the fungus have been reported in soils of Malaysiain which four *Fusarium* species were identified namely, *F. solani, F. semitectum, F. equiseti* and *F. oxysporum* (Latiffah *et al*., 2007). Another study of soils from Lesotho, Nigeria and Zimbabwe showed the occurrence of *F. oxysporum, F. equiseti, F. solani, F. moniliforme, F. compactum, F. nygamai,* and*F. chlamydosporum*.In addition, there were eight other*Fusarium* species isolated *viz**F. merismoides, F. polyphialidicum, F. graminearum, F. subglutinans, F. sambucinum, F. longipes, F. semitectum*and*F. lateritium*(Nwanwa and Nelson, 1993; Fawole and Olowonihi, 2005). Soils of Mahlanya, Swaziland, were also studied and showed the occurrence of only two speciesviz.*Fusarium moniliforme* and *Fusarium thapsinum*(Mansuetus *et al*., 2000). A similar study ofsoils of South Africashowed the presence of nineteen species.These were*F. chlamydosporum, F. merismoides, F. lateritium*, *F. culmorum, F. compactum, F. dlamini, F. poae, F. proliferatum, F. verticillioides*, *F. scirpi, F. polyphialidicum,F. graminearum*, *F. sambucinum*, *F. napiforme, F. oxysporum, F. equiseti, F. semitectum*, *F. nygamai* and *F. solani*. Of these *F. oxysporum, F. equiseti, F. semitectum*, *F. nygamai*

and *F. solani* were the most frequently isolated(Jeschke *et al.*, 1990).Soils of Kenya showedthe presence of 26 species of *Fusarium*(Maina *et al*., 2009).

The present study hasshown that there are significant differences (p=0.05) in the occurrence of *Fusarium* spp. among the farms sampled and the measured interactions between farms and sites. The occurrence of*Fusarium* spp. indicated that soilsof some sites and farms were favorable for growth and survival of the fungus. This could be due to the differences in soil textural types (Larkin *et al*., 1993; Amir and Alabouvette, 1993), moisture content (Senthilkumar *et al*., 2011), pH (Hargreaves and Fox, 1977;Oritsejafor, 1986) and organic matter content (Wakelin *et al.*, 2008).Medium-textured soils (sandy-clay-loam) generally support the occurrence of *Fusarium* spp. while heavier clay soils (loam and clay-loam) suppress their existence (Scher and Baker, 1980; Yang *et al*., 2000). Soil organic matter content has also been reported to be responsible for the high numbers of *Fusarium* spp. in soil by Fawole and Olowonihi (2005).

The high occurrence of *Fusarium* isolates in soils from LNF1 may be attributed to its favourable sandy soil texture (sandy-clay-loam),soil moisture (19.2%) and high organic matter content (2.30%) as reported by Larkin *et al*., (1993), Fawole and Olowonihi, (2005)and Senthilkumar *et al*., (2011).The farms with lowest occurrence of *Fusarium*isolates were LNF2, LSF3 and LWF3 despite showing favourable conditions for growth and survival of the fungus. This could be due to the inhibitory effects of certain soil borne antagonistic fungi such as *Trichoderma* species (Inam-ul-haq *et al*.,

2009). These species thrive in sandy-clay-loam and clay loam soils (Inam-ul-haq *et al*., 2009).

The species diversity and richness results obtained for the studied farms did not differ markedly from each other as C.V. values were low(0.8 and 0.7, respectively). However, significant interactions between farms and sites were noted for species diversity and richness of *Fusarium* spp. This indicates thatsoils of some farms and sites were suitable for theexistence and survival of a variety of *Fusarium* spp. Species diversity and richness at LSF2 was highest and least at LNF2 and LWF3 (Table 7 and Table 9).Thismay be due to the variation in physical andchemical propertiesof these soils, and the crop types grown. These factors have been shown to affect the occurrence and recovery of *Fusarium* spp. by Bumbieris and Lloyd (1967), Mullins *et al*. (1990),Ocamb and Kommedahl (1994), Alabouvette, (1999), Mauseth, (2008), Rousk *et al*.(2010) and McGuire *et al*.(2012). It is reported that soils with high species diversity have high species richness (Rosenzweig, 1995; Stirling and Wilsey, 2001).This trend is clearly indicated by the results of the present study.

Diversity of *Fusarium* spp. and other organisms have been reported to contribute to the availability of essential elementsin the soil for plant growth (Kaufman and Blake, 1973; Regalado *et al*., 1997; Lozovaya *et al*., 2006; Sagar and Singh, 2010; Thion *et al*., 2012).Of the three distribution types of organisms that occur in nature, the clumped distribution of *Fusarium* species in the soil was found to be the most common type which has also been reported by Veech *et al.* (2003), Ormerod and Vaughan (2005)and Mauseth (2008).

The results of this study demonstrate that *Fusarium oxysporum*(48%) and *F. solani*(31%) are the most predominant species(Table 4). This may bedue to their resilience to a wide range of soils (Amir and Alabouvette, 1993; Weber *et al*., 2006).The resilience of*Fusarium oxysporum* and *F. solani* is indicated by their occurrence as soil pathogens and also as saprophytes(Nemec, 1987;Larkin and Fravel, 1998; Larkin and Fravel, 2002). The plant pathogenic representatives of *Fusarium oxysporum*are involved in a variety of plant diseases such as vascular wilts, damping-off, crown rots and root rots (Jarvis and Shoemaker, 1978; Summerell and Rugg, 1992).

The results of this study clearly elucidate the presence of a variety of *Fusarium* spp. in the arable soils around Lusaka District. Some of these species may cause plant diseases while others may occur as non-pathogens responsible for a variety of health problems.

6.0. CHAPTER SIX: CONCLUSION AND RECOMMENDATION

The study has shown that *Fusarium* species occur in arable soils of Lusaka District.A total of 105 isolates of *Fusarium* species were isolated from soils collected from 12 farms and four sites from the study area. These were characterized and identified in 8 distinctspecies of *Fusarium*. The study has also shown that of these eight species, *Fusarium oxysporum* and *F. solani*were the most predominant species with regards to the frequency of occurrence. There was significant differences (p=0.05) in interactions between farms and sites sampledwith regards to species occurrence, diversity and richness. The study also demonstrates that the clumped distribution type of *Fusarium* spp. is the most common type. The studyprovides the baseline information on the occurrence, diversity and distribution of *Fusarium* spp. in agricultural soils of Zambia. These findings suggest that farmers should avoid cultivating of *Fusarium*-susceptible crops in such soils.

RECOMMENDATION

Following this investigation, it is apparent that this research on soil *Fusarium* species must be extended to major farming blocks around Zambia so as to provide a good working collection for the study of variability and population dynamics of various species of *Fusarium* indigenous to Zambian soils.

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Appendix 1: The crops and location of collected soil samples

	Farm Area	UTM/UPS Coordinates	Hdddmmss Coordinates	Crops in Field	Crops grown previously
Mahesh	Lusaka South	Ele: 1246m 35L 0634398 UTM 8282316	S15^{o} 31' 59.1'' E028^{o} 15' 11.5''	Wheat	Soy Beans
Mt Makulu	Lusaka South	Ele:1229m 35L 0634063 UTM 8280701	S15^{o} 34' 51.7'' E028^{o} 15' 00.7''	None	Maize
Thandiwe	Lusaka South	Ele:1258m 35L 0636344 UTM 8282499	S15^{o} 31' 52.9'' E028^{o} 16' 16.7''	Wheat	Soy Beans
Sunlight	Lusaka West	Ele: 1242m 35L 0623626 UTM 8295213	S15^{o} 25' 01.4'' E028^{o} 09' 07.7''	Wheat	Maize
Kaypi	Lusaka West	Ele:1241m 35L 0621718 UTM 8295605	S15^{o} 24' 49.0'' E028^{o} 08' 03.6''	Wheat	Soy Beans
Okapi	Lusaka West	Ele: 1221m 35L 0621189 UTM 8296805	S15^{o} 24' 10.1'' E028^{o} 07' 45.6''	Wheat	Soy Beans
Morning side	Lusaka North	Ele:1140m 35L 0620841 UTM 8342901	S14^{o} 59' 10.0'' E028^{o} 07' 26.0''	Potatoes	Cabbage, Tomatoes
GART Research Field 1	Lusaka North	Ele:1144m 35L 0618331 UTM 8344724	S14^{o} 58'11.2'' E028^{o} 06'01.7''	Wheat	Soy Beans
GART Commercial field 2	Lusaka North	Ele: 1175m 35L 0616870 UTM 8348608	S14^{o} 56' 06.0'' E028^{o} 05' 12.1''	Wheat	Soy beans

Appendix 1 continued: The crops and location of collected soil samples.

Farm Name	Farm Area	UTM/UPS Coordinates	Hdddmmss Coordinates	Crops in Field	Crops grown previously
Anviona	Lusaka East	Ele:1308m 35L 0651356 UTM 8287517	S15^{o} 29' 06.3'' E028^{o} 24' 38.8''	Vegetables (Lettuce, potatoes, rape)	Vegetables (carrots, onions,cabbage)
Silver Rivers	Lusaka East	Ele: 1186m 35L 0656354 UTM 8293828	S15^{o} 25' 40.1'' E028^{o} 27' 25.7''	Wheat	Soy Beans
Sable Walkover	Lusaka East	Ele: 1166m 35L 0653448 UTM 8301772	S15^{o} 21' 22.2'' E028^{o} 25' 46.8''	Wheat	Soy Beans

Appendix 2: Morphological Characteristics of *Fusarium* species isolated from 12 farms around Lusaka District of Zambia.

Species	Microconidia	Macroconidia	Conidiophore	Chlamydospores	Colony color on PDA
Fusarium crookwellense	Not observed	Falcate with dorsal side more curved than ventral, usually 5-septate, pronounced foot shaped basal cell, curved and tapering apical cell	Not Observed	Smooth walled in chains and clusters	White colony with red pigments
Fusarium solani	Oval, 0-1 septate	Straight to slightly curved, 3-7 septate, poorly developed basal cell	Long monophialides	Smooth in pairs and chains	White sparse mycelium
Fusarium verticillioides	Oval to club shaped, 0 septate in long chains	Not observed	Monophialides	Not observed	Purple-white, violet pigmentations
Fusarium badinda	Fusiform,0-septate	Falcate,foot shaped basal cell,3- septate, hooked-curved apical cell	Not observed	Singly in pairs and chains	White- violet, violet pigmentation
Fusarium denticulatum	Long oval , fusiform,0-3 septate	Slender and slightly curved, 3-5 septate, foot shaped basal cell, beaked apical cell.	Finger-like polyphialides	Not observed	White and brown pigments
Fusarium proliferatum	Club shaped, 0- septation in short to moderate lengthed chains	Relatively straight, 3-septate, poorly developed basal cell, curved apical cell	Not observed	Not observed	Purple- violet colonies, colorless pigmentation

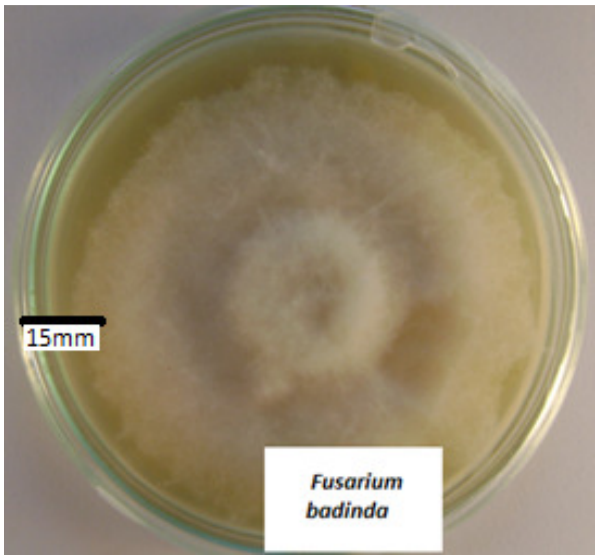
Appendix 2 continued: Morphological Characteristics of *Fusarium* species isolated from 12 farms around Lusaka District Zambia

Species	Microconidia	Macrocondia	Conidiophore	Chlamydospores	Colony color on PDA
Fusarium oxysporum	Oval, elliptical,0-septate	Medium length, slightly Curved,3-septate,foot shaped basal cell, curved apical cell	Short monophialides	Smooth or rough walled formed singly and in pairs	Floccose white and pale brown colonies dark violet and no pigmentation
Fusarium nelsonii	Straight, 0-3 septate	Straight and curved, 3 septate, foot shaped basal cell, curved apical cell.	Polyphialides	Verrucose and pale brown in chains and clusters	Floccose colony with red pigmentation

PLATES 1 to 8

Morphological and conidial types of 8 *Fusarium* species identified in this study.

PLATE 1*: Fusarium badinda*

a 

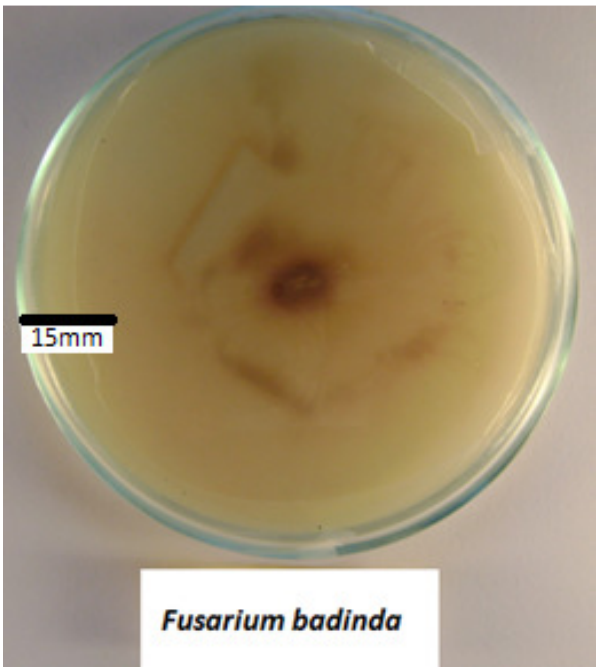
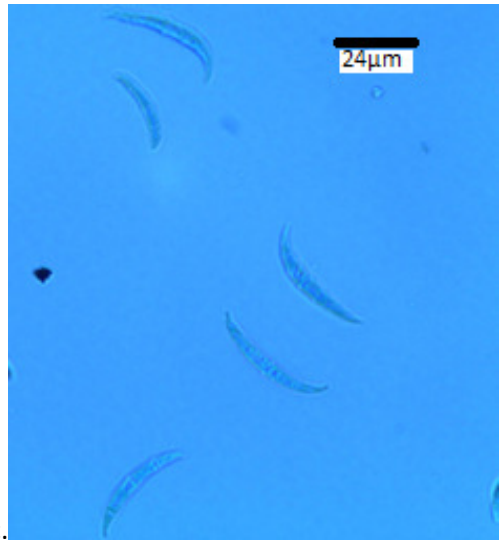
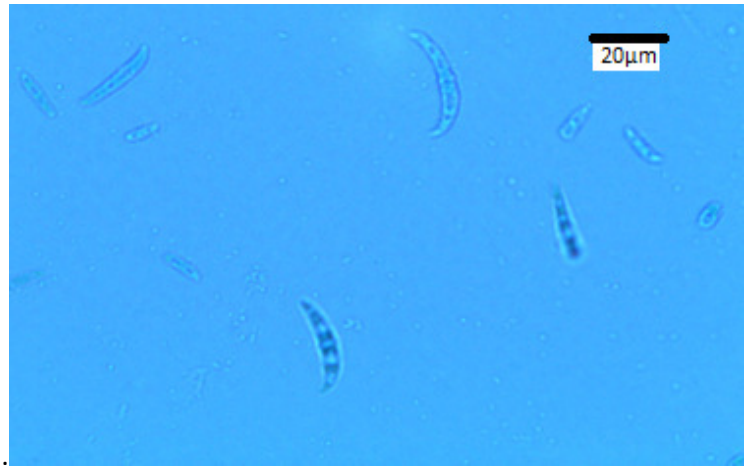
b. 

Plate 1 continued on page 71.

c. 

d. 

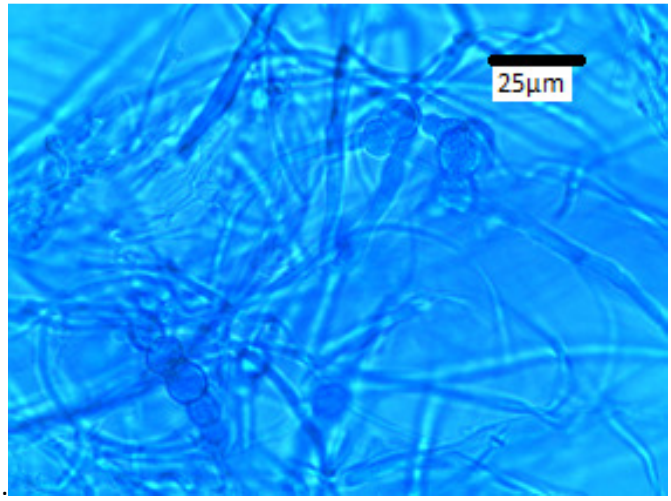
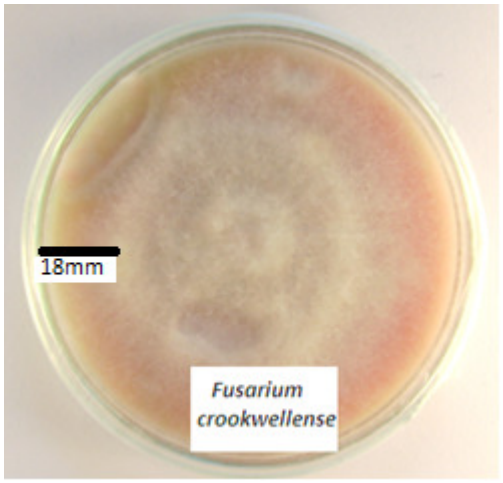
e. 

Plate 1.*Fusarium badinda*, (a), white-pale purple colony on PDA; (b), pale purple pigments in center on PDA; (C), macroconidia (falcate with hooked- curved apical cell, 3 septate 30-40 x 2-3 micrometersin size) ; (d), microconidia (fusiform,0-1 septate,7-13 micrometers in size); (e), chlamydospores produced singly, in pairs, clusters and chains.

PLATE 2: *Fusarium crookwellense*

a.



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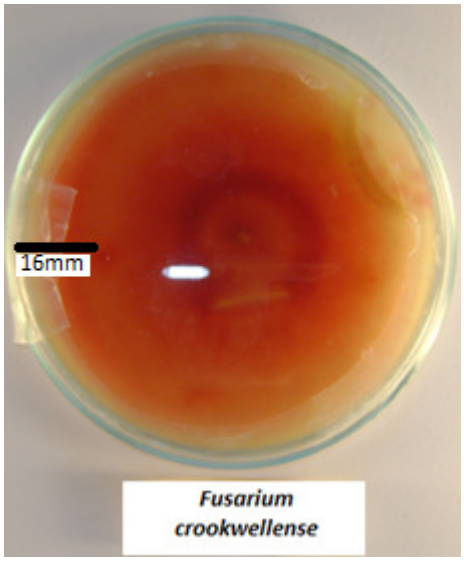

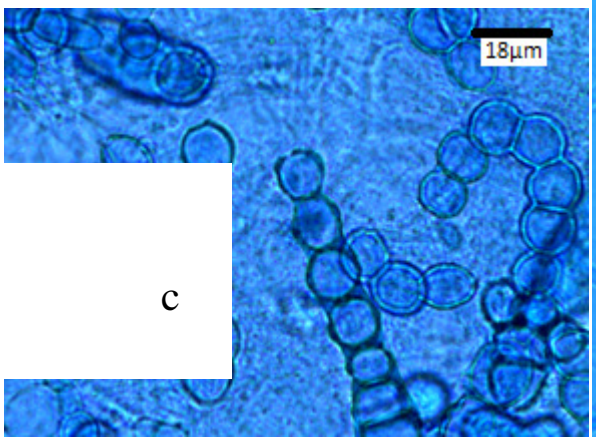

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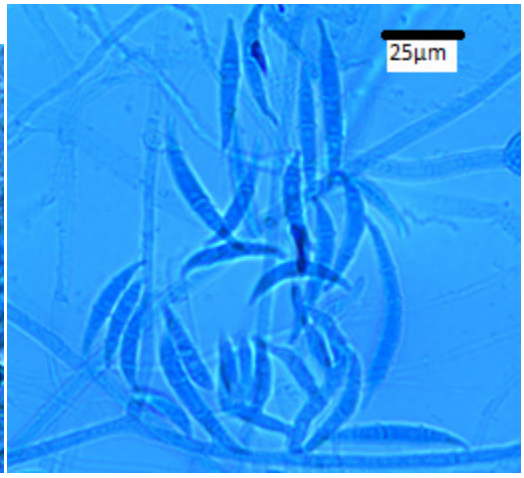

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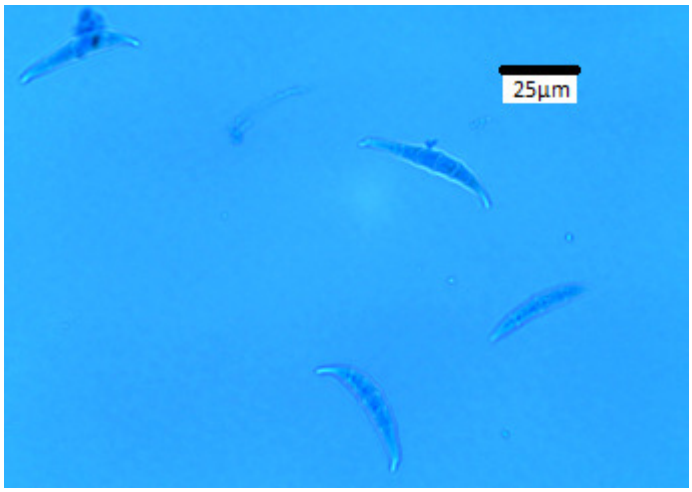
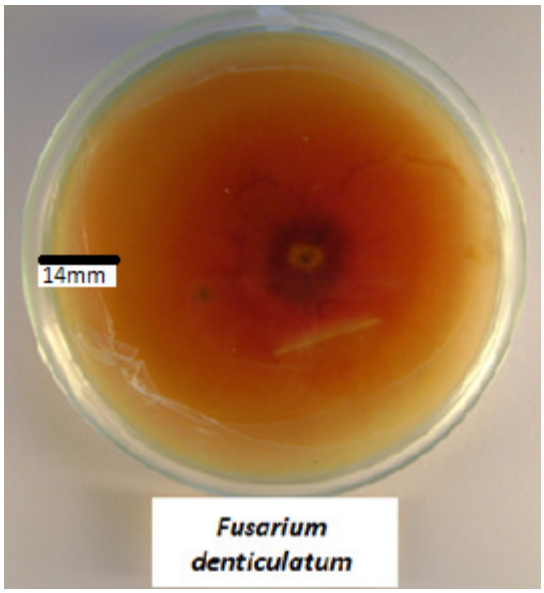
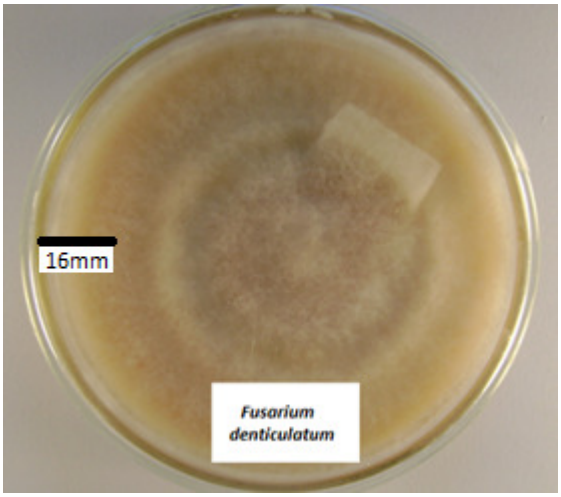
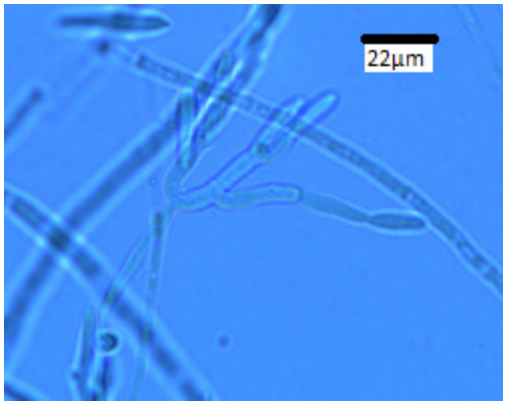
e 

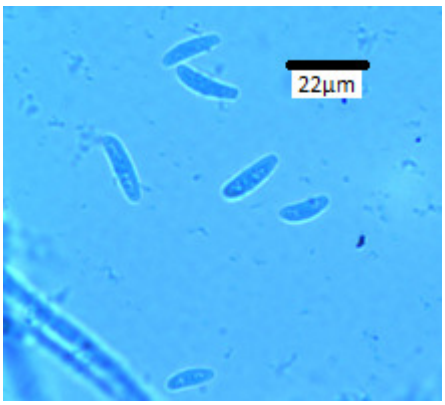
Plate 2.*Fusarium crookwellense*, (a), white-reddish colony on PDA; (b), produces red pigments in PDA; (c), chlamydospores produced in chains and clusters; (d-e), macroconidia, falcate with dorsal side more curved, 3-5 septate, 35-40 x 3-4 micrometers in size.

PLATE 3: *Fusarium denticulatum*

a. 
14mm
Fusarium
denticulatum

b. 
16mm
Fusarium
denticulatum

c. 
22µm

d. 
22µm

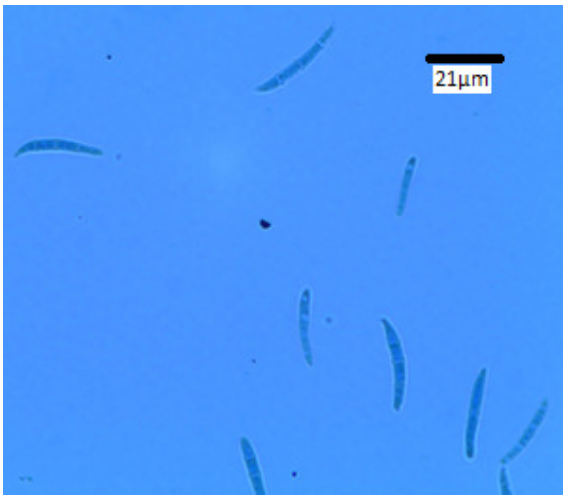
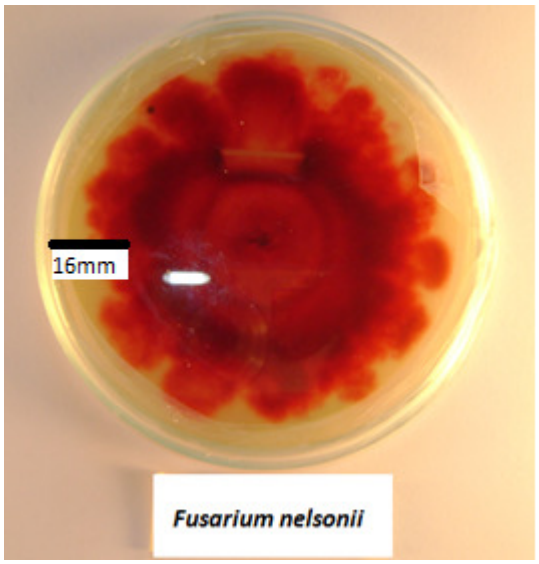
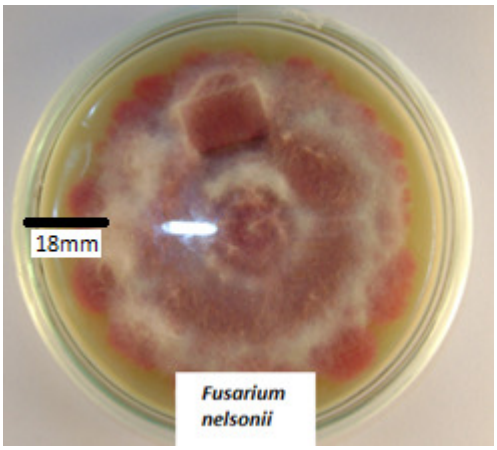
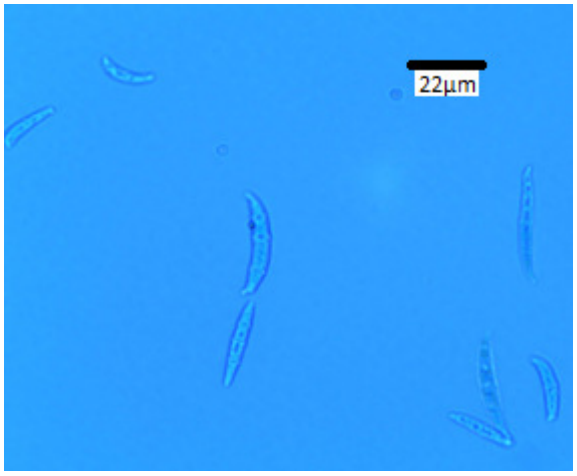
e. 

Plate 3. *Fusarium denticulatum*, (a), produces orange-brown pigments in center In PDA; (b), colony whitish –brown color on PDA; (c), finger-like polyphialides; (d), microconidia oval, long, 0-1 septate 6-18 x 2-3 micrometers in size; (e),macroconidia, slightly curved, 3-5 septate, 25-30 x 2-3 micrometers in size.

PLATE 4:*Fusarium nelsonii*

a. 

b. 

c. 

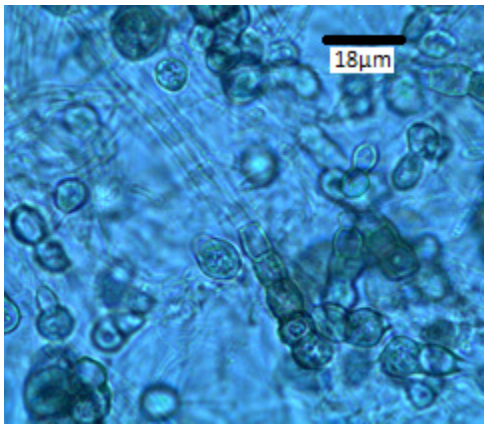
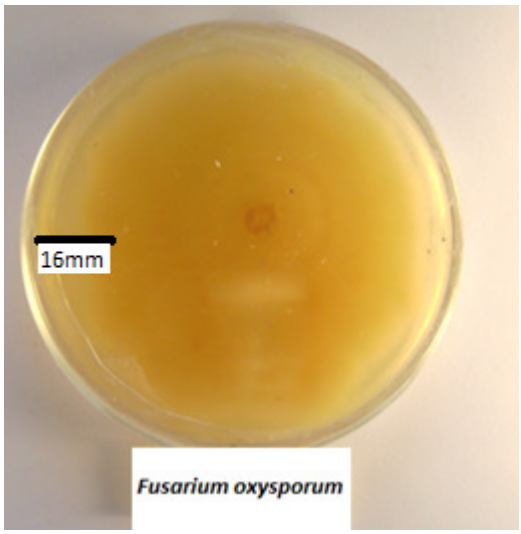
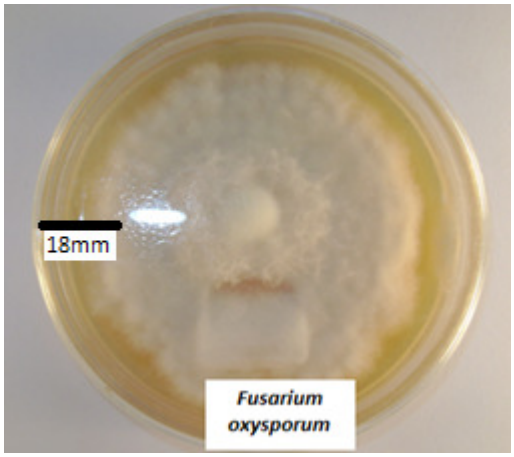
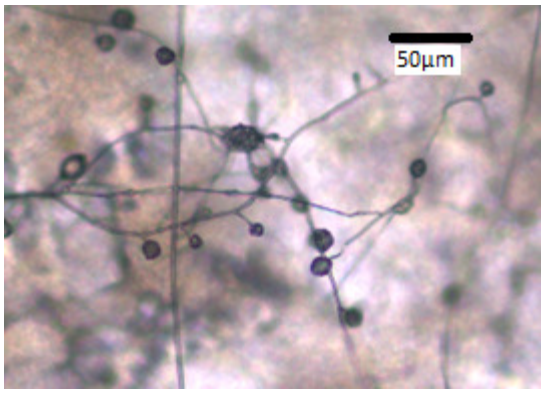
d. 

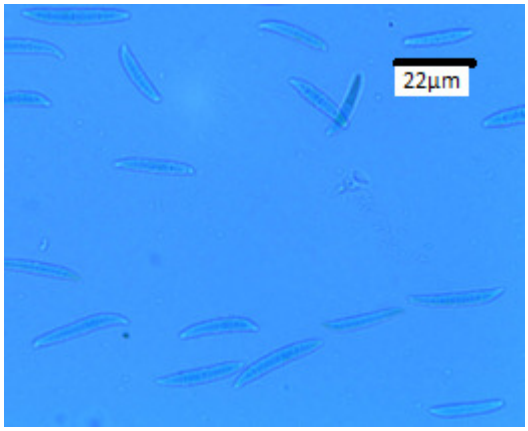
Plate 4.*Fusarium nelsonii*, (a), producing red pigments in PDA; (b), floccose white- red colony; (c), macroconidia and microconidia straight and slightly curved, 3- septate, 25-40 x 3-5 micrometers in size; (c) chlamydospores brown in color, rough and occurring singly, in pairs and short chains.

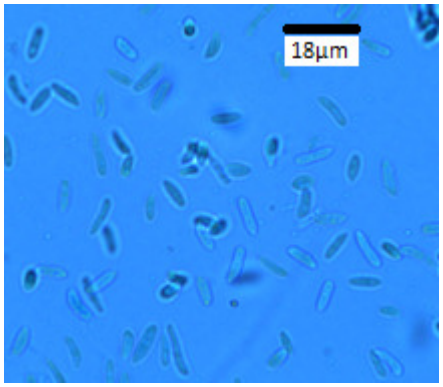
PLATE 5 ***:Fusarium oxysporum***

a. 

b. 

c. 

d. 

e. 

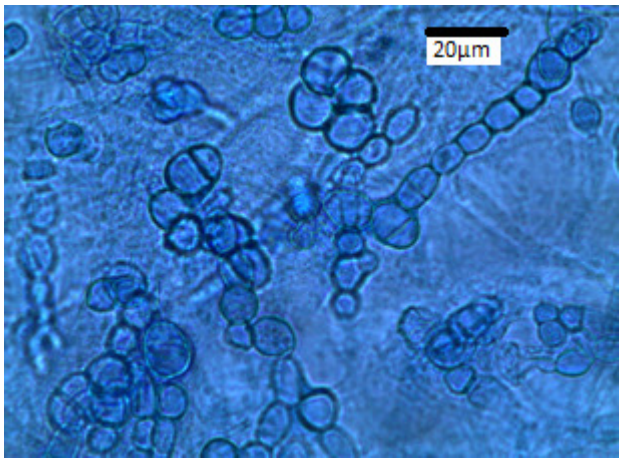
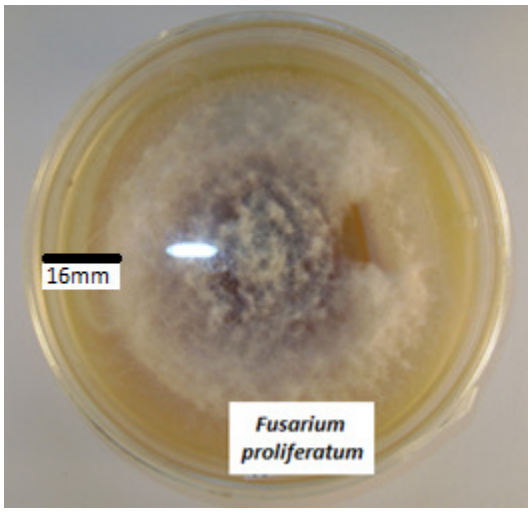
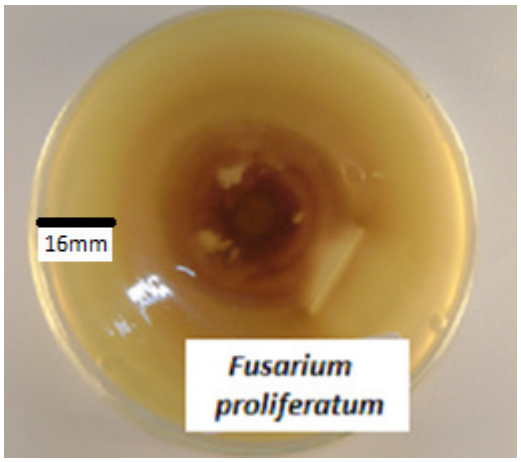
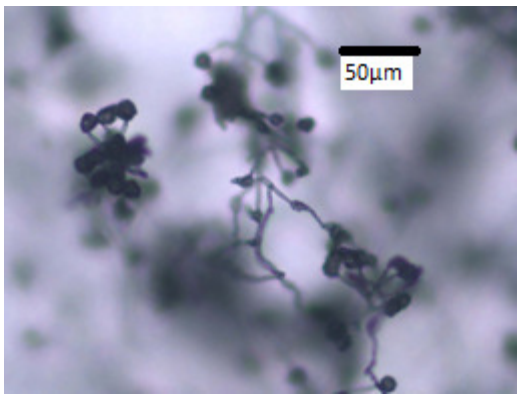
f. 

Plate 5. *Fusarium oxysporum*, (a), colony with no pigmentation in PDA; (b), floccose white colony on PDA;(c), short monophialides; (d), macroconidia 3-septate, slightly curved, 27-46 x 3-5 micrometres in size; (e), microconidia, oval-ellipsoid, 0- septate, 7-11 x 2-3 micrometres ; (f), chlamydospores, globose and formed in clusters and chains.

PLATE 6*: Fusarium proliferatum*

a. 

b. 

c. 

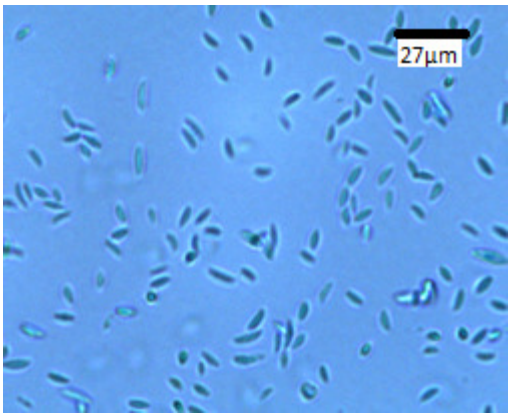
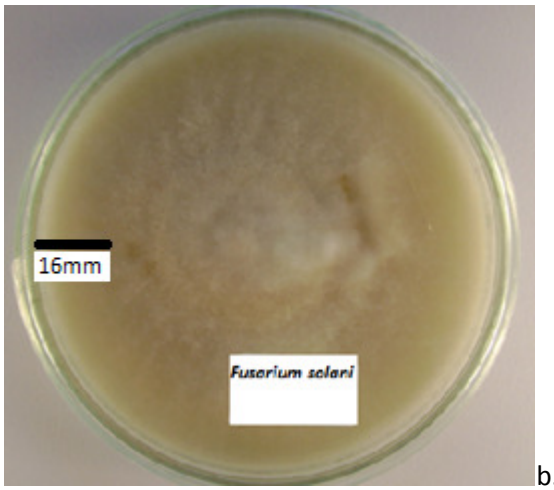
d. 

Plate 6.*Fusarium proliferatum*, (a), purple white colony on PDA; (b),colony produces purple pigments in PDA; (c), microconidia in short chains on monophialides and 'rabbit ears' polyphialides; (d), microconidia club shaped, 0-septate, 5-11x 1-2 micrometers in size.

PLATE 7: *Fusarium solani*

a. 

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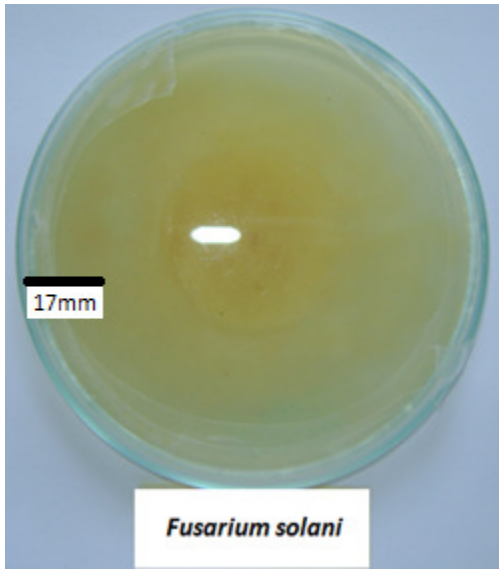
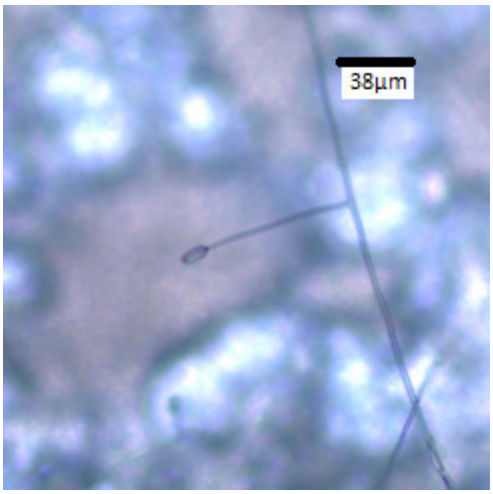
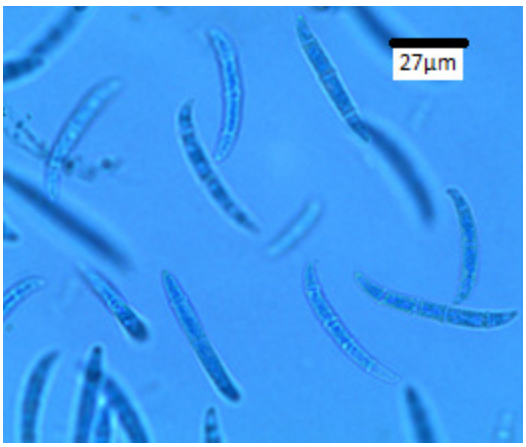
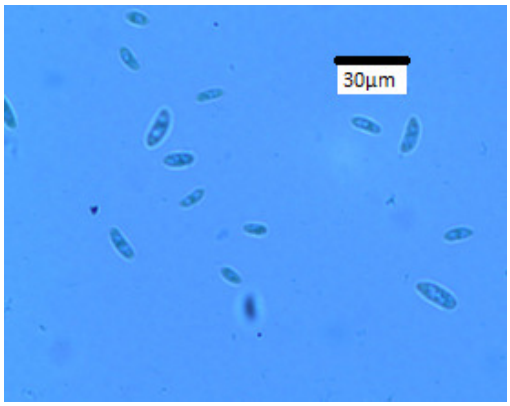


c. 

d. 

e. 

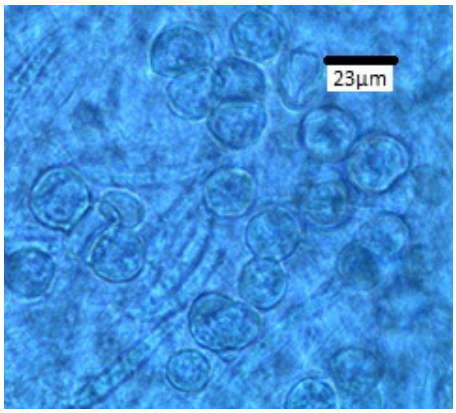
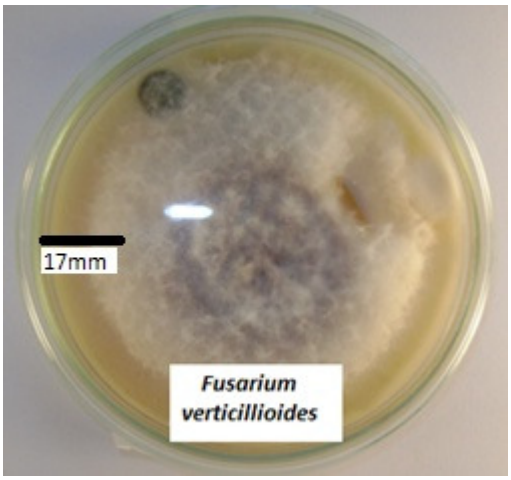
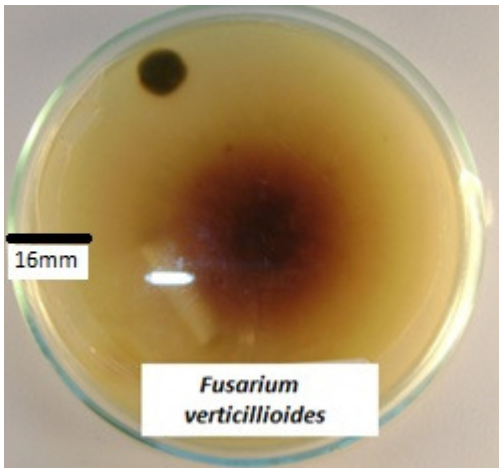
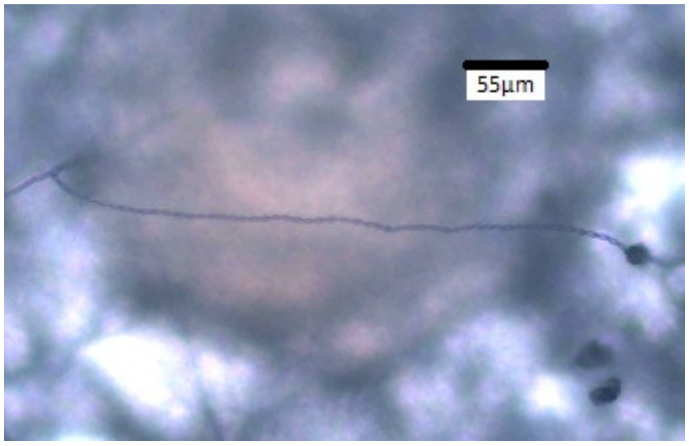
f. 

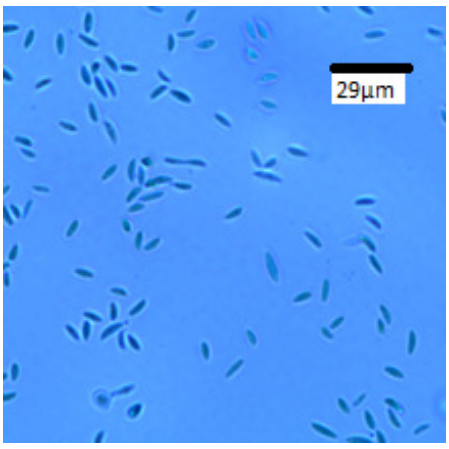
Plate 7. *Fusarium solani*, (a), cream white colony with sparse mycelium on; (b), no pigments produced in PDA; (c), long monophialides; (d), macroconidia slightly curved, 3-7 septate, 35-50 x 4-5 micrometers in size; (e), microconidia oval, ellipsoid and fusiform, 0-2 septa, 8-16 x 2-4 micrometres in size; (f),globose to oval chlamydospores produced singly and in pairs.

PLATE 8: *Fusarium verticillioides*

a. 

b. 

c. 

e. 

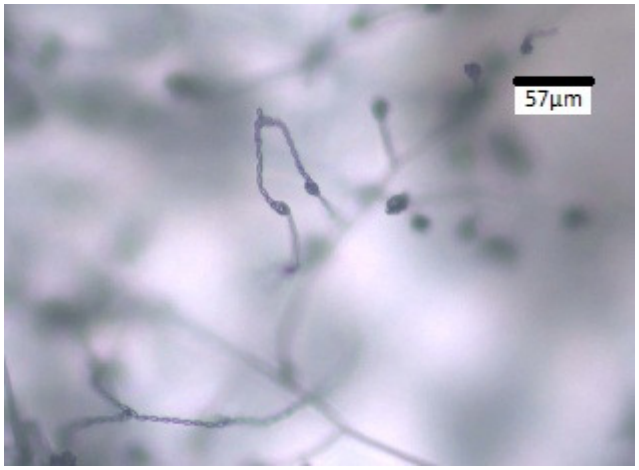
d. 

Plate 8. *Fusarium verticillioides*, (a), purple white colony on PDA; (b), dark purple pigments in PDA ; (c-d), long chains of microconidia on monophialides; (e), 0-septate, oval to club shaped microconidia, 5-11 x 1-2 micrometres in size.