IMPACT OF LAND USE ON DISTRIBUTION AND DIVERSITY OF *Fusarium* species IN NGANGAO FOREST, TAITA TAVETA DISTRICT IN KENYA AND THE ADJACENT FARMLANDS

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156/10844/04

A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY) OF KENYATTA UNIVERSITY

February 2010
DECLARATION

Candidate’s declaration
I declare that this thesis is my original work and has not been presented for award of a degree in any other University or any other award.

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This work is dedicated to my wife Ann and our two children Osteen and Clarette for their love, prayers, and encouragement during the entire period of my studies.
ACKNOWLEDGEMENTS

First of all I give thanks to God, by whose grace this work was accomplished. This work could not have been possible without the assistance of several institutions and individuals whom I sincerely acknowledge. I am greatly indebted to my supervisors, Dr. E. Monda, Dr. S. Okoth, and Dr. N. Njoroge who invested valuable time to guide me through the studies and research. I pay special tribute to the CSM-BGBD project for financial assistance that enabled me to carry out the research. I benefited a lot from the support I got from the staff of microbiology and molecular genetics laboratories, of University of Nairobi, to whom I am sincerely grateful. I would like to thank my fellow students, Mutuku, Margaret, Elizabeth, Ruto, and Joan for their invaluable assistance. I must also thank all my fellow MSc students in the CSM-BGBD project especially Margaret Mwangi and Elizabeth Siameto for being available whenever their assistance was sought. Special thanks to Peter Wachira who participated and coordinated all field work activities and record keeping. Joseph Mungatu is duly appreciated for letting me draw from his immense experience in research and scientific writing. I pay special tribute to the farmers in Umingu and Werugha locations of Taita Taveta district, who gave us access to their farms to obtain soil samples.
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ACRONYMS AND ABBREVIATIONS

AP-PCR  Arbitrary Primed Polymerase Chain Reaction
CEC    Cation Exchange Capacity
CLA    Carnation Leaf Agar
ddH$_2$O double distilled water
DNA    Deoxyribonucleic acid
dNTPs  deoxy Nucleotide Triphosphates
EDTA   Ethylene diamine tetraacetic acid
h      hour
HCl    Hydrochloric acid
KCl    Potassium chloride
L      litre
LUTs   Land Use Types
MgCl$_2$ Magnesium Chloride
ng     nanograms
NO     Nitrogen Oxide
N$_2$O Dinitrogen oxide
PCR    Polymerase Chain Reaction
PDA    Potato Dextrose Agar
PDB    Potato Dextrose Broth
pH     potential of Hydrogen
PPA    Peptone Pentachloronitrobenzene Agar
PTC    Programmable Thermalcycler
RAPD   Random Amplified Polymorphic-DNA
SNA    Synthetic Nutrient Agar
RFLP   Restriction Fragment Length Polymorphism
rRNA   ribosomal Ribonucleic Acid
SDS    Sodium dodecyl sulphate
µl     microlitre
UPGMA  Unweighted pair group method with arithmetic means
ABSTRACT

The effect of current Land Use Types (LUTs) and soil depth on distribution and diversity of soil *Fusarium* species was carried out in Taita Taveta district, Kenya. Sixty sampling points were chosen along a land use gradient to represent six different LUTs: maize, horticulture, napier, fallow/shrub farmlands, planted and indigenous forests. Soil samples were collected at the depths of 0 to 10 and 10 to 20 cm. *Fusarium* species were isolated from soil samples by dilution plate technique using *Fusarium*-selective media (PPA). A total of 1865 isolates were recovered which resulted into 26 *Fusarium* species. *Fusarium oxysporum*, *F. solani* and *F. sporotrichioides* were the dominant species in this study area. *Fusarium oxysporum* and *F. solani* isolates were recovered from all LUTs. *Fusarium compactum* had the lowest frequency of isolation with its isolates recovered from fallow/shrub LUT only. The study also revealed that there was significant difference in *Fusarium* species abundance and richness across the LUTs ($P<0.001$) and soil depths ($P<0.05$). There was significant difference in abundance ($P=0.039$) and richness ($P=0.041$) between the upper and the lower soil layers. It was also observed that *Fusarium* species diversity across the LUTs was significant ($P<0.001$) with horticulture being the most diverse while planted forests being the least diverse. *Fusarium* species diversity between the two soil depth levels was not significant ($P=0.063$). Regression analysis revealed that there was significant positive correlation ($P<0.001$) between abundance, richness and diversity of soil Fusaria with soil phosphorus and pH levels. Significant positive correlation ($P<0.05$) between soil Fusaria abundance ($r=0.25$) and diversity ($r=0.0988$) and soil Mg was also observed. Soil K was also noted to be positively correlated with soil *Fusarium* species richness ($r=0.1688$) and diversity ($r=0.403$). However, there was significant negative correlation between exchangeable soil acidity and soil Fusaria abundance ($r=-0.605$), richness ($r=-0.1317$) and diversity ($r=-0.16$) ($P<0.05$). Principal Component Analysis based on the relative *Fusarium* species abundance differentiated the LUTs with 79.69 % variance explained by the first and second components. Genetic relatedness among the twenty six *Fusarium* species was assessed using Random Amplified Polymorphic DNA (RAPD) assay where six primers were used. Amplification products were examined and presence or absence of each size class of bands was scored as 1 and 0, respectively. The resulting matrix was used to compute Jaccard’s similarity coefficients and UPGMA cluster analysis using computer package NTSYS-pc. Jaccard’s similarity coefficients ranged from 0.257 to 0.583 among the *Fusarium* species studied indicating high genetic diversity. The UPGMA cluster analysis clearly grouped *Fusarium* species into two main clusters of 17 and 9 species, respectively. *Fusarium avenaceum* and *F. nygamai* depicted the highest genetic similarity of 58.3 %. The results indicate that there is a higher abundance and diversity of soil *Fusarium* in the more disturbed lands than in the forests, indicating that agricultural intensification increases soil *Fusarium* populations. This understanding can aid in devising ways of managing this potentially pathogenic fungus.

**Key words**: Distribution, Diversity, RAPD, Land Use Types, Primers
CHAPTER 1

INTRODUCTION

1.1 Background

*Fusarium* is one of the most ubiquitous, abundant, and important genera of soil microfungi. The genus contains many species of environmental, agricultural and human health importance (Martino *et al*., 1994; Vismer *et al*., 2002). The notoriety of this genus mainly results from pathogenicity towards a wide range of plants. Plant diseases caused by *Fusarium* species include vascular wilts, seedling damping-off, and rots of stems, crowns, and roots. Some species of *Fusarium* also produce mycotoxins resulting in food contamination.

*Fusarium* species are distributed worldwide as important plant pathogens, as well as opportunistic colonizers of plant and agricultural commodities, or as saprophytes on debris and cellulolytic plant materials. They are known to occur throughout the world in a variety of climates, in the soil and soil debris as well as on many plant species as epiphytes, parasites or pathogens, yet ecological and geographical information about them is not well documented in literature. Several species cause a range of plant diseases such as vascular wilt, root and stem rot, seedling blight, cereal ear rot and fruit rot. *Fusarium* species are also the major cause of storage rot of fruits and vegetables and are frequently associated with cereal and legume grains, which they usually colonize before harvest. Some *Fusarium* strains can synthesize several mycotoxins, which may accumulate in infected plants before harvest or in stored or processed agricultural commodities.

The occurrence of *Fusarium* mycotoxins particularly in cereal grains is of great concern in all cereal-growing areas, and their occurrence in processed feeds and foods is often associated with mycotoxicoses in human and domesticated animals. The most common *Fusarium* mycotoxins are trichothecenes, zearalenones, and fumonisins. In addition, moniliformins, beauvericins and fusaproliferin may occasionally present problems. The predominance of *F. xylarioides*, *F.
Verticilliioides and F. graminearum in Kenyan maize (Kedera et al., 1999) is cause for concern because most isolates of these species produce mycotoxins that can cause equine leucoencephalomalacia, porcine pulmonary edema and experimental liver cancer in rats. In addition to being strongly plant-pathogenic, some Fusarium can also have beneficial attributes. Fusarium oxysporum, for example, is one of the most economically destructive species of Fusarium (Leslie and Summerell, 2006), and yet has also been reported to increase plant growth (Pung et al., 1992) and even to suppress plant disease (Larkin et al., 1993).

Given the high abundance and ubiquitous distribution of Fusarium species in the soil, their role in saprophytic decomposition – especially cellulolytic activity- is a key process in the cycling of nutrients in many terrestrial ecosystems. Trophic-level links between methyltrophic bacteria and Fusarium species have been revealed (Lueders et al., 2004). Following application of $^{13}$C methanol to soils, a substantial amount of labelled C was shown to be present in eukaryotic DNA, which was dominated by Fusarium species. Some Fusarium species, in particular, F. oxysporum, have been found to have an important role in soil denitrification (Laughlin and Stevens, 2002; Takaya et al., 2002) having the ability to reduce NO, N$_2$O and even fully N$_2$ (Shoun et al., 1992). Fusarium fungi are therefore a functionally important biological component of terrestrial soils. Understanding how land use and management practices affect soil Fusarium communities will have tangible benefits beyond plant disease control. Plant diseases caused by Fusarium species in the tropics are becoming more significant with the introduction of intensive, high-yielding production systems and genetically uniform cultivars (Waller and Brayford, 1990).

The genus Fusarium was introduced by Link in 1809, and is now approaching its third century as a genus that contains many plant-pathogenic fungi. The members of this genus can incite, directly diseases in plants, humans, and domesticated animals, (Martino et al., 1994; Kremery et al., 1997;
Mselle, 1999; Vismer et al., 2002). In addition, Fusarium spp. produce an intriguing array of secondary metabolites that are associated with plant disease, as well as cancer and other growth defects in humans and domesticated animals. Some of these secondary metabolites are used commercially either directly or as the starting material for chemical syntheses of plant and animal growth promoters. Allegations of the use of mycotoxins produced by some of these fungi as biological weapons also have been made (Rosen and Rosen 1982; Mirocha et al., 1983; Heyndrickx et al., 1989). Naturally occurring outbreaks of Fusarium toxicoses directly affecting human beings have occurred historically. Thus, Fusarium has always been a visible genus with many strains, species and metabolites of an importance that transcends just science and agriculture.

The direction and rate of current land-use change is a global concern (Vitousek, 1994). In the tropics, the change has been initiated by deforestation and accelerated by its subsequent phase of agricultural intensification, which results in a gradient of land-use intensity (Ruthenberg, 1980; Swift, 1997). Agricultural intensification is believed to have an impact on biodiversity and the functioning of the ecosystem (Giller et al., 1997) as the system gets more intensive, biodiversity decreases and its ecological function and sustainability may be affected. According to Okoth (2004), there is a clear need to study the biodiversity of soil microorganism in Kenya and evaluate the effect of external factors including agricultural intensification on soil biodiversity and ecosystem function at various temporal and spatial scales. It is estimated that, the current assault on the tropical forest could lead to the disappearance of about four hundred thousand fungal species in the next twenty-five years (Hawksworth, 1991; Lal, 1991). The possibility of using the biodiversity of soil microorganism populations, as a tool for monitoring soil health needs to be explored (Crossley et al., 1992).
The use of microorganism as bio-indicators of environmental impact is a well-established concept. Microorganisms are excellent indicators of soil health. *Fusarium* is a key indicator organism since it is relevant ecologically, abundant and easily enumerated and identified. Elmholtts (1996) demonstrated the use of *Fusarium* species as bioindicators for disturbed ecosystems. The results showed some species to be very promising as bioindicators, especially *F. solani* (Martius) Appel and Wollenweber emend Snyder and Hansen, *F. equiseti* (Corda) Saccardo, and *F. culmorum* (W.G. Smith) Saccardo.

This study was conducted in Ngangao forest, of Taita Taveta district, and the adjacent farmlands. The area has unique agro-ecological zones that dominantly favour agricultural land use and has diverse land use cover types representative of the whole country. Ngangao forest is unique within Kenya and in the world in general since it is a treasure house of flora and fauna occurring nowhere else in the world and has been placed eighth globally as biodiversity hotspot areas (Newmark, 1998). It has both indigenous natural forests and exotic plantations. This forest is however, fast disappearing due to pressure for arable land, felling of trees and destruction by fire. In the adjacent agricultural farmlands, the dominant land use type is intensive agriculture. The aim of this study was to establish the impact of some land use practices and soil depth on distribution and diversity of *Fusarium* species in the soil.

### 1.2 Problem Statement

With three quarters of this country’s land being uncultivable, the cultivable lands are under intensive agricultural production and this brings with it the negative effects reported for modern methods of intensified land management practices including increase in pathogenic species of *Fusarium* (Luque *et al.*, 2005). An increase in use of water soluble mineral phosphorous and
potassium fertilizers and chemosynthetic crop protection products, for example, fungicides, herbicides, in crop production have an effect on soil *Fusarium* populations.

The human population has risen steadily over time and this coupled with scarcity of arable land has led to increased pressure on agricultural systems to provide for the high population. This has resulted into dynamic changes in land uses, for example, deforestation and intensification of agriculture. Agricultural intensification has become a real phenomenon (Okoth, 2004) and the effects of these changes in land use have not been fully documented in terms of distribution and diversity of soil microorganisms. Effects of agricultural practices on *Fusarium* are less well documented and understood. This study sought to investigate how patterns of Land Use Types influences the distribution and diversity of *Fusarium* species in the soil, given the practical and economic importance of this genus.

1.3 Justification

Scientific knowledge related to function and value of belowground biodiversity in quantitative terms is not as strong as that related to aboveground biodiversity (Anderson and Cairney, 2004). Soil biodiversity and its linkages with sustainable agriculture and natural resource management in the tropics, makes strengthening knowledge related to soil biodiversity important. Agricultural intensification is believed to have impact on biodiversity and the functioning of ecosystems (Giller *et al.*, 1997; Swift *et al.*, 1997). The effects of deforestation and subsequent agricultural intensification have not been thoroughly assessed or well documented in Kenya.

*Fusarium* species are generally known to occur throughout the world (Nelson *et al.*, 1990) but their general distribution and diversity in Kenyan soils is still not well documented. Economically, these fungi cause billions of dollars of damage each year worldwide through direct crop yield loss (Windels, 2000), and losses from contamination of otherwise apparently sound foodstuffs with
mycotoxins that can be harmful to both humans and domesticated animals. This fungus is generally associated with diseases (such as vascular wilts, damping off, and ear blight) of a wide range of crop plants including maize, rice, sorghum, millet, pineapples, mangoes, bananas and cotton (Leslie et al., 2005).

*Fusarium* species are ubiquitous in soils and can serve as good ecological indicators (Elmholts, 1996) of disturbed ecosystems. According to Benyon et al., (2000), the most informative studies on systematics, population, and evolutionary biology of *Fusarium* should be those that focus on isolates from natural ecosystems and from fields tended by indigenous, subsistence farmers, an approach this study takes. Taita Taveta is the main agricultural area at the coastal region (Mwanyumba and Mwang’ombe, 1999) and therefore agricultural output is important in feeding the coastal population. Control of *Fusarium* diseases is therefore important and so the reason for choice of site. The soil depth of 0 to 20 cm selected was because propagule numbers of *Fusarium* species are higher in the upper top soil than in other portions of the soil profile (Lim and Varghese 1997).

The inventory obtained can be used in future for purposes of comparison to what has been done in other parts of the world and possibly used in policy formulations.

### 1.4 Research Hypotheses

(i) Different land use practices impact on distribution and diversity of soil *Fusarium* species.

(ii) There is a significant difference in *Fusarium* species distribution and diversity in relation to soil depth.

(iii) There is a significant genetic relationship among soil *Fusarium* species.
1.5 Research Questions

In order to focus the study on the problem, the following questions were formulated.

(i) What is the effect of land use practices on the distribution and diversity of soil *Fusarium* species?

(ii) What is the impact of soil depth on distribution and diversity of *Fusarium* species?

(iii) What is the extent of genetic relationship among soil *Fusarium* species?

1.6 General Objective

To determine the distribution, diversity and the genetic relationship of soil *Fusarium* species in relation to different land use categories and soil depth.

1.7 Specific Objectives

(i) To determine the effect of land use on distribution and diversity of *Fusarium* spp. from soils of Ngangao forest and the adjacent farmlands.

(ii) To determine the distribution and diversity of *Fusarium* spp. in different soil depth levels from Ngangao forest and the adjacent farmlands.

(iii) To determine genetic relationship of *Fusarium* spp. isolated from soils of Ngangao forest and the adjacent farmlands.
CHAPTER 2

LITERATURE REVIEW

2.1 Soil Biodiversity

Biodiversity is defined as the variety and variability of living organisms and the ecosystem in which they occur. The soil represents a favourable habitat for microorganisms and is inhabited by a wide range of them (Davet and Rouxel, 2000). Although not generally visible to the naked eye, soil is one of the most diverse habitats on earth and contains one of the most diverse assemblages of living organisms (Giller et al., 1997). A typical, healthy soil might contain several species of vertebrate animals, several species of nematodes, hundreds of species of mites, fifty to a hundred species of insects, hundreds of species of fungi and perhaps thousands of species of bacteria and actinomycetes.

Nowhere in nature are species so densely packed as in soil communities (Pugh and Boddy, 1988). One gram of productive soil may contain over a hundred million microorganisms representing over a thousand species (Nannipieri et al., 1990). From some of the available estimates, the numbers of species presently described of selected soil biota are still preliminary and much lower than the estimated total numbers of species within each group. For example, the described number of soil dwelling fungal species ranges from eighteen to thirty-five thousand, while the projected number may be greater than a hundred thousand (Hawksworth, 1991).

An increasing interest has emerged with respect to the importance of microbial diversity in the soil as a habitat. The extent of the diversity of microorganisms in soil is seen to be critical to the maintenance of soil health and quality, as a wide range of microorganisms are involved in important soil functions. Microorganisms in soil are critical to the maintenance of soil functions in
both natural and managed agricultural soils. Soil is a habitat for vast numbers of diverse species of microflora, some of which are yet to be known. Over geological time, soil microorganisms have had great opportunity to become distributed worldwide. Wind, water, and animal transport, in existence since before the advent of humans, foster this worldwide distribution. Thus, almost any microbial species has opportunity for ubiquitous distribution.

Typically, microorganisms decrease with depth in the soil profile, as does organic matter. Generally, the population density does not continue to decrease to extinction with increasing depth, nor does it necessarily reach a constant or very slowly declining density. Fluctuations in density commonly occur at lower horizons. Most fungal species show preference for the upper profile although *Chrysosporium* has been found proportionately more numerous at the 30-cm depth than at 10 cm (Clark, 1969).

According to Hawksworth (1991), there are over one and a half million species of fungi and one thousand are being discovered each year. According to Tiessen *et al.* (1984), there is more bioactivity in macroaggregates than in microaggregates, as the former have enhanced nutrient content and hospitable microclimate, providing a domain more favourable for organisms. The intensity of the aggregate-associated bioactivity, however, is less than that associated with two other domains, that of the plant root and its immediately adjacent soil and that provided by plant residues when returned to soil (Clark and Paul, 1970). Plant root systems provide substrate material for microbial growth. The sum total of phenomena occurring at or near the root-soil interface has great impact both on plant welfare and on the soil biota.

Barber and Martin (1976) stated that the number of soil microorganisms at successive distances from the root surface is inversely correlated with increasing distance. In natural ecosystems and in
most managed forests and grasslands, the major portion of the net primary production goes to the litter. The litter biota varies with depth and with stage of decay. In the studies carried out by Clark and Paul (1970), the frequency of observance of *Fusarium* species in grass litter was proportionately lower as compared to that in the underlying humus.

The management practices that change soil properties or plant cover affects the soil biota. There are innumerable practices, any of which may variously affect soil water content, temperature, aeration, and pH regimes, and organic carbon and nitrogen levels. These practices include soil tillage, addition of agrochemicals, clear-cutting and controlled burning. The continued environmental disruption caused by bringing more land into cultivation and deforestation for agricultural purposes in many areas has implications beyond the loss of genetic diversity. Over the years, Kenyan soils have become severely degraded through activities like deforestation, soil nutrients depletion, use of agrochemicals and erosion of both soil and genetic resources. Studies suggest that the loss of a single plant species results in elimination of up to fifteen other species of plants unable to survive without that plant (Schumann, 1991). This in turn affects the dynamic balance of soil microorganisms.

Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from soil microorganisms (Giller *et al*., 1997). In particular, they play an active role in soil fertility because of their involvement in nutrient cycling, soil aggregation, breakdown of toxic compounds, and inorganic transformations (Crossley *et al*., 1992). They are responsible for the decomposition of the organic matter entering the soil and therefore in the recycling of nutrients in soil (Okoth, 2004). Other beneficial effects of soil microorganisms include soil aggregation, breakdown of toxic compounds, both metabolic by-products of organisms and agrochemicals, inorganic transformations that make available nitrates,
sulphates, and phosphates as well as essential elements such as iron and manganese and nitrogen-fixation into forms usable by higher plants (Anderson, 1994). The activity of soil microorganisms is responsible for the colour, structure and even the smell of soil. The earthly odour most evident after soil disturbance, such as ploughing, is a volatile chemical (geosmin) produced by a group of filamentous bacteria, the actinomycetes (Clark, 1969).

The different groups of soil biota clearly influences soil properties and processes, including pedogenesis, nutrient and water cycles and availability, decomposition, aggregation and biological processes (Ruthernberg, 1980). It has however been difficult to identify their roles in plant production since it is difficult to determine the interactions between above and below ground biodiversity (Anderson, 1994). Through changing the physical and chemical environment mainly by agricultural practices, for example, through addition of lime, fertilizers and manures, through tillage practices, the use of pesticides and so forth, the ratio of different organisms and their interactions is significantly altered (Crossley et al., 1992; Sanchez, 1997). Many soil microorganisms are detrimental to plant production. Bacteria and actinomycetes cause some plant diseases but it's fungi that account for most soil-borne diseases such as wilts, root rot, club rot, and blight cause most damage (Leslie et al., 2005). Some organisms may compete for nitrogen with higher plants and, under conditions of poor drainage, soil organisms may compete for limited oxygen (Anderson, 1994).

Agricultural practices as well can have significant positive and negative impacts. For example, high external input agriculture can overcome specific soil constraints by use of inorganic fertilizers, pesticides, and other amendments, in order to meet plant requirements (Sanchez, 1997). Although these practices have led to considerable increases in overall food production worldwide, they also tend to decrease or disregard the potential benefits of soil biological activities in
maintaining soil fertility and enhancing plant production. Furthermore, the misuse or overuse of these practices has led to soil and environmental degradation and decline in productivity in certain areas of the world. In addition, most of the farmers do not have access to, or cannot afford, the external inputs necessary to apply the principles and practices of high external input agriculture (Vandermeer et al., 1998).

*Fusarium* species are commonly and easily isolated from natural ecosystems as well as arable soils. They are cosmopolitan group that exhibit parasitic, saprotrophic and pathogenic behaviour under different circumstances. According to Wilberforce et al., (2002), *Fusarium* species abundance in the soil varied between disturbed and undisturbed ecosystem, with disturbed sites being several times greater than the undisturbed sites.

### 2.2 Taxonomic Description of *Fusarium*

The genus *Fusarium* belongs to the kingdom Fungi, phylum Ascomycota, class Hyphomycetes, order Hypocreales and family Hypocreaceae. This genus was introduced by Link in 1809, validated by Fries in 1821 in terms of the International Botanical Code. The taxonomy of *Fusarium* has undergone a number of changes during the last 100 years. The concept of a species within the genus has varied greatly between broader and narrower concepts. The basis for all modern taxonomic systems in *Fusarium* is the work of Wollenweber and Reinking. Prior to this landmark publication, there were around a thousand *Fusarium* species described. Wollenweber and Reinking provided a sub-generic system based on 16 sections, 65 species and 77 subspecific varieties and forms. Snyder and Hansen (1940; 1941; 1954), reduced the number of species within the genus to nine and introduced the concept of single spore cultures in identification of species. Nelson et al., (1983) produced an identification manual that recognized a larger set of species than
the nine species of Snyder and Hansen. Identification of *Fusarium* species in this study was done based on this manual, which employs morphological species concept.

*Fusarium* is now approaching its third century as a genus that contains many plant-pathogenic fungi. *Fusarium* taxonomy has been plagued by changing species concepts with as few as nine or well over a thousand species being recognized by various taxonomists during the past hundred years, depending on the species concept employed (Summerell *et al*., 2003). There are currently three different basic species concept employed, that is, morphological, biological and phylogenetic (Leslie *et al*., 2001). Since the 1980s, the number of recognized species has increased gradually, with the number of recognized species now greater than eighty. DNA sequences have been used to generate characters that usually are treated cladistically to form phylogenies (Abd-Esalam *et al*., 2003; Abdel-Satar *et al*., 2003).

The taxonomy of *Fusarium* is difficult (Mehortra, 1990). This is because this genus consists of species that are highly variable and require specific conditions to form their optimal morphologies (Saremi, 2003). They tend to mutate rapidly causing further difficulties in identification. *Fusarium* consists of species that are frequently the conidial states of the genera *Gibberella*, *Nectria*, *Calonectria* and *Micronectriella*, belonging to the Hypocreals (Booth, 1971).

### 2.3 General Characteristics of *Fusarium*

*Fusarium* species are filamentous, saprophytic fungi that are widely distributed on plants and in the soil. They grow rapidly on Sabourand Dextrose Agar at 25 °C and produce woolly to cottony, flat, spreading colonies. The only slow growing species is *F. dimerum* Penzig. From the front, the colour of the colony may be white, cream, tan salmon, cinnamon, yellow, red, violet, pink or
purple. From the reverse, it may be colourless, tan, red, dark purple or brown. Sclerotium, which is the organized mass of hyphae that remains dormant during unfavorable conditions, may be observed macroscopically and is usually dark blue in colour. Sporodochium, the cushion-like mass of hyphae bearing conidiophores over its surface is usually absent in cultures and when present, it may be observed as cream to tan or orange colour except for F. solani, which gives rise to blue-green or blue sporodochia (Sutton 1998; de Hoog, 2000).

Hyaline septate hyphae, conidiophores, phialides, macroconidia and microconidia can be observed microscopically (Larone, 1995; Sutton, 1998; de Hoog, 2000). Phialides are cylindrical, with a small collarette, solitary or produced as a component of a complex branching system. Monophialides and polyphialides (in heads or in chains) may be observed. Macroconidia are produced from phialides on unbranched or branched conidiophores. They are two- or more celled, thick-walled, smooth, and cylindrical or sickle-shaped. Macroconidia have a distinct basal foot cell, pointed distal ends, and tends to accumulate in balls or rafts and they form the main basis of identification (Larone, 1995). Microconidia, on the other hand are formed on long or short simple conidiophores. They are one-celled (occasionally two- or three- celled), smooth, hyaline, ovoid to cylindrical, and arranged in balls (occasionally occurring in chains) (Larone, 1995; de Hoog, 2000).

In addition to these basic elements, chlamydospores are also produced by some Fusarium spp. Chlamydospores, when present, are sparse, in pairs, clumps or chains. They are thick-walled, hyaline, intercalary or terminal (Larone, 1995; Sutton 1998; de Hoog, 2000) with a warty appearance and light colouration, usually yellow-brown. Chlamydospores are more common in older cultures than in younger ones and often occur more abundantly on media such as Soil Agar (Summerell, 2003). Macroscopic and microscopic features, such as, colour of the colony, length
and shape of the macroconidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are key features for the identification of *Fusarium* species (de Hoog, 2000). Molecular methods, such as 28S rRNA gene sequencing may be used for rapid identification of *Fusarium* strains to species level (Nicholson *et al*., 1998; Abd-Elsalam *et al*., 2003).

### 2.4 Pathogenicity and Clinical Significance of *Fusarium*

*Fusarium* species are able to cause disease on nearly all kinds of economic plants (Booth, 1971). They are known to cause great economic losses (Windels, 2000; Rosa-Marque and Guillermo, 2004). Particularly, the wilt and root rot diseases cause great losses to farmers (Hillocks, 1992) and *Fusarium* species are the most common fungi causing these diseases. *Fusarium* wilt is a major cause of seedlings death in many countries (Minton and Garber, 1983). In Egypt, *Fusarium oxysporum* Schlechtendahl emend. Snyder and Hansen, is frequently isolated from cotton seedlings infected with damping off (Aly *et al*., 1996). *F. culmorum*, *F. graminearum*, *F. avenaceum* (Fries) Saccardo and *F. Poae* (Peck) Wollenweber are the species most commonly associated with ear blight, brown foot rot and seedling blight (Parry *et al*., 1995).

*Fusarium* species from agricultural crops have been well studied with respect to mycotoxins production and genetic diversity while similar studies of communities from non-agricultural plants are much more limited (Leslie *et al*., 2004). Phylogenetic comparisons among *Fusarium* species indicate that patterns of distribution and diversity of mycotoxins within the genus are complex and that associations with plant host and pathogenicity are not obvious (Moretti *et al*. 1997; Fotso *et al*., 2002). Some metabolites such as beauvericin, fusarins and moniliformin appear to be distributed more or less throughout the genus (Desjardins, 2006). *Fusarium* species produce a diverse array of known mycotoxins and secondary metabolites, including gibberellic acid,
moniliformin, fumonisins, fusaric acid beauvericin and fusaproliferin (Leslie et al., 2004; Leslie et al., 2005) that can contaminate animal and human feed and foods. These, when they contaminate the grains, may have adverse effects on health (Macdonald and Chapman, 1997; Chulze et al., 2000).

The predominance of F. verticillioides (Saccardo) Nirenberg (formerly F. moniliforme) in Kenyan maize (Kedera et al., 1999), is cause for concern because most isolates of this species produce fumonisins, mycotoxins that can cause equine leucoencephalomalacia, porcine pulmonary edema and experimental liver cancer in rats. Fumonisins have been detected in maize and maize-based foods and feeds in North America, South America, Europe, Asia, and South Africa (Thiel et al., 1991; Beardall and Miller, 1994; Doko et al., 1995)

As a social phenomenon, Fusarium plant diseases have had several major impacts. One was the near devastation of the commercial banana industry in the 1960s by panama wilt caused by Fusarium oxysporum f. sp. cubense (Ploetz, 1990). The recent losses of several billion dollars (Windels, 2000) by many wheat and barley farmers to Fusarium head scab in the upper Midwest of the United States has shifted cropping strategies and bankrupted many farmers in the region. At the same time, the causal agent of Fusarium head scab can be used in commercial fermentations to produce the precursor for one of the most widely used commercial cattle growth promotants (Hidy et al., 1977). Recent problems caused by strains of Fusarium which may have originated from endophytes or pathogens of native Gossypium species (Wang et al., 2004), are threatening the future of the cotton industry in Australia while simultaneously demonstrating the relatedness of native and agricultural populations and suggesting new avenues for understanding how these fungi evolve.
Many plants have at least one *Fusarium*-associated disease. Records reveal that over 81 of the 101 economically important plants have at least one associated *Fusarium* disease (Leslie and Summerell, 2006). As these fungi also may grow as apparently symptomless endophytes under many conditions, the claim that, “if it is green, there is some *Fusarium* that can grow on it, in it, or with it” probably is not too far removed from the truth. The types of diseases induced are quite varied, as is their severity, and may include root or stem rots, cankers, wilts, fruit or seed rots, and leaf diseases (Leslie et al., 2005).

Results of different experiments on cropping systems (Martinson and Hartwig, 1993) and soil tillage management show that cultural practices greatly affect disease development, especially those caused by soil-borne pathogens such as *Fusarium*. Windels and Wiersma (1992) have shown that conservation tillage do increase disease incidence and propagule density in *Fusarium* species of small grains such as wheat and rye. Doran, (1980) has shown that reduced and no-till regimes foster microbial activity at or near the soil surface. For the 0 to 7.5 cm depth, total fungal counts were 1.57 times higher in no-till than in conventional tillage while counts for total fungi were significantly reduced for 7.5 cm to 15 cm depth. Fungicides, fumigants, and other eradicant-type chemicals cause much greater disruption of the soil biota.

As well as being a common plant pathogen, *Fusarium* spp. are causative agents of superficial and systemic infections in humans. Infections due to *Fusarium* are collectively referred to as fusariosis. The mortality rate for human patients with systemic *Fusarium* infections is greater than 70 % (Kremery *et al*., 1997), and HIV-infected patients are susceptible to such *Fusarium* infections as well (Mselle, 1999; Guarro *et al*., 2000). The most virulent *Fusarium* spp. is *F. solani* (Mayayo *et al*., 1999). Trauma is the major predisposing factor for development of cutaneous infections due to *Fusarium* strains. Disseminated opportunistic infections on the other hand, develop in immuno-
suppressed people particularly in neutropenic and transplant patients (Boutati and Anaissie, 1997; Austen et al., 2001). *Fusarium* is one of the most drug resistant fungi and among the *Fusarium* spp. *F. solani* in general tends to be most resistant of all (van Cutsem 1989; Arikan et al., 1999; Arikan et al., 2001).

*Fusarium* species have therefore, practical and economic significance in several fields, which include;

(i) Plant Pathology- Traditionally, *Fusarium* has mostly been studied in the context of its ability to cause diseases of many economically important crop plants (Macdonald and Chapman, 1997). The diseases include wilts, ear blights, root rots, and cankers of important plants, including trees.

(ii) Food Microbiology/mycotoxicology- Some *Fusarium* species colonize stored cereal grains and crops in the field and produce mycotoxins, for example, fumonisins, zearalenone, and trichothecenes (Kedera et al., 1999; Langevin et al., 2004; Leslie et al., 2004). However, most of the species do not grow well at reduced water activity, hence generally regarded as field fungi rather than storage fungi.

(iii) Medical Mycology- Some *Fusarium* species cause opportunistic infections of human and animal eyes, skin or nails. Systemic infections may also occur in immunosuppressed patients (Abramowsky et al., 1974; Kremery et al., 1997; Mselle, 1999; Guarro et al., 2000).

(iv) Mycoprotein production- Some species are used to produce mycoproteins, as secondary metabolites. For example, Quorn that is produced from *Fusarium graminearum* Schwabe and *F. venenatum* Nirenberg.
Biodegradation- Members of this genus play a role in the biodegradation of organic materials as well as biodeterioration of industrial products such as pharmaceutical solutions, cosmetic products and machine cooling fluids. *Fusarium solani* is an important contaminant and biodeteriogen (Anon, 1971; Onions *et al*., 1981).

Biocontrol- Entomogenous *Fusarium* species are known to occur in ten out of the twelve sections of the genus. *Fusarium lateritium* Nees has been reported to be pathogenic to the cereal leaf beetle and the scale insect (Grove and Pople, 1980). *F. polyphialidicum* Marasas, Nelson, Toussoun and van Wyk has been tested for use as an insect control agent (Hajek *et al*., 1997).

Growth Promoters- Some strains of this genus produce secondary metabolites that are used commercially either directly or as the starting material for chemical syntheses of plant and animal growth promoters (Tomasini *et al*., 1997; Shukla *et al*., 2003; Shukla *et al*., 2005).

Biological Weapons- Allegations of the use of mycotoxins produced by some of the species in this genus, as biological weapons, have been made (Rosen and Rosen, 1982; Miracho *et al*., 1983).

2.5 Distribution of *Fusarium*

The genus *Fusarium* has a cosmopolitan distribution with representative occurring in most parts of the world (Saremi, 2003). It is common in both agricultural and native environments (Thrall *et al*., 1997) and is widely distributed in soil and organic substrates. The members of this genus have been isolated from permafrost of arctic, tropical rainforest and from the sands of the Sahara (Mandeel, 1996). This fungus is found as normal mycoflora of commodities, such as rice, bean, soybean, and other crops (Marasas *et al*., 1984). Some species are almost ubiquitous, others have a
more restricted distribution, occurring in temperate regions or the (sub) tropics (Leslie and Summerell, 2006). *Fusarium* species are mainly soil borne and are isolated also from a very wide range of plants colonizing the roots, stems or leaves (Burgess et al., 1993).

The spores produced by most *Fusarium* species are formed in a slimy matrix facilitating dispersal by means of water rather than air. This makes *Fusarium* relatively uncommon members of the air mycoflora (Leslie and Summerell, 2006). They are abundant in cultivated soils both in temperate and tropical regions (Mehortra and Aneja, 1990, Krupinsky et al., 2002). *Fusarium* species in the tropics are quite diverse in terms of numbers of species distribution, host range and virulence just as with the temperate regions (Gordon, 1960). *F. culmorum* is often predominant in cooler regions, such as Northwest of Europe (Parry et al., 1995).

Saremi (2003) has confirmed an empirical association between climate and distribution of *Fusarium* species. In his survey undertaken in five areas with a range of climates in Australian soils, he found that, cosmopolitan species, that is, *F. oxysporum*, *F. solani* and *F. equiseti* (Corda) Sacc. sensu Gordon, were recovered from all areas. *F. compactum* (Wollenweber) Gordon and *F. nygamai* Burgess and Trimboli were recovered from warmest areas while *F. crockwellense*, *F. culmorum*, *F. tricictum* and *F. sporotrichioides* were recovered only from coolest areas. *F. sambucinum* Fuckel was recovered frequently from cool areas. *F. proliferatum* (Matsushima) Nirenberg, *F. chlamydosporum*, *F. graminearum* and *F. scirpi* were isolated from moderate to warm areas, whereas *F. anthophilum*, *F. lateritium*, *F. merismoides* and *F. babinda* tended to occur in wet temperate areas. Species such as *F. acuminatum*, *F. avenaceum* and *F. semitectum* did not show distinctive patterns in their distribution within the region surveyed. Nelson et al., (1990) reported that some *Fusarium* species have restricted distribution. For example, *F. sambucinum* was reported to be common in cold-temperate and alpine soils but uncommon in the tropics unlike *F. 
quseti, which is common in warm and subtropical areas. *F. oxysporum* and *F. solani* were reported to occur in both tropical and temperate areas. In another related study, *F. proliferatum* was frequently isolated from cool areas than in hot areas (Wong and Jeffries, 2006).

Other studies indicate that *Fusarium* species distribution and diversity decreases with increasing soil depth (Rodriguez-Molina *et al.*, 2000; Vujanovic *et al.*, 2006). Rupe *et al.*, (1999) quantified the vertical and temporal distribution of the *F. solani* in the soil and found that, the soil numbers of *F. solani* were greatest in the top 15 cm of the soil. Soil temperature also affects relative abundance of *Fusarium* species. According to a survey carried out by Burgess and Backhouse, (1999) propagule density of *F. compactum* and *F. solani* were greatest at high temperature. *Fusarium torulosum* (Berk. and Curt.) Nirenberg and *F. acuminatum* Ellis and Everhart, showed high propagule density at low temperatures. Stubble burning of wheat reduces the incidence of *F. graminearum* (Burgess *et al.*, 1993). *Fusarium* spp. population structure is also strongly influenced by soil phosphate level, soil dehydrogenase activity and soil calcium levels (Mandeel, 1996).

Soil moisture affects soil *Fusarium* (Gott *et al.*, 1994). In their studies, they found that greatest range of soil *Fusarium* species was greatest in moist soils. They also found that the type of vegetation growing in a certain region affects distribution of *Fusarium* spp. in the soil with greatest range of species recovered in grassed sites while fewest being recovered from cycad and palms sites. The *Fusarium* infections in crops are significant when stubble is retained on the soil surface compared to when it is incorporated into the soil using disc plough (Swan *et al.*, 2000). This may be due to conservation of more water in the soil. According to Lim (1972), *Fusarium* population in paddy soils showed considerable variation in size but was generally small. Only three species, *F. oxysporum*, *F. roseum* and *F. solani* were found in paddy soils in West Malaysia, with *F. roseum* most widely distributed. Factors other than water and temperature are also influential (Backhouse
and Burgess, 1995). In the tropics, *Fusarium* wilt of bananas is mostly restricted to those soils containing the clay smectite (Clark, 1969).

Vertical distribution of *Fusarium* species also correlates with organic carbon level (Juzwik et al., 2002). The distribution of *Fusarium* hyphae and spores in soil usually is irregular both spatially and temporally (Rupe et al., 1999). The spatial distribution usually varies across a particular site and down the soil profile (Gott et al., 1994). In general, propagule abundance decreases with soil depth, with samples from depths greater than 30 cm from the surface yielding relatively less fungus (Rodriguez-Molina et al., 2000). Plant rhizospheres often contain a wider range of *Fusarium* species (Juzwik et al., 2002). The distribution of *Fusarium* in the soil is considerably affected by the method of straw disposal and depth and method of tillage (Skoglund and Brown, 1988; Bateman et al., 1998; Chulze et al., 2000; Rheeder et al., 2006). Deeper sampling (15 to 25 cm) showed that ploughing resulted in a more even, but diluted distribution of *Fusarium* species. Steinkellner and Langer (2005) studied the influence of several long-term conventional and conservation tillage treatments on the incidence and the diversity of *Fusarium* spp. in the soil and observed that there was significant difference in *Fusarium* spp. diversity between different tillage regimes employed. There was a higher diversity of *Fusarium* spp. in conservation tillage than in plough-based tillage. Mouldboard plough treatments resulted in a lower diversity of *Fusarium* spp. than the chisel plough and rotary tiller treatments.

2.6 Molecular Characterization by use of RAPD-PCR

Molecular methods are universally applicable. Two important technical advances have stimulated the use of molecular methods. These are the advent of Polymerase Chain Reaction (PCR) and the selection of oligonucleotides primers specific to a specific fungus. The development of the PCR has revolutionized the analysis of nucleotide sequences (Mullis et al., 1986). This technique allows
the *in vitro* amplification of DNA. It involves the annealing of oligonucleotides to homologous sequences in template DNA, followed by DNA polymerization primed by these oligonucleotides using deoxyribonucleotide triphosphates as substrates (Williams *et al*., 1990). DNA amplification is brought about by repeated temperature recycling through denaturation of double-strand DNA, annealing of primer to single-strand DNA target sequences and extension of primer using target DNA as template (Brown, 1995). Each amplification product is expected to result from two annealing sites in inverted orientations, 3’ ends facing each other, within amplifiable distance (Williams *et al*., 1990).

Since its development, PCR has revolutionized molecular biological techniques with modifications of the original procedure being tailored to suit a range of needs and offering alternative strategies for generating molecular markers. The first experiment with PCR used the Klenow fragment of DNA polymerase 1 from *Escherichia coli*. However, Klenow activity is denatured by the high temperatures needed for denaturing the template DNA necessitating addition of new enzymes at the start of each cycle. The technique was revolutionized by the discovery of a heat stable polymerase (*Taq* polymerase) isolated from thermophilic bacteria, *Thermus aquaticus* (Saiki *et al*., 1988). PCR is an extremely powerful procedure that allows one to make million fold copies of a selected DNA sequences in a genome (Welsh and McClelland, 1990). The random amplified polymorphic DNA (RAPD) technique also called Arbitrary Primed PCR (AP-PCR) represents a source of variation for generating a species class of molecular markers (William *et al*., 1990; Welsh and McClelland, 1990).

The RAPD is a good assay for studying polymorphisms (Hadrys *et al*., 1992). The assay is based upon the observation that a single short oligonucleotide of a random nucleotide sequence, when mixed with any DNA and *Taq* polymerase then subjected to thermal cycling conditions similar to
those of PCR will prime the amplification of several segments of the DNA (William et al., 1990). DNA amplification with random primers has been shown to be a sensitive method for revealing polymorphisms randomly distributed throughout any given genome (Sabir, 2006).

The RAPD maker method detects DNA polymorphism that result in the loss or gain of amplification product at a locus (Welsh and McClelland, 1990). Amplification can be lost owing to substitution within a primer-binding site or a large insertion between two primer–binding sites. Amplification can be gained from substitution or insertion, that create new primer binding sites hence bring them within PCR amplifiable range of each other. Hence, there are typically only two possible alleles at a RAPD marker locus represented by the amplified product (the present allele) and no band (the absence or null allele). According to Welsh and McClelland (1990), polymorphism therefore, could be caused by differences in nucleotide sequences at the priming sites (such as point mutation), or by structural rearrangements within amplified sequences (for example, insertions, deletions or inversions). Williams et al., (1990) described the RAPD reaction in details.

The banding pattern on agarose gel have been used in derivation of molecular taxonomy and in the study of populations genetics of various organisms. The analysis of RAPD patterns have been utilized in identification and differentiation of species and strains of various organisms. RAPD analysis is a fast, PCR-based method of genetic typing based on genomic polymorphisms. The technique is highly sensitive to nucleotide differences and can assay single nucleotide differences (Williams et al., 1990; Welsh and McClelland, 1990). RAPD produces DNA profiles of varying complexity, depending on the primer and template used. Random markers as products of the PCR-RAPD technique (Williams et al., 1990; Hadrys et al., 1992) have been developed to differentiate numerous fungi. RAPD analysis has been applied widely in the detection and genetic
characterization of phytopathogenic fungi including race differentiation in several formae speciales of *F. oxysporum* (Manulis *et al*., 1994; Bentley *et al*., 1995; Migheli *et al*., 1998). RAPDs are already widely used as a diagnostic tool in many laboratories alongside, or as alternative to, Restriction Fragment Length Polymorphism analysis in *Fusarium* studies (Amoah *et al*., 1996). Random PCR approaches are being increasingly used to generate molecular makers, which are useful for taxonomy and for characterizing *Fusarium* populations. RAPD assay have been used extensively to define *Fusarium* populations at species, intraspecific, race, and strain levels (Sabir, 2006). The use of molecular markers based on the PCR for species identification and as diagnostic tool became very popular during the last decade (Sabir, 2006). Nijs *et al*., (1997) studied variations in RAPD patterns within *Fusarium* species from cereals from various parts of Netherlands while Hering and Nirenberg, (1995) used RAPD technique to differentiate *Fusarium* into species. On the other hand, Gherbawy and Abdel-zaher (1999) used RAPD technique to analyze different formae speciales of *F. oxysporum*. Moller *et al*., (1999) studied fungal populations of *F. verticillioides* and *F. subglutinans* using RAPD technique. Gherbawy *et al*., (2002) used RAPD technique for identification of *F. subglutinans*, *F. proliferatum*, and *F. verticillioides* isolated from maize in Australia. Pasquali *et al*., (2003) characterized isolates of *F. oxysporum* pathogens on *Argyranthemum frutescens* L. using RAPD technique.

Abd-Elsalam *et al*., (2003) used RAPD markers to study inter- and intra-specific variation of twelve *Fusarium* species isolated from cotton-growing areas in Egypt. Various workers have used RAPD-PCR as a method for analyzing genetic variation within and between *Fusarium* species (Walker *et al*., 2001; Khalil *et al*., 2003; Beladid *et al*., 2004). RAPD analysis has been used effectively to distinguish between species of *Fusarium* (Voigt *et al*., 1995; Yli-Mattila *et al*., 1996; Jana *et al*., 2003). RAPD analysis has also successfully delineated groups within *Fusarium* species including *F. avenaceum* (Yli-Mattila *et al*., 1996), *F. graminearum* (Quellet and Seifert, 1993) and
F. verticillioides (Voigt et al., 1995). It is therefore a useful maker in detecting genetic relatedness between Fusarium spp. The RAPD assay offers a non-labour intensive, time saving method for demonstrating genetic relatedness among individuals (William et al., 1990).

This technique has been used in many studies of Fusarium both in genetic linkage mapping and population genetic applications. RAPD assay has many advantages as a means of characterizing genetic variability such as speed, low cost, minimal requirement for DNA and lack of the use of radioisotopes (Jana et al., 2003). As a technique, the RAPD procedure offers several advantages over established RFLP methods (Khalil et al., 2003). Results are obtained rapidly and, thus, it offers the ability to process large numbers of isolates for survey studies. Furthermore, there is no need for a species-specific gene library or any form of clone required as a probe. All that is required is one or several suitable 10-mer primers (William et al., 1990).

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted in Taita-Taveta district, in coast province, 200 km northwest of Mombasa town and 360 km southeast of Nairobi city (Fig. 1). The district covers an area of 16,975 km². There are 48 forests which have survived on hilltops in the district of which 28 are gazetted. The Taita hill forests (Latitudes 3° 20’ and Longitude 38° 15’) are located in southeastern part of Kenya, about 150 km inland from Coast and 25 km west of Voi town in the middle of the parched plains of Tsavo West National Park. These hills cover an area of 1000 km² and form the northern
most part of the Eastern Arc Mountains (Lovett, 1986). From the level of 600 m above sea level, the hills rise to a maximum elevation of 2228 m above sea level (Beentje and Ndiangu'i, 1988).

While much of the surroundings plains are relatively low-lying, the peaks, which climb to a height of 2228 m above sea level, experience a distinct microclimate. The climate of the area is under the influence of Inter Tropical Convergence Zone, with a bimodal rainfall incidence. The mean annual rainfall is 1500 mm, though there is high year-to-year variability. There are two rainy seasons in the area, March-May/June and October-December. The Taita hills hold a series of tropical montane cloud forests that are a global biodiversity hot spot (Mittermeier et al., 1998). Because of long isolation combined with climatic stability, the Eastern Arc forests have evolved a distinctive flora and fauna with very high levels of endemism (Beentje and Ndiangu'i, 1988).
Among the plants, they include; a wild coffee species, *Coffea fadenii*, *Psychotria taitensis*, *Saintpaulia taitensis*, an invertebrate *Taitastrepsus flavipes*, reptile *Amblyodipas teitana*, fungus *Meliola taitensis* and a bird *Zosterops poliogastra* among others. These are among the highest priorities for biodiversity conservation in Kenya (Brooks et al., 1998).

These forest hills have been fragmented for a long time but large-scale fragmentation has mainly occurred since the 1960’s (Brooks et al., 1998). They have been identified as one of the habitats in urgent need of inventorization and protection by the Strategy Conference for Management and Protection of Kenya’s Plant Communities, held in 1984 (Beentje and Ndiangu’i, 1988). The once extensive indigenous forests have suffered substantial vegetation loss and degradation leaving only small remnants on hilltops while the formerly denuded hilltops have been reforested with exotic trees (Beentje and Ndiangu’i, 1988).

Although clearing of the forests for cultivation purposes seems to have occurred for a long time, plantation of exotic trees are a more recent phenomena (Muya et al., 2005). This is compounded
by the fact that the importance of the forests biodiversity is still less appreciated by the custodians, decision makers and stakeholders (Wass, 1995). These forests are an example of extreme habitat fragmentation and only small remnants now remain on the hilltops (Mwanyumba and Mwang’ombe, 1999).

While fragmentation might not be a recent phenomenon, it has reached critical levels in recent years (Brooks et al., 1998). To date less that 400 ha of original forests is retained in a scatter of three larger remnants, Chawia (50 ha), Ngangao (92 ha) and Mbololo (220 ha) and nine tiny remnants, embedded in a mosaic of human settlements, smallholder cultivation plots and exotic plantations. Taita hills forests are the only part of the Eastern Arc forests found in Kenya. Eastern Arc forests run from southeast Kenya to Usambara region of Tanzania. There exist thirteen taxa of plants and nine taxa of animals endemic to these forests, in addition to 37 species of plants of different genera that are rare both nationally and globally (Newmark, 1998). Despite the small sizes of the remaining forests, disturbance continues with little control. The landscape is now a mosaic of habitats: scattered forest fragments of different sizes and disturbed levels, separated by agricultural land and plantations of exotic trees.

Taita soils are composed of a high-humic A-horizons overlying pinkish acid sandy loam. These sandy loams are generally deep with high infiltration rates, a low pH (3 to 5), and low water holding capacity and are low in nutrients due to excessive leaching (Muya et al., 2005). The soils are also characterized by the presence of high aluminum levels, low calcium levels, and unavailable potassium, causing a low cation exchange capacity (Muya et al., 2005). The soils are mainly haplic Acrisols, eutric Cambisols, chromic Luvisols and Regosols (Muya et al., 2005). Most of the land under agriculture is on the hill slopes and valley bottoms (Plate 1). The crops
planted mainly are maize, beans, sweet potatoes, cassava, bananas, fruit trees and horticultural crops like tomatoes, kales, cabbage, lettuce, which are limited to valley bottoms. These crops have been planted since 1967 to the present and farm inputs have largely remained the same (Muya et al., 2005). Agriculture contributes 95 % of household income in this area and majority of the farmers here are small-scale farmers (Muya et al., 2005).

3.2 Sampling Design and Sample Collection

Soil samples were collected along a land use gradient from the study area. Allocation of sample plots within the study site was done in a systematic grid. A grid system of plot allocation ensured better coverage of most of the land use types thus reducing the chance of any stratum being under sampled. To avoid auto-correlation the recommended distance between sample plots was 200 m. Two grids of 55 regularly spaced points (200 m mesh size) spanning all LUTs were established at the study site. A total of hundred and ten points were marked. Sampling points were geo-referenced using a GPS 38 Personal Navigator Positioning System. From these a hundred and ten points, sixty points were selected randomly to represent six major land use types, namely; Horticulture, Maize based farming, Fallow (Shrub land), Napier grass based farming, Planted forests of (Pines and Cypress) and Indigenous forests. Some of the LUTs, from which soils were sampled are shown in Plate 1.
Sampling was done once during the month of February 2006. Cylindrical soil corer, 10 cm in depth and 6 cm in diameter, was used to collect soil samples. At each of the sampling point, twelve soil cores were collected from 0 to 10 cm soil depth level as shown (Fig. 2). The twelve sub-samples were thoroughly mixed in sterile containers, put in sterile paper bags, and labeled. These formed the composite samples for each of the point. This procedure was repeated for the lower soil depth level (10 to 20 cm).

Fig. 2 Schematic representation of the twelve soil sampling points which comprised one main sampling point.

The soil corer was sterilized by dipping in 70 % ethanol between sampling points and soil depth levels to avoid cross contamination. The 0 to 20 cm soil depth level covered in this study was selected due to mycological considerations since it contains majority of soil micro fungi found in the soil (Lim and Varghese, 1977; Skujins, 1984). Samples were then placed in ice cube boxes and taken to mycology laboratories of University of Nairobi, for further analysis. Soil samples were
air-dried in a laminar flow hood for 48 h before storing them at 5°C in paper bags. This was done to minimize microbial activity in the soil samples before they were processed.

3.3 Isolation of *Fusarium* species from the Soil

The composite soil samples for each point of the two depth levels were divided in halves and one-half was stored at 5°C for any further use. For the other halves, soils were assayed using serial dilution plating method (Burgess *et al.*, 1988) with 0.1% Tap Water Agar (Brayford, 1993). The small amount of agar in the solution was necessary because it made it easier to disperse the soil particles. *Fusarium*-selective modified Pentachloronitrobenzene-Peptone Agar (PPA) (Burgess *et al.*, 1998) was used to recover *Fusarium* isolates from the soils. This isolation media were prepared in 2 L Erlenmeyer-flasks, autoclaved for 15 min at 120°C and allowed to cool to 45°C in a water bath. Fifteen milliliters of the isolation media were aseptically dispensed into 9 cm diameter plastic petri dishes. Plates containing media were all stored at 5°C for at least 3 days prior to inoculation. The dry media quickly absorbed excess water from the soil suspension after inoculation, which helped to minimize bacterial contamination.

From each of the two levels of soil depth, in each of the sampling point, 10 g of air-dried soil were removed and added to 90 ml of sterile 0.1% Tap Water Agar. The mixture was vigorously agitated (200 rpm for 60 s on a Lab-Line Orbital shaker, Melrose Park, IL) and 10 ml of the resulting suspension were pipetted into a flask containing 90 ml of sterile distilled water. This procedure was repeated up to the third ten-fold dilution. Then, 1-ml aliquots from second and third dilutions, in three replicates, were aseptically pipetted on to the petri dishes containing PPA medium and spread evenly across the agar surface with a sterilized bent glass applicator. The petri dishes were then incubated in an alternating temperature regime, 25°C day/20°C night, at 65% relative humidity, under cool white fluorescent lights (Philips TL 40W/80 RS F40BLB) with a 12 h
photoperiod, for 7 to 10 days. Observations were made from the third day onwards for developing colonies. The PPA medium used was selective for *Fusarium* species. It is highly inhibitory to most other fungi and bacteria but allows slow growth of *Fusarium*, which forms small colonies of 5 to 10 mm diameter after 5 to 7 days.

### 3.4 Purification and Identification of *Fusarium* species

Colonies from PPA media were transferred to Synthetic Nutrient Agar (SNA) media. This is because most species on PPA do not form distinctive colonies and sporulates poorly with abnormal conidial morphology. SNA is a weak nutrient agar and limits cultural degenerations as well as promoting uniform sporulation and good conidiogeneous cell development. For each of the colonies growing on PPA plates, a well-defined and shaped colony was chosen and a small piece at the edge of the colony was carefully transferred onto a separate SNA medium petri dish and incubated at 25°C for 5 days. Subsequently, in order to obtain monosporic cultures of each colony formed on SNA, from which identification was based, very dilute inocula, of 5 to 10 spores per drop of suspension (when viewed at low power magnification), were prepared and spread on 2% Tap Water Agar plates. These were then incubated for 15 h for germination. Germlings were then subcultured on different media, that is, SNA, Carnation-Leaf-Agar (CLA) and Potato-Dextrose-Agar (PDA) media plates, for growth and identification. Many species of *Fusarium* readily formed sporodochia with robust, uniform macroconidia on the CLA that were very useful for identification. PDA cultures were primarily used to assess pigmentation and gross colony morphology. Cultures grown on SNA were evaluated for microconidia that are more abundant and diverse on this medium, and for chlamydospores, which often are more common and produced rapidly on this medium.
All the pure isolates sub cultured on PDA, CLA and SNA were incubated for ten to twenty days at 25 °C under fluorescent lamps (Sylvania cool white tubes) with a 12 h photoperiod. Fusaria were identified to the species level where morphological characters were used as the basis of identification (Nelson et al., 1983). Identification was made according to Nelson et al., (1983), Burgess et al., (1988), Brayford (1993) and Leslie and Summerell (2006). The following morphological characters were used in identification; colony colour on PDA, macroconidia characteristics on CLA and microconidia characteristics on SNA. Data on all isolates were entered in data recording sheets, in order to collate the resulting observations. The data entered included; colony colour and agar pigmentation on the PDA (Nelson et al., 1983; Summerell et al., 2003), the shape, size and manner of macroconidia formation on CLA, the shape, presence or absence of microconidia and their manner of formation on CLA and SNA, the size, shape and type of conidiogenous cells on SNA, size of mesoconidia on CLA and SNA, and the absence or presence of chlamydospores and their formation on CLA and SNA media.

The above procedure was followed for each of the two soil depth levels, that is, 0 to 10 cm and 10 to 20 cm, in each of the land use category identified. After identification, the single spore cultures were stored in agar slants of SNA in screw cap bottles at 4 °C and in sterilized soil in screw cap bottles using the standard techniques (Windels et al., 1993).

3.5 Soil Chemical Characteristics

The remaining soil samples from each point, of each of the two levels of depths, were analyzed to measure the following characters of the soil. The soil pH was determined in distilled water and IN KCl (1:2.5, soil: solution ratio). Total Nitrogen was determined by the catalytic oxidation of organic and chemically combined nitrogen and subsequent alteration to NH4 by the micro Kjeldahl process. Cation Exchange Capacity (CEC) was determined after a first exchange with 1N
ammonium acetate at pH 7, and a second exchange with 1N KCl. Exchangeable Calcium, Magnesium and Potassium were extracted with 1N ammonium acetate, and determined by atomic absorption spectroscopy for Calcium and Magnesium, and by emission spectroscopy for Potassium. Soil Phosphorus content was analysed by the method of compartmental analysis of the kinetics of isotopic exchange of phosphate ions in soil-solution systems maintained in a steady state. Other variables like Cu, Fe, Mn, K, and exchangeable acidity were determined using Mehlich method (Hinga et al., 1980). Total Organic Carbon (TOC) was estimated by oxidation using sulphuric acid and titrating the unused residue against ferrous sulphate (Nelson and Sommets, 1975).

3.6 Molecular Analysis

RAPD-PCR technique was used to determine genetic relatedness among Fusarium species identified. In order to obtain DNA from each of the identified Fusarium species, single-spore Fusarium species were grown for six days at 25 °C, in 50 ml of potato dextrose broth (PDB) (Difco), in a rotary shaker at 150 rpm. The mycelia were harvested from the cultures by pouring the liquid media containing fungal growth through a sterilized non-gauze milk-filter using filter funnels. The culture filtrate was allowed to drain briefly for 30 minutes and the filtered mycelia were washed twice with sterile distilled water, while still in the filter funnel. The washed mycelia samples were removed from the funnel with a clean sterile spatula, onto a pad of clean sterile paper towels, and blotted dry. The mycelia samples were then freeze-dried overnight, before storing them at -20 °C wrapped in aluminum foils.

To extract DNA, frozen mycelia samples for each of the identified Fusarium species, were ground with mortar and pestle in liquid nitrogen. DNA from the ground mycelia was extracted according to the method described by Liu et al., (2000). To a 1.5 ml Eppendorf tube containing 500 µl of
lysis buffer {400 mM Tris HCl [pH 8.0], 60 mM EDTA [pH 8.0], 150 mM NaCl, 1 % Sodium Dodecyl Sulphate} a small lump of mycelia was added by using a sterile toothpick, with which the lump of mycelia was disrupted. The tube was then left at 25 °C temperature for 10 minutes. After adding 150 µl of potassium acetate (pH 4.8, which was made of 60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic Acid and 28.5 ml of distilled water), the tube was vortexed briefly and centrifuged at 13,000 X g for 1 minute. The supernatant was transferred to another 1.5 ml Eppendorf tube and centrifuged again as described above. After transferring the supernatant to a new 1.5 ml Eppendorf tube, an equal volume of isopropyl alcohol was added and the tube mixed briefly by inversion. Then the tube was centrifuged at 12,000 X g for 2 minutes and the supernatant discarded.

The resultant DNA pellet was washed in 300 µl of 70 % ethanol. The pellet was centrifuged at 10,000 X g for 1 minute and the supernatant discarded. The DNA pellet was then air-dried by inverting the tube on a tissue paper before it was dissolved in 100 µl of TE buffer. DNA was quantified using spectrophotometer. Fifteen microlitres of each sample were added to 735 µl TE and mixed well. The optical density (OD) of a dilution of each DNA sample was measured at 260 nM to determine the concentration of each DNA sample using Beckmann DU-65 Spectrophotometer. The concentration of each DNA sample was adjusted with TE to 50 ng/ µl and stored at -20 °C. The OD<sub>260</sub> and OD<sub>280</sub> was also read to determine the purity of each DNA sample. This procedure was followed for all DNA samples obtained from all the 26 Fusarium species.

3.7 RAPD Assessment and Primer screening

The RAPD technique as described by Williams et al., (1990) was used as starting point for setting up a standard protocol suitable for Fusarium species. In order to identify primers that generate
informative arrays of PCR Products, five *Fusarium* species were selected from the entire panel of species. Ten different 10-mer primers (Invitrogen Corporation, San Diego, CA, USA) with a G/C content of 60 to 70 % (Table 1) were screened for amplification of template DNA. Those that provided informative RAPD patterns were selected.

**Table 1.** The codes and sequences of the ten primers screened for RAPD-PCR

<table>
<thead>
<tr>
<th>Primer Code</th>
<th>5’-Sequence-3’</th>
<th>(G +C) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS01/06</td>
<td>GTGACGTAGG</td>
<td>60</td>
</tr>
<tr>
<td>FS02/06</td>
<td>TGCCGAGCTG</td>
<td>70</td>
</tr>
<tr>
<td>FS03/06</td>
<td>AGTCAGCCAC</td>
<td>60</td>
</tr>
<tr>
<td>FS04/06</td>
<td>AATCGGGGCTG</td>
<td>60</td>
</tr>
<tr>
<td>FS05/06</td>
<td>AGGGGTCTTG</td>
<td>60</td>
</tr>
<tr>
<td>FS06/06</td>
<td>GGTCCCTGAC</td>
<td>70</td>
</tr>
<tr>
<td>FS07/06</td>
<td>GAAACGGGCTG</td>
<td>60</td>
</tr>
<tr>
<td>FS08/06</td>
<td>CAGGCGCTTCA</td>
<td>60</td>
</tr>
<tr>
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<td>GGTAACGCC</td>
<td>70</td>
</tr>
<tr>
<td>FS09/06</td>
<td>GTGATCGCAG</td>
<td>60</td>
</tr>
</tbody>
</table>

Primarily, concentration of template DNA, MgCl$_2$, *Taq* polymerase and the thermal cycling profile were varied independently to define reaction conditions which generated reproducible and scorable RAPD profiles. It was noted that template DNA concentrations from one to 100 ng/µl had no significant effect on changing the RAPD pattern. However, concentrations above 100 ng/µl reduced the number of amplified fragments and with 1 ng/µl of template DNA, no products were formed. This finding was verified with various primers and with template DNA of different species. Furthermore, varying the annealing temperature from 28 to 38 °C had no obvious influence on the RAPD patterns. In contrast, only a narrow range of MgCl$_2$ concentration (2 to 4 mM) resulted in constantly reproducible patterns. The established protocol (Table 2) was then utilized to generate informative RAPD patterns.
All working solutions including primers and buffers were prepared in advance. RAPD-PCR procedures were carried out as described by Khalil et al., (2003). All experiments were done using the same reagents and high double distilled water (ddH$_2$O) to ensure repeatability and consistent results. Aliquots of the working stocks were prepared for each experiment and stored frozen (-20 °C). All manipulations were carried out with dedicated DNA-free pipettes in a sterile hood to minimize risk of contamination. The amplification reactions were performed in 25 microlitre volumes in thin-walled PCR tubes after optimization.

PCR reactions were performed in a PTC-100 (Programmable Thermal Controller), programmed for an initial cycle of 2 minutes at 94 °C, 30 seconds at 31 °C and 2 minutes at 68 °C, followed by 35 cycles of 30 seconds at 94 °C, 30 seconds at 31 °C and 2 minutes at 68 °C. There was a final extension step of 5 minutes at 68 °C followed by a cooling to 4 °C until samples were recovered. Tubes containing all reaction components except DNA were used as negative controls. RAPD reactions were performed twice for 4 replicates of each species.

Amplicons were analyzed by electrophoresis in a 2% Agarose gel with 1 X TBE buffer (0.09 M Tris-borate, 0.002 M EDTA, 1 M EDTA [Ph 8]), stained with ethidium bromide. Five microlitres

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock solution</th>
<th>Volume</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddH$_2$O</td>
<td>14.8 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dNTP mix</td>
<td>10 mM</td>
<td>2 µl</td>
<td>0.8 mM</td>
</tr>
<tr>
<td>Taq Buffer</td>
<td>10 X</td>
<td>2.5 µl</td>
<td>1 X</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>25 mM</td>
<td>2.5 µl</td>
<td>2.5 mM</td>
</tr>
<tr>
<td>Primer</td>
<td>5 U/ µl</td>
<td>0.2 µl</td>
<td>1 U</td>
</tr>
<tr>
<td>DNA</td>
<td>50 ng/µl</td>
<td>2.5 µl</td>
<td>5 ng</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>25 µl</td>
<td></td>
</tr>
</tbody>
</table>
of the loading buffer (bromophenol blue) were added to each reaction and mixed well before taking 12 µl of the mixture and loading into the submerged slots in the gel. Gels were allowed to run at 100 volts, constant voltage, for 2 hr. They were then recovered, slid onto a UV transilluminator and photographed with a Polaroid apparatus. The loading buffer was used to trace the migration of PCR products in the gels. A 50-bp ladder (Product code S7025, Sigma, USA,) was used as a molecular maker in determining the sizes of the amplification products. Presence or absence of each size class was scored as 1 and 0, respectively. The resulting matrix was used to compute Jaccard’s similarity coefficients (Sneath and Sokal, 1973) and a dendrogram constructed from the distance matrices employing the Unweighted Pair Group Method with Arithmetic Means (UPGMA) using a Computer package NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) version 1.80 (Rohlf, 1993). Molecular analysis of the identified *Fusarium* species was performed in the molecular genetics laboratories of University of Nairobi.

### 3.8 Statistical Analysis

The data obtained showing distribution and diversity of *Fusarium* species in the soil in relation to various land use types and soil depth levels were analyzed using Biodiversity analysis R package (Kindt and Coe, 2005) and GenStat computer package, discovery edition. Data on abundance were analyzed on log- transformed data and all the other analyses were carried out on untransformed data. Means found to be significantly different were separated using Tukey test at $P<0.05$. Regression analyses were performed to determine the effect of soil chemical characteristic on soil *Fusarium* abundance, richness and diversity at $P=0.05$ level of significance.
Chapter 4
RESULTS AND DISCUSSION

4.1 Results

4.1.1 Abundance of *Fusarium* with respect to Land Use Type (LUT) and soil depth

From the sixty soil sampling points across the six LUTs, 1865 isolates of *Fusarium* were recovered. The identification of these isolates resulted into 26 *Fusarium* species (with one unidentified species) (Table 3). Morphological characters of some *Fusarium* species are shown in Plate 2. The abundance of *Fusarium* isolates obtained was compared for the six LUTs and the two soil depth levels. Data obtained on abundance were transformed using logarithm (log abundance+1), so as not to violate normality assumptions. The results obtained showed that maize LUT contributed 51.6 \% of all *Fusarium* isolates recovered, followed by horticulture LUT with a proportion of isolation of 33.2 \% (Table 3). Therefore, the two LUTs contributed 84.8 \% of all *Fusarium* isolates obtained, with both forests contributing only 5.9 \% (Table 3).

From the results obtained, it is very clear that *Fusarium oxysporum* was the predominant species isolated from soils in this study area, with isolates mainly recovered from maize LUT (Table 3). *Fusarium oxysporum*, *F. solani* and *F. sporotrichioides* had the highest isolation frequencies of 37.9 \%, 10 \% and 7.2 \% respectively, (Table 3). Isolates of these three species accounted for 55.1 \% of all *Fusarium* isolates recovered. *Fusarium oxysporum* and *F. solani* represented 49.9 \% and 45.8 \% of *Fusarium* population in maize and horticulture LUTs, respectively.
Plate 2. Morphological characters of spores of *Fusarium* species isolated from soils in Taita Taveta district, Kenya; 1- polyphialides of *F. denticulatum*, 2- microconidia of *F. solani*, 3-, 4- and 5-chlamydospores of *F. oxysporum*, 6- mycelia and chlamydospores of *F. compactum*, 7- macroconidia of *F. scirpi*, 8- macroconidia of *F. graminearum*, 9- conidiophores of *F. graminearum*.

(Scale bar=50μm).

Table 3. Frequency of isolation of *Fusarium* species in different Land Use Types from both soil depth levels in Taita Taveta district, Kenya.
<table>
<thead>
<tr>
<th><strong>Fusarium</strong> species</th>
<th>Maize</th>
<th>Fallow/shrubland</th>
<th>Napier</th>
<th>Horticulture</th>
<th>Planted forest</th>
<th>Indigenous forest</th>
<th>Overall Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F. oxysporum</strong></td>
<td>36.9</td>
<td>24.4</td>
<td>28.1</td>
<td>42.5</td>
<td>34.4</td>
<td>35.6</td>
<td>37.9</td>
</tr>
<tr>
<td><strong>F. solani</strong></td>
<td>13</td>
<td>12.2</td>
<td>28.1</td>
<td>3.3</td>
<td>7.8</td>
<td>9.6</td>
<td>10.0</td>
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<td><strong>F. sporotrichioides</strong></td>
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<td>14.5</td>
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<td>17.8</td>
<td>9.4</td>
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<td>4.0</td>
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<td><strong>F. verticillioides</strong></td>
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<td><strong>F. diami</strong></td>
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<tr>
<td><strong>F. nelsonii</strong></td>
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<td>0</td>
<td>8.2</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>F. beomiforme</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>F. compactum</strong></td>
<td>0</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Overall frequency of isolation (%)</strong></td>
<td>51.6</td>
<td>6.5</td>
<td>2.8</td>
<td>33.2</td>
<td>2.3</td>
<td>3.6</td>
<td>100</td>
</tr>
</tbody>
</table>

*Fusarium compactum* had the lowest frequency of isolation at 0.2 % (Table 3). Its isolates were recovered only from fallow/shrub LUT. Isolates of *Fusarium acuminatum* Ellis and Everhart were less prevalent in the soil and were only isolated from fallow and horticulture LUTs (Table 3).
The following species were recovered only from maize LUT: F. graminearum, F. decemcellulare Brick, F. heterosporum Nees and Fries, F. xylarioides, F. phyllophilum Nirenberg and O’Donnell, and F. verticillioides (Saccardo) Nirenberg (Table 3). The upper soil layer produced most of the isolates of these species. Isolates of F. lateritium were obtained from soils only from two LUTs namely, planted forests and horticulture (Table 3).

*Fusarium avenaceum* isolates were recovered from maize and horticulture LUTs while F. redolens, F. beomiforme and F. torulosum species were recovered at lower elevations, such as valley bottoms where horticulture was practiced (Table 3). An unidentified *Fusarium species*, F. beomiforme and F. compactum had a very low prevalence (Table 3).

Isolates of the unidentified *Fusarium* species were recovered in the lower soil level in the indigenous forest. *Fusarium semitectum* isolates were recovered from napier, horticulture and indigenous forest LUTs (Table 3). This species comprised a higher percentage of *Fusarium species* in indigenous forests than in napier and horticulture LUT. *Fusarium dlamini* isolates were limited to sites under maize and horticulture. *Fusarium sporotrichioides* isolates were recovered in high numbers from horticulture although they were still isolated from fallow/shrub lands and planted forests LUTs (Table 3). *Fusarium polyphialidicum* isolates were mainly recovered from indigenous forests and maize LUT although they were still isolated from, napier and planted forests LUTs but in low counts (Table 3).

*Fusarium nygamai* isolates were recovered from maize, napier and horticulture LUTs and were absent in the forests while *F. chlamydosporum* isolates were mainly recovered from horticulture, napier and fallow/shrub lands, but absent from forest lands (Table 3). *Fusarium scirpi* and *F. compactum* isolates were recovered only from fallow/shrub lands (Table 3). *Fusarium nelsonii* was
Fusarium denticulatum isolates were not present in soils in fallow/shrub, napier and planted forests LUTs. Fusarium poae isolates were recovered from maize and horticulture based farmlands only. Fusarium lateritium isolates were recovered from horticulture and planted forests LUTs (Table 3).

In the fallow/shrub lands, Fusarium chlamydosporum was the most abundant species while in napier based farmlands the three frequently isolated species were Fusarium nygamai, F. solani and F. oxysporum all with a similar frequency of isolation of 28% (Table 3).

There was significant difference ($P<0.001$) in abundance of Fusarium across the six LUTs (Table 4). Maize LUT had the highest abundance (mean log (abundance +1) of $3.2\pm0.24$) followed by horticulture LUT (mean log (abundance + 1) of $3.11\pm0.32$). Planted forests had the lowest abundance of Fusarium isolates (mean log (abundance +1) of $1.07\pm0.31$) (Table 4).

Table 4. Effects of Land Use Type on abundance, richness and diversity of Fusarium species in Taita Taveta district, Kenya

<table>
<thead>
<tr>
<th>LUT</th>
<th>Mean log (abundance + 1)</th>
<th>Mean richness</th>
<th>Mean shannon index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigenous forests</td>
<td>1.31±0.27</td>
<td>0.92±0.22</td>
<td>0.23±0.08</td>
</tr>
<tr>
<td>Planted forests</td>
<td>1.07±0.31</td>
<td>0.70±0.22</td>
<td>0.09±0.07</td>
</tr>
<tr>
<td>Fallow</td>
<td>1.99±0.31</td>
<td>1.56±0.33</td>
<td>0.42±0.12</td>
</tr>
<tr>
<td>Napier</td>
<td>1.22±0.29</td>
<td>0.95±0.32</td>
<td>0.27±0.11</td>
</tr>
<tr>
<td>Maize</td>
<td>3.2±0.24</td>
<td>4.91±0.82</td>
<td>1.07±0.18</td>
</tr>
<tr>
<td>Horticulture</td>
<td>3.11±0.32</td>
<td>5.38±0.90</td>
<td>1.21±0.15</td>
</tr>
<tr>
<td>$P$ value</td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
</tr>
</tbody>
</table>

Fusarium abundance also varied significantly with soil depth ($P=0.039$) (Table 5). However, the mean standard error bars in Fig. 3 indicate that variability
within each soil depth level was not big. The top soil layer had a higher value (mean log (abundance +1) of 2.23±0.21) than the lower soil layer (mean log (abundance +1) of 1.65 ±0.18). The top soil layer accounted for 79.8 % of all *Fusarium* isolates recovered (Table 5).

**Table 5.** Effects of soil depth on frequency of isolation, abundance, richness and diversity of soil *Fusarium* in Taita Taveta district, Kenya

<table>
<thead>
<tr>
<th>Soil depth level</th>
<th>Frequency of isolation (%)</th>
<th>Mean log (abundance +1)</th>
<th>Mean richness</th>
<th>Mean shannon</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10 cm</td>
<td>79.8</td>
<td>2.23±0.21</td>
<td>2.87±0.45</td>
<td>0.65±0.10</td>
</tr>
<tr>
<td>10 – 20 cm</td>
<td>20.2</td>
<td>1.65±0.18</td>
<td>1.75±0.30</td>
<td>0.41±0.08</td>
</tr>
<tr>
<td><em>P</em> value</td>
<td><em>P</em>=0.039</td>
<td><em>P</em>=0.041</td>
<td><em>P</em>=0.063</td>
<td></td>
</tr>
</tbody>
</table>

*Fig. 3.* *Fusarium* isolates mean abundance from different soil depths from Taita Taveta district, Kenya
4.1.1.2 Species Richness with respect to Land Use Types and soil depth

A total species richness of 26 was recorded for the six LUTs (n =120). Difference in species mean richness in relation to the six land use types was significant (P< 0.001) (Table 4). Horticulture was the richest LUT with a mean richness of 5.38±0.9, followed by maize with a mean richness of 4.91±0.82. Planted forests had the lowest richness (mean richness of 0.7±0.22) (Table 4). With respect to LUT, species accumulation curves demonstrated that species accumulated more quickly in horticulture and maize LUTs while they accumulated slowest in planted forest LUT (Fig. 4). Detection of *Fusarium* species increased with increase in number of the soil samples processed. However, in horticulture and fallow LUTs, all available species were captured with fewer samples as opposed to indigenous and maize LUTs where there was need to process more soil samples in order possibly recover all available *Fusarium* species.
Fig. 4. Species accumulation curves of *Fusarium* species isolates with respect to the Land Use Types isolated from Taita Taveta district, Kenya

*Fusarium* species richness varied significantly with soil depth ($P=0.041$) (Table 5). The top layer of the soil was richer in *Fusarium* species (mean richness of $2.87\pm0.45$) than the lower one (mean richness of $1.75\pm0.30$) (Table 5). Detection of *Fusarium* species increased with increase in number of soil samples taken. Species accumulated more quickly in the upper soil layer than the lower one (Fig. 5).
Fig. 5. Species accumulation curves of *Fusarium* species isolates with respect to the two soil depth levels, isolated from Taita Taveta district, Kenya

4.1.2 Diversity of *Fusarium* species across the Land Use Types and soil depth

The diversity of *Fusarium* species varied significantly with the LUTs ($P<0.001$) (Table 4). Diversity indices calculated for *Fusarium* species indicate that overall, horticulture LUT had the greatest species diversity ($H=1.21 \pm 0.15$) followed by maize ($H=1.07 \pm 0.18$) and fallow/shrub ($H=0.42 \pm 0.12$) LUTs. Both forests had low *Fusarium* species diversity, with planted forests being the least diverse ($H=0.09 \pm 0.07$) (Table 4).

Renyi’ diversity ordering technique were used since they provide enough information to allow one to conclude that one LUT or depth is more diverse than the other (Kindt and Coe, 2005). Renyi’ diversity profiles of *Fusarium* species in the six land uses shows that disturbed ecosystems exhibited a greater diversity than the undisturbed ecosystems (Fig. 6).
Evenness of species was highest in fallow/shrub LUT and lowest in horticulture LUT. Evenness profiles show two distinct categories in the study area (Fig. 7). The evenness profiles in the less disturbed LUTs were distinct and above those of the more disturbed LUTs which included maize and horticulture LUTs.

Fusarium species diversity did not vary significantly with soil depth ($P=0.063$) (Table 5). However, the top soil layer had a higher diversity of *Fusarium* species (mean Shannon of 0.65±0.10) than the lower one (mean Shannon of 0.41±0.08) (Table 5). The upper soil layer had a higher evenness than the lower soil profile.
Evenness profiles of *Fusarium* species isolated from soil under different Land Use Types in Taita Taveta district, Kenya.

4.1.3 Analysis of difference in species composition

Data on abundance were transformed with logarithm to diminish the influence of the dominant species on the results before calculation of ecological distance using Bray-Curtis distance statistic. Distance matrix generated gave a dendrogram of two major clusters. The results indicated that the area under study had more similar species (Fig. 8). The first cluster was comprised of maize and horticulture LUTs showing the two are more similar in species composition than with the other four LUTs. The second cluster of fallow/shrub, napier, planted and indigenous forests LUTs indicated that they had more similar species (Fig. 8). However, fallow/shrub LUT formed a subcluster on its own in this second cluster. These results indicate that there were differences in *Fusarium* species composition between highly disturbed and less disturbed LUTs.
**Fig. 8.** Dendrogram showing analysis of *Fusarium* species similarity from soils under different Land Use Types in Taita Taveta district, Kenya.

### 4.1.4 Effects of Soil Chemical Characteristics on *Fusarium* species Abundance, Richness and Diversity

Differences in soil chemical characteristics across the LUTs were significant ($P<0.05$) (Table 6). Cultivated soils had higher levels of soil P, K, and Mg but low in N, C, Fe, and exchangeable acidity compared to forest soils, which recorded highest levels of C, N, and Fe (Table 6). Horticulture and maize based farmlands had the highest pH levels of 5.0 and 4.7, respectively. Forest ecosystems had the lowest levels at 3.5 and 3.9 for planted and indigenous forests, respectively (Table 6). Forest soils were more acidic than soils from other LUTs with planted forest recording the highest level of acidity. Total organic C levels (%) observed ranged from a low of 1.7 (Napier LUT) to a high of 2.9 (Indigenous forests LUT) (Table 6). The total N levels (%) ranged from 0.2 in horticulture LUT to 0.5 in indigenous forests LUT (Table 6). Phosphorous
levels (ppm) ranged from a low of 5.8 in planted forest LUT to a high of 45.0 in horticulture LUT (Table 6). Fallow/shrub and napier based farms did not show extreme values for these soil variables (Table 6).

Table 6. Mean values of soil chemical characteristics across the Land Use Types in Taita Taveta district, Kenya

<table>
<thead>
<tr>
<th>Land use type</th>
<th>pH</th>
<th>Acidity</th>
<th>N (%)</th>
<th>C (%)</th>
<th>P (ppm)</th>
<th>K (m.e%)</th>
<th>Ca (m.e%)</th>
<th>Mg (m.e%)</th>
<th>Mn (m.e%)</th>
<th>Cu (ppm)</th>
<th>Fe (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>4.7</td>
<td>0.4</td>
<td>0.2</td>
<td>1.8</td>
<td>37.8</td>
<td>0.4</td>
<td>2.6</td>
<td>2.9</td>
<td>0.6</td>
<td>1.4</td>
<td>40.7</td>
</tr>
<tr>
<td>Fallow/shrub</td>
<td>4.3</td>
<td>0.8</td>
<td>0.2</td>
<td>1.8</td>
<td>12.6</td>
<td>0.5</td>
<td>2.9</td>
<td>2.1</td>
<td>0.4</td>
<td>1.1</td>
<td>61.1</td>
</tr>
<tr>
<td>Napier</td>
<td>4.4</td>
<td>0.8</td>
<td>0.2</td>
<td>1.7</td>
<td>19</td>
<td>0.4</td>
<td>2.7</td>
<td>2.1</td>
<td>0.5</td>
<td>1.9</td>
<td>33.0</td>
</tr>
<tr>
<td>Horticulture</td>
<td>5.0</td>
<td>0.2</td>
<td>0.2</td>
<td>1.8</td>
<td>44.9</td>
<td>0.6</td>
<td>2.7</td>
<td>2.8</td>
<td>0.6</td>
<td>0.8</td>
<td>52.3</td>
</tr>
<tr>
<td>Planted forests</td>
<td>3.5</td>
<td>2.0</td>
<td>0.4</td>
<td>2.3</td>
<td>5.8</td>
<td>0.2</td>
<td>2.4</td>
<td>1.2</td>
<td>0.7</td>
<td>1.1</td>
<td>89.3</td>
</tr>
<tr>
<td>Indigenous forests</td>
<td>3.9</td>
<td>1.1</td>
<td>0.5</td>
<td>2.9</td>
<td>19.9</td>
<td>0.3</td>
<td>2.7</td>
<td>0.8</td>
<td>0.6</td>
<td>1.1</td>
<td>76.9</td>
</tr>
<tr>
<td>P value</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.019</td>
<td>0.002</td>
<td>0.018</td>
<td>0.001</td>
<td>0.006</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Regression analysis of soil chemical characters against fungal abundance, richness and diversity indicated that soil P and pH had a significant positive effect on abundance, richness and diversity of soil Fusaria ($P<0.001$). Soil K had a weak positive effect on soil *Fusarium* richness ($r=0.1688$) and diversity ($r=0.403$) whereas Mg had a significant positive effect on abundance ($r=0.25$) and diversity ($r=0.0988$) of soil Fusaria ($P<0.05$). Results also indicated that exchangeable acidity levels had a strong significant negative effect on the abundance ($r=-0.605$), richness ($r=-0.1317$) and diversity ($r=-0.316$) of soil Fusaria ($P<0.05$). There was also a significant negative correlation between soil N levels and *Fusarium* richness ($r=-0.294$) and diversity ($r=-0.67$) ($P<0.05$). The results further indicated that other soil parameters like C, Ca, Mn, Fe and Cu had no significant effect on the abundance, richness and diversity of soil *Fusarium* species ($P>0.05$).
Redundance analysis revealed that all *Fusarium* species were positively correlated with soil P, pH, Mg, K, and Ca, but negatively correlated with soil Mn, C, N, exchangeable acidity Cu and Fe (Fig. 9). The results further indicated that the following species were strongly positively correlated; *Fusarium graminearum*, *F. solani*, *F. avenaceum*, *F. xylarioides*, and *F. verticillioides* and although associated with maize LUT, their abundances were strongly influenced by soil P (Fig. 9). *Fusarium poae* was strongly correlated with the above species in this LUT but its abundance was positively influenced by soil pH (Fig. 9).

*Fusarium oxysporum* was positively correlated with the above-mentioned species as well as those commonly isolated from horticulture LUT. Its abundance was strongly positively influenced by soil K (Fig. 9). The *Fusarium* species in maize LUT were less correlated with those found commonly in horticulture LUT namely, *F. torulosum*, *F. chlamydosporum*, *F. acuminatum*, and *F. sporotrichioides* (Fig. 9). These species were found to be positively correlated with soil Ca levels (Fig. 9).
Fig. 9. Relationship between soil *Fusarium* species and soil chemical parameters in Taita Taveta district, Kenya.

Principal Component Analysis based on the relative *Fusarium* species abundance differentiated the LUTs with 79.69 % variance explained by the first and second components (Fig. 10a). The two components produced three main groups of *Fusarium* species (Fig. 10a). The distribution pattern of these species followed the different LUTs (Fig. 10a and Fig 10b). The first component explained 47.59 % variance while the second component explained 32.1% variance observed. Results revealed that the first component separated maize LUT from the rest while the second component separated maize and horticulture from the other four LUTs (Fig. 10b)
Fig. 10a. Effects of F1 and F2 on distribution of *Fusarium* species in Taita Taveta district, Kenya.

Fig. 10b. Effects of F1 and F2 on distribution of LUTs in Taita Taveta district, Kenya.

4.1.5 RAPD Analysis

4.1.5.1. Identification of informative primers
RAPD analysis was used in this study to exploit the potential of a molecular technique for the study of genetic variability in *Fusarium* species. Out of 10 primers screened for amplification, six were selected (Table 7) since they produced bands that were highly reproducible and consistently well amplified. The choice of selected primers was based on the number of bands generated as well as the quantity of different and reproducible patterns yielded. Tubes containing all reaction components except DNA were used as negative controls. The negative controls without the template DNA produced no amplifications.

**Table 7.** Sequences of oligonucleotide primers used for RAPD analysis

<table>
<thead>
<tr>
<th>Primer Code</th>
<th>5'-Sequence-3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS01/06</td>
<td>GTGACGTAGG</td>
</tr>
<tr>
<td>FS02/06</td>
<td>TGCCGAGCTG</td>
</tr>
<tr>
<td>FS04/06</td>
<td>AATCGGGCTG</td>
</tr>
<tr>
<td>FS05/06</td>
<td>AGGGGTCTTG</td>
</tr>
<tr>
<td>FS06/06</td>
<td>GGTCCCTGAC</td>
</tr>
<tr>
<td>FS08/06</td>
<td>CAGGCCTTCA</td>
</tr>
</tbody>
</table>

4.1.5.2 Genetic relationship among *Fusarium* species

The amount of genetic variation was evaluated by PCR amplifications with a set of six primers. The six primers generated 702 scorable bands. Amplification products were examined and fragment size determined. The size of the DNA fragments ranged from 230 to 2,950 bp and the
number of markers generated by each primer ranged from 83 for primer FS08/06 (Plate 3) to 146 for primer FS04/06. Jaccard’s similarity coefficients ranged from 0.257 to 0.583 among the *Fusarium* species indicating a high genetic diversity. The similarity coefficients were then used to construct a dendrogram using the UPGMA. The dendrogram in Fig. 11 was produced by combining data from all the six primers and its the result of UPGMA cluster analysis of 702 bands for 26 *Fusarium* species.

The UPGMA cluster analysis clearly grouped *Fusarium* species into two main clusters with 25.7 % genetic similarity (Fig. 11). The first main cluster comprised of seventeen species with genetic similarity of 26.2 %. The species in this cluster are; *F. oxysporum*, *F. beomiforme*, *F. semitectum*, *F. scirpi*, *F. avenaceum*, *F. nygamai*, *F. poae*, *F. redolens*, *F. verticillioides*, *F. graminearum*, *F. heterosporum*, *F. chlamydosporum*, *F. sporotrichioides*, *F. decemcellulare*, *F. compactum*, *F. spp.*, and *F. torulosum*. The genetic similarity values between species of this cluster ranged from 26.2 to 58.3 % (Fig. 11). The species belonging to this cluster were again grouped into two subclusters (Fig. 11). The first subcluster comprised of twelve species with genetic similarity values ranging from 32.83 to 58.3 %. The second subcluster comprised of five species with genetic similarity values ranging from 38.53 to 45.89 %. *Fusarium avenaceum* and *F. nygamai* depicted the highest genetic similarity of 58.3 %, followed by *F. verticillioides* and *F. graminearum* with a genetic similarity value of 57.3 % (Fig. 11).

The second major cluster comprised of nine species namely; *F. acuminatum*, *F. dlamini*, *F. nelsonii*, *F. xylarioides*, *F. denticulatum*, *F. lateritium*, *F. polyphialdicum*, *F. solani* and *F. phyllophilum*, with genetic similarity values ranging from 29.5 to 56.34 % (Fig. 11). This cluster was further separated into two subclusters at 29.5 % genetic similarity. The first subcluster comprised of six species with genetic similarity range of 31.52 to 56.34 %. The second subcluster consisted of three species with a genetic similarity value ranging from 30.67 to 34.13 % (Fig. 11).
The dendrogram showed that there is little correlation between some of the clusters of *Fusarium* species and LUT (Fig. 11). For example, *F. solani* isolated from all LUTs were placed in the same subcluster with *F. phyllophilum*, which was isolated from maize LUT only (Fig. 11). In the other hand, *F. sporotrichioides*, *F. decemcellulare*, *F. compactum*, *F. torulosum* and *F. sp* representing all the LUTs except napier LUT occurred in the same subcluster. However, the dendrogram indicate a little correlation between one subcluster consisting of *F. dlamini*, *F. nelsonii*, *F. xylarioides* and *F. denticulatum* and maize and horticulture LUTs.

![Dendrogram](image)

**Fig. 11.** UPGMA dendrogram showing genetic relationships among *Fusarium* species based on Jaccard’s similarity coefficients from RAPD data.
4.2 Discussion

4.2.1 Distribution and diversity

This study has revealed that *Fusarium* species are widespread in occurrence in the targeted LUTs. It is also evident that all the sampled LUTs were different in terms of frequency of isolation of *Fusarium* populations (Table 3) with intensively cultivated lands having a higher frequency of isolation. One of the interesting observations made from this study was the rarity of *Fusarium* species in the forests soils compared to that of cultivated soils. It is clear from the study that cultivated soils contained not only large populations of *Fusarium*, but also more species than uncultivated soils. These results are quite similar to those obtained by Lim (1974) and Wilberforce *et al.*, (2002), who found that there were low Fusaria population in natural vegetation soils. In their work Sarathchandra *et al.*, (2005), found that soil *Fusaria* populations were significantly lower in undisturbed soils compared to highly disturbed soils. *Fusarium* species are ecological members of both agricultural and native communities.

The distribution of soil *Fusaria* is known to be influenced by vegetation and crop grown, by cultural practices, by moisture content of soils and by other inherent characters exhibited by the species. Most of the species isolated are usually found in or on soil, where they exist as colonizers of living plant parts or plant residues within the soil or adjacent to the soil surface (Booth, 1971; Burgess, *et al.*, 1975). In the absence of suitable substrate, these fungi persist as resistant or dormant hyphae in plant residues colonized parasitically or saprophytically, or as discrete propagules such as chlamydospores and resistant conidia. Therefore, these populations have been regarded as soilborne fungi.
In contrast to forest LUTs, cultivated soils showed considerable large populations of *Fusaria*, although the individual *Fusarium* species varied in population size according to the LUT (Table 3). Among the 26 species of *Fusarium* found in the soils, *F. oxysporum* and *F. solani* were found to be occurring in large numbers of isolates (Table 3) and widest in distribution. Kasel *et al.*, (2008) suggested that soil *Fusarium* community is more susceptible to disturbance such as land use change. In their work in Australia, there was clear and consistent evidence of land use effects on soil fungal community composition. Soils from sites of same land use despite geographical separation had same species while different land uses of same location were clearly separated despite being close.

Results from this study demonstrated that agricultural land mosaics of Taita Taveta were higher in *Fusarium* species diversity and abundance than forest ecosystems (Table 4). The low levels of *Fusarium* in these soils may suggest that these undisturbed ecosystems may be more resilient to and suppressive of these fungi than the cultivated farmlands. The results suggest *Fusarium* species may have been suppressed in indigenous forests soils by other predominant saprophytic fungi present or are selected against through lack of suitable or susceptible host roots in these ecosystems, as work by Clark, (1969) and Wilberforce *et al.*, (2002) suggests. It could be argued that considerable ground leaf litter formed an environment not conducive for Fusaria to become established in the forests soils. Some trees are known to produce root exudates that reduce Fusaria populations in the soil (Lim, 1974). Moreover, root systems have their own specialized root associations explaining absence of some *Fusarium* species in forest soils (Lim, 1974).

Management practices such as land clearing, burning of vegetative debris, continuous tillage, monoculture, crop rotation, organic residue inputs, retention or removal of crop residues and use of agrochemicals have been shown to be among causes altering soil *Fusarium* population structure.
In these LUTs, farmers use both inorganic fertilizers and pesticides to increase yields and control pests (Muya et al., 2005). Consequently, these practices may contribute to high diversity and abundance of *Fusarium* observed (Kasel et al., 2008). Other factors such as food availability and habitat preference factors have been indicated as factors, which may explain differences in abundance and species composition of soil microorganisms (Jones et al., 1989). Findings of lower diversity and abundance, particularly in natural forests of Taita Taveta are inconsistent with the expectations of pristine forests holding a higher abundance and diversity (Leslie and Summerell, 2006).

The difference in *Fusarium* abundance between the top and the lower soil depth layer was significant ($P=0.039$) (Table 5). The graph on *Fusarium* isolates mean abundance demonstrated that variability in abundance within each soil depth level was low (Fig. 3). However, the top soil level had a higher abundance than the lower level (Table 5). These results are in agreement with those of Lim and Varghese, (1977) who observed that population of *Fusarium* isolates decreased with increasing depth in Malaysian soils. The probable reason could be the fact that most roots of crops are found in the top layer where there is more moisture and nutrients. The increased resource base created by higher root growth in the top soil layer would create environmental conditions in which a greater number of *Fusarium* isolates could be supported (Thrall et al., 1997). The other probable reason could be due to difference in soil temperatures, with the lower level having relatively lower temperatures. Burgess and Backhouse, (1999) observed that higher soil temperature regimes increased the relative abundances of *Fusarium* isolates in the soils, while abundance decreased with decreasing soil temperatures found in lower soil horizons.

Higher diversity is generally attributed to high competition, causing niche restriction, or low competition due to predation (Huston, 1979), for example, the grazing by soil organisms of extra-
radical mycelium. With respect to LUT, species accumulation curves demonstrated that species accumulated more quickly in horticulture and maize LUTs while they accumulated slowest in planted forest LUT (Fig. 4). This is a clear indication of how abundant *Fusarium* isolates are in these more disturbed LUTs unlike the other less disturbed LUTs. These findings are similar to those reported by Kasel *et al.*, (2008) in their study of the effect of land use on soil fungal community in Australia. A faster rate in *Fusarium* species accumulation in top soil than the lower soil layer (Fig. 5) suggests, fewer soil samples would be taken to isolate all the *Fusarium* species present. In their study of Spain and United Kingdom soils, Wong and Jeffries, (2006) observed that top soil layer had a higher species accumulation rate than the lower one. They suggested that the top soil layer had more air required for respiration by *Fusarium* than the lower one.

*Fusarium* species populations pathogenic to crops, for example, *Fusarium oxysporum* and *Fusarium solani*, were found to be higher in cultivated soils. These results are consistent with those obtained by Rheeder and Marasas (1998) in Transkei region of South Africa, where they observed significantly high populations of *Fusarium* in soils from maize growing areas compared to native lands. This could be attributed to presence of more air in disturbed soils and presence of suitable hosts (Juzwik *et al.*, 2002).

The change from natural forests to intensive agriculture could account for the increase in *Fusarium* species known to infect crops (Giller *et al.*, 1997). The selective effect of the crops is probably the most significant factor in determining *Fusarium* abundance in the soils. Wardle *et al.*, (2004) found the plant species composition and diversity to be the drivers of belowground diversity. They suggested that, greater aboveground diversity and composition resulted in greater belowground diversity.
Tillage conditions and previous crop may have an impact on inoculum density by creating a greater reservoir of primary inoculum (Steinkellner and Langer, 2005). Clear LUT related differences in abundance, richness and diversity were revealed among soil Fusaria. Understanding the reasons for LUT differences in this *Fusarium* is crucial to understanding the consequences of disturbance for the soil *Fusarium* populations. Having the ruderal characteristic of producing large numbers of asexual spores, and not relying on extensive mycelial networks (Pugh and Boddy, 1988), members of this genus are likely to be favoured by agricultural perturbations such as ploughing coupled with availability of soil moisture (Sarquis and Borba, 2007). In their report, Alabouvette et al., 1993 observed that it is in cosmopolitan fungi, such as *Fusarium*, which are capable of non-pathogenic colonization of many hosts that LUT difference in abundance appear to be manifested.

*Fusarium oxysporum* had the highest isolation frequency (37.9 %) of all *Fusarium* species. Nelson et al., (1990) reported a higher recovery of *F. oxysporum* in relation to other species like *F. acuminatum, F. avenaceum, F. semitectum F. verticillioides* and *F. solani*. This species exists in soil as a vigorous saprophyte and survives as chlamydospores or mycelial fragments, either free in the soil matrix or in organic residues in or on soil (Leslie and Summerell, 2006). This species is known to develop better at higher soil temperatures (above 20°C) (Joffe and Palti, 1977), and has been shown to be not only a vascular parasite but also an aggressive colonizer of moribund tissues at warm temperatures (Sangalang et al., 1995). Higher soil temperatures stimulate microbial activity and thereby hasten decomposition of plant materials, which in turn increases chances for survival of strains of *Fusarium oxysporum* because of its high competitive saprophytic ability (Leslie and Summerell, 2006).

At higher temperatures, *Fusarium oxysporum* may be pathogenic to roots of many plants (Warren and Kommedahl 1973), especially if roots are wounded or if other *Fusarium* species are also
present. Waipara and diMienna (2002) reported high levels of pathogenic *F. oxysporum* in cultivated soils more than in the uncultivated soils. The higher soil temperatures therefore may favour root infection by, and therefore survival of *Fusarium oxysporum* in the soil. This fungus has the characteristic of producing large numbers of asexual spores and hence is likely to be favoured by agricultural perturbations (Reeleder et al., 2006).

*Fusarium solani* was also abundant as it was isolated from all the land use types and the two soil depths. This species has a cosmopolitan distribution. Literature indicates that it has been isolated in numerous native soils (Burgess and summerell, 1992; Summerell et al., 1993). *Fusarium solani* is recorded as a pathogen on a vast and diverse range of host plants in the tropics especially legumes and trees (Nelson et al., 1981). The fungus does well under hot soil conditions although its abundance is more strongly dependent on water availability than temperature (Joffe and Palti, 1977). This fact may explain its high numbers in this region. Resistant spores, chlamydospores are produced abundantly making them able to persist in the soils for long (Booth, 1971). *Fusarium sporotrichioides* Sherbakoff was also abundant especially in the horticulture based farmlands as well as fallow/shrub land use type. It is found in the soil on a variety of substrates (Leslie and Summerell, 2006). It abundantly forms resistant spores that enhances its survival in the soil (Mandeel, 1996).

*Fusarium graminearum*, *F. decemcellulare* Brick, *F. heterosporum*, *F. xylarioides*, *F. phyllophilum* Nirenberg and O’Donnell, and *F. verticillioides* were only isolated from maize based farmlands. *F. verticillioides* as a pathogen of maize is found throughout the world wherever maize is grown. This species is adapted to desert soils (Joffe and Palti, 1977) and this may account for its abundance in this hot regions. The fungus occurs as an endophyte but is also well adapted to air dispersal. It persists in host residues on the soil surface or in the soil, following mechanical incorporation and can survive for up to 900 days in dry conditions (Liddell and Burgess, 1985). This fungus was not found as frequently in the soil as *Fusarium oxysporum* probably because it
has to survive as hyphae in bits of plant debris. Information on geographic distribution for this species is too sparse to enable generalization regarding to its environment requirements. As maize is the most important cereal in Kenya (Kedera et al., 1999), the predominance of *Fusarium verticillioides* in Kenyan maize is cause for concern because most isolates of this species also produce fumonisins, mycotoxins.

On the other hand, although *Fusarium heterosporum* has one of the widest documented geographic ranges for any *Fusarium* species, being recovered from subantarctic soils and from both relatively dry as well as wet areas (McMullen and Stack, 1983; Rheeder et al., 1990; Preece et al., 1994), it had low prevalence in this study area. These results are in agreement with those obtained by Lim and Varghese, (1977) who obtained low levels of this species from soils in Malaysia. Due to the fact that this fungus is a weak pathogen of grasses infected by *claviceps* species (Preece et al., 1994), its low occurrence could be explained by probably lack of suitable hosts in this region. The occurrence of *F. decemcellulare* in maize based farms could be explained by their pathogenicity on tropical fruit trees, which were found in this region (Darvas and Kotze, 1987).

*Fusarium nygamai* Burgess and Trimboli was isolated from maize, napper and horticulture based farmlands only. This fungus is commonly found in hot dry areas. It can dominate in the soil of some native grassland (Burgess and Summerell, 1992). It has been associated with root rot of beans, asparagus, cotton, maize, millet, rice and sorghum (Balmas et al., 2000; Kurmut et al., 2002; Leslie et al., 2005). *Fusarium beomiforme* Nelson, Toussoun and Burgess had a very low isolation frequency. The fungus was only isolated from horticulture based farms in very low counts. This fungus has been isolated in soil and soil debris in tropical area of Queensland, Australia, Papua New Guinea and Natal, South Africa (Nelson et al., 1990) and consequently may be adapted to the higher temperatures occurring in Taita Taveta, Kenya. The reasons for the low
abundance of this fungus may be partly due to their characteristic sparse chlamydospore formation, their lack of pathogenicity on plants as well as prevailing dry conditions in the study area. The fungus has not been reported to be plant pathogenic and probably is a saprophyte.

The abundance of *Fusarium dlamini* Marasas, Nelson and Toussoun was equally low. The fungus was only isolated from maize and horticulture based farmlands. It was first described from cultures isolated from plant debris in soil from maize fields in South Africa but has since been reported from other maize growing regions of Argentina and Norway (Marasas *et al.*, 1984).

*Fusarium redolens* Wollenweber was isolated only in Horticulture based farmlands. The isolates of this fungus were relatively low in number. The fungus is mainly isolated from soils in temperate areas. This may probably account for their low counts in this region, which is generally dry and hot. This fungus has been associated with root rot disease of a large number of plant species in temperate regions especially asparagus, beans, carnation, peas, roses and spinach (Booth, 1971). It also have been reported from native grasslands (McMullen and Stack, 1983).

*Fusarium denticulatum* Nirenberg and O’Donnell isolates were only obtained from maize and horticulture based farmlands and indigenous forests. This fungus lacks resistant spores and probably this explains their low numbers in the soils. The fungus is known to be pathogenic to sweet potatoes (Clark, *et al.*, 1995), crops that are grown in the study area. *Fusarium chlamydosporum* was isolated from fallow/shrub lands, napier and horticulture based farmlands although it was mainly isolated from soils under horticulture. This species is commonly found in arid and semi arid regions in the soil and as a saprophyte on a variety of substrates (Burgess and Summerell, 1992; Sangalang *et al.*, 1995). Chlamydospores also form abundantly and rapidly increasing their survival chances. These reasons may explain the high isolation frequency in this region.
Fusarium poae (Peck) Wollenweber had a low abundance and was only isolated from maize and horticulture based farmlands. This fungus is commonly found in temperate regions and this explains its low abundance in this region. The other probable reason is the fact that formation of resistant chlamydospores in the soil is very infrequent (Leslie and Summerell, 2006). This species is regularly recovered from a variety of grains and seeds of numerous native and domesticated plant species (Inch and Gilbert, 2004; Kosiak et al., 2004). The presence of this fungus in the soil may be as a result of spread by mites (Siteroptes graminum) that usually feeds on them or by airborne microconidia. It is usually considered a weak pathogen (Doohan et al., 1999).

Fusarium compactum (Wollenweber) Gordon ranked last on frequency of isolation (0.2 %) for all species. It was only isolated in the fallow/shrub lands. This species is commonly recovered from grassland and desert soils in dry, hot climates (Burgess and Summerell, 1992; Mandeel, 1996). This fungus has low competitive ability (Sangalang et al., 1995) and this may explain their rarity in this regions. Although it has a high capacity for survival in the soils, it prefers soil temperatures above 30\(^0\)C. It rarely occurs, if at all, in cooler areas (Backhouse and Burgess, 1995). This species is mainly regarded as a soil saprophyte. Fusarium scirpi Lambotte and Faut. was only isolated from fallow/shrub lands with an isolation frequency of 0.5 %. This fungus is adapted to wind dispersal (Burgess et al., 1985). This species have been recovered in arid and semi arid regions in the soil (Burgess and Summerell, 1992; Gott et al., 1994). The results obtained coincides with experiments of Burgess and Backhouse (1999), which suggested that this fungus is rare in areas that are too cold or in the hot summer rainfall areas of the tropics.

Fusarium acuminatum was less prevalent in the soil compared to other species isolated from this area. It was only isolated from fallow and horticulture based land use types. Although pathogenicity was not tested, these species have been reported as root pathogens in literature
especially of legumes, pumpkin and wheat. *Fusarium acuminatum* is more common in temperate areas, often in grassland and cultivated soils living as a soil saprophyte as well as a root pathogen. It is less common in tropical areas (Backhouse and Burgess, 1995). It survives poorly at higher temperatures (Sangalang *et al.*, 1995) and presumably, this is one reason why this fungus was not found in abundance in this region where temperatures are generally higher. This fungus is also a poor competitor at temperatures ranging from 18 to 30 °C (Saremi and Burgess, 2000).

*Fusarium avenaceum* (Fries) Saccardo is predominantly soilborne and common in temperate regions throughout the world (Summerell *et al.*, 1993), as a saprophyte and as a pathogen of legumes (Kellock *et al.*, 1978), carnations, vegetables and various perennial species. This species has been recovered from soils from temperate forests (Summerell *et al.*, 1993) and from desert soil in Israel (Joffe and Palti, 1977). The fungus was only isolated from maize and horticulture based farms. The fact that it does not form resistant chlamydospores (Leslie and Summerell, 2006) may account for its low numbers in the soils of this region.

*Fusarium graminearum* are cosmopolitan but primarily found with maize, wheat and barley, but also known from other annual and perennial plants. The fungus was isolated from maize based farms only. It requires high temperatures to form spores explaining their high numbers in this region. However, this fungus does not survive as chlamydospores. Microconidia are absent and formation of chlamydospores variable (Leslie and Summerell, 2006).

*Fusarium torulosum* was only isolated from horticulture based farmlands. This fungus is found in the soil and on a variety of plant species especially in the temperate regions although it can still survive in high temperatures. It has been recovered in the roots of a number of plants including
cereals, tomatoes, beetroot and trees (Nirenberg, 1995). Microconidia are very rare and this may explain its restricted distribution. *Fusarium lateritium* has a worldwide distribution occurring in oil and woody plants. In trees it causes wilt, tip or branch die-back or cankers (Booth, 1971; Marasas, *et al.*, 1984). This fungus was only isolated from horticulture based farmlands and planted forests. Microconidia are generally absent. Since this fungus prefer cool weather, its abundance in this region is low.

*Fusarium nelsonii* Marasas and Logrieco was only isolated from maize and horticulture based farmlands in low numbers. This fungus has been isolated in South Africa from plant debris in soil, *Medicago* roots and sorghum malt. Little information is available on ecology of this fungus. *Fusarium polyphialidicum* Marasas, Nelson, Toussoun and van Wyk has been isolated from soil and plant debris from southern (Marasas *et al.*, 1986; Onyike and Nelson, 1993) and western (Onyike and Nelson, 1993) Africa, Italy and arid portions of central Australia (Gott *et al.*, 1994; Sangalang *et al.*, 1995). In this study the species was isolated from all other land uses except horticulture based farmlands and fallow/shrub lands. The geographic and host range of this fungus is not well defined.

*Fusarium semitectum* Berkeley and Rav. is common in soils but also may be dispersed in the atmosphere and by colonization of plant parts. This fungus is widely distributed and particularly common in tropics (Burgess *et al.*, 1988) and is generally regarded as secondary colonizer of plant tissues. It is commonly isolated from diverse aerial plant parts in tropical and sub-tropical areas especially on bananas and other fruits. It has been isolated in the desert soil (Joffe and Palti, 1977). It is believed to survive as mycelium in plant residues. This fungus is not generally regarded as an important plant pathogen. Microconidia that are abundantly formed which are easily dislodged by
wind and dispersed in the atmosphere by wind and this might be the reason responsible for the occurrence of this fungus in the soils of this region.

The results obtained in this study also indicated that diversity of Fusarium species across the LUTs was significant ($P<0.001$). According to Gomez et al., (2007), the management of the soil influences fungal propagules population diversity. High diversity is generally attributed either to high competition, causing niche restriction or to low competition due to predation (Huston, 1979). Disturbed ecosystems, with diverse plants growing, would provide capacity to support a higher diversity of competing species. Planted forests had the lowest diversity ($H=0.09$) as only species capable of colonizing the plants here would be present. Overall, there appears to be a relationship of diversity of Fusarium species and the type of vegetation present. A greater diversity of vegetation is presumed to support a greater diversity of Fusarium species as the data indicates. These findings are in agreement with those of Wakelin et al., (2008). In their work, they found that soil Fusarium communities were primarily affected by plant residue inputs. Incorporation of stubble increased significantly the relative abundance of soil Fusaria. They further noted that $F. verticillioides$ was absent when stubble was burned but present if it was retained. However, crop rotation was noted to cause a significant change in relative abundances of Fusaria.

Analysis of difference in species composition indicated that maize and horticulture had more similar Fusarium species than with the other four LUTs (Fig.8). These results indicate that there were differences in Fusarium species composition between highly disturbed and less disturbed LUTs. Fallow/shrub LUT formed a subcluster on its own indicating it had Fusarium species occurring only in this LUT.
It has been demonstrated conclusively that differences in *Fusarium* populations between LUTs do exist. Soil variables may be one of the mechanisms by which management affects soil *Fusarium* populations. Key soil characters varied significantly across LUTs ($P<0.05$) and influenced the distribution and diversity of *Fusarium* species (Fig. 9). Maize and horticulture LUTs had the highest levels of soil pH, P, Mg, and K. This may account for the highest abundance, richness and diversity of soil Fusaria observed in these LUTs. These results are consistent with the findings by Jones *et al.*, (1989) who reported that increase in soil P resulted in increase in soil Fusaria.

The use of phosphatic fertilizers in these intensively cultivated farmlands (Muya *et al.*, 2005) could be a probable reason for high occurrence of *Fusarium* populations. High exchangeable acidity has been found to have a negative correlation with *Fusarium* populations in the soil. However, soil pH positively influenced abundance, richness and diversity of soil Fusaria. These results may suggest that *Fusarium* isolates are intolerant to high acidity. The low abundance, richness and diversity of Fusaria observed in the less disturbed ecosystems could be explained by the high levels of soil N and exchangeable acidity found in these ecosystems. Rezacova *et al.*, (2005) observed that higher N fertilizer applications significantly suppressed the abundance of soil Fusaria. In their work, Kasel *et al.*, (2008) observed that there were correlations between soil fungal community composition and soil chemical variables.

Principal Component Analysis based on the relative *Fusarium* species abundance differentiated the LUTs with 79.69 % variance explained by the first and second components (Fig. 10a). The two factors produced three main groups of *Fusarium* species (Fig. 10a). The distribution pattern of these species followed the different LUTs (Fig 10a and Fig. 10b). The first component explained 47.59 % variance while the second component explained 32.1 % of the variance observed. As
shown in Fig. 10b, the first component separated maize LUT from the rest of the LUTs while the second component separated maize and horticulture from the other four LUTs. These results indicate that maize contributed substantially to abundance of soil Fusaria. The first component was therefore presumed to be host type. Strong species-selective effect of maize towards some Fusaria is evident. The study also revealed that maize LUT had more points with positive observations for Fusaria than the other LUTs.

The second component was presumed to be level of intensification. Both maize and horticulture LUTs experience a higher level of perturbations including tillage, application of fertilizers, burying of plant residues in the soil, crop rotation, application of fungicides, and irrigation. All these are known to alter the abundance and diversity of *Fusarium* species in the soil (Wakelin *et al.*, 2008). Since horticultural crops as well as maize have shorter lifecycles unlike the trees, nappier and shrubs, which are perennials, they lead to increased manipulations of the soils. Growth of these crops also involves application of chemicals in the soil, and incorporation of residues into the soil. These practices have an overall effect of increasing soil Fusaria populations (Steinkellner and Langer, 2005). It is clear from the results that *Fusarium* is highly favoured by increased intensification in agriculture.

Plant diseases caused by *Fusarium* species in the tropics are becoming more significant with the introduction of intensive, high-yielding production systems and genetically uniform cultivars (Waller and Brayford, 1990). Wilberforce *et al.*, (2003) observed that agricultural disturbance has a deleterious effect on the diversity of culturable root-endophytic fungi and it favours potentially pathogenic species. *Fusarium oxysporum* and *Fusarium solani* are the most common plant pathogens in this region. These species are also widely dispersed saprophytic soil inhabitants throughout the tropics and the vascular wilts they cause are the most economically important
diseases caused by *Fusarium* in the region. An increase in dominance of these potentially pathogenic fungi may have significant consequences for the susceptibility of a plants community to fungal diseases. *Fusarium oxysporum* features as the first and still the most important *Fusarium* species causing diseases on economically important crops.

4.2.2 Genetic Relatedness

In this study, six primers were used for amplification, yielding 702 polymorphic bands. A dendrogram showing genetic similarities among *Fusarium* species was constructed based on the 702 polymorphic bands using UPGMA cluster analysis (Fig. 11). Jaccard’s similarity coefficients ranged from 0.257 to 0.583 among *Fusarium* species indicating a diverse gene pool in the genus. The results obtained demonstrate that high genetic variability exists among the recovered *Fusarium* species as revealed by RAPD markers. The polymorphisms observed for RAPD markers revealed a high degree of genetic diversity in *Fusarium* species in this study. Clustering analysis clearly separated the species into distinct RAPD classes. The dendrogram obtained separated the 26 species into 2 main clusters, one comprising of 17 species and the other 9 species.

A low genetic similarity value for the species under study was observed (GS= 25.7 %). Of all taxa examined *Fusarium avenaceum* and *F. nygamai* showed the greatest genetic similarity (GS=58.3). *Fusarium xylarioides* and *F. phyllophilum* displayed very little genetic resemblance with each other although both were recovered from maize LUT. *Fusarium solani* were closest genetically to *F. polyphialidicum* with genetic similarity of 34.13 %. *Fusarium solani* was isolated from all LUTs while *F. polyphialidicum* isolates were recovered from most of LUTs. These results are similar with those reported by Khalil *et al.*, (2003). The results are also consistent with the findings of Szecsi and Dobrovolsky (1985) who found a low genetic similarity between *Fusarium* species especially between *F. solani* and *F. oxysporum*. In their experiments, Beladid *et al.*, (2004) found a
higher genetic variability among *Fusarium* species using RAPD analysis for Algerian isolates. Walker *et al.*, (2001) also demonstrated high levels of genotypic diversity among *Fusarium* isolates using RAPD analysis. However, these results contrasts the findings reported by Fiona *et al.*, (2000) that found a strong genetic relationship between *Fusarium torulosum* and *Fusarium avenaceum*.

The dendrogram showed that there is little correlation between some the clusters of *Fusarium* species and LUT although most species in the subclusters of the second major cluster were isolated from maize and horticulture LUTs. *Fusarium graminearum* and *F. verticillioides* were both isolated from maize LUT and indicated a relatively higher genetic relatedness of 57.3 %. The results obtained in this study are in agreement with those obtained by Abd-Elsalam *et al.*, (2002), that indicated lack of clear relationship between RAPD profiles of *Fusarium* species across LUTs in Egypt. Sabir (2006) used RAPD analysis on isolates of *F. sambucinum* and could not detect any correlation between RAPD patterns and geographical location where the isolates were obtained. Pasquali *et al.*, (2003) also could not identify any correlation between geographical origin and the formation of clusters of strains of *F. oxysporum* isolated in Egypt.

High genetic diversity may indicate sexual reproduction is occurring within species that have sexual state. Therefore, new genotypes are constantly being produced and diversity remains high even in a limited spatial area. Bowden and Leslie (1999) hypothesized that high levels of genotypic diversity occurring in a small spatial area indicate high levels of sexual recombination. A higher genetic diversity observed could also be explained by mutations, which might have taken place within *Fusarium* species. Snyder and Hansen, (1954) suggested that mutations play a great role in causing variations and speciation in this genus. Nevertheless, migration and sexual recombination may be likely contributing to the maintenance of genetic diversity. The disturbance
resulting from agricultural activities may cause a shift in reproductive strategy towards asexual (clonal) reproduction at the expense of sexual reproduction and as such affect intraspecific and interspecific diversity.

The genetic variability between species may simply reflect their taxonomic distinction or, alternatively be a function of habitat difference. The distinct groups found in RAPD analysis (Fig.11) suggest the usefulness of this method for revealing interesting phylogenetic patterns in *Fusarium* species. This study has also demonstrated that RAPD-PCR can be employed effectively as a reliable DNA fingerprinting technique to study the genetic variability among *Fusarium* species. However, the efficacy of this method depends on various factors but the optimal concentrations of template DNA, MgCl₂ concentration and the annealing temperature were the most important parameters for obtaining the best resolutions.

This study has shown that the method of Liu et al., (2000) is very efficient for extraction of DNA from *Fusarium* species. The results represented here demonstrate that 2 % agarose gel gives a satisfactory resolution of PCR amplification products. In recent years, numerous DNA-based fingerprinting methods that reveal the genetic diversity of similar organisms have arisen. The advent of these DNA-based molecular methods has provided useful tools with which to study the phylogeny of *Fusarium* and to differentiate species, formae speciales, races and strains (Khalil et al., 2003). RAPDs are already widely used as diagnostic tools in many laboratories along side or as an alternative to RFLP analysis in determining genetic variability among *Fusarium* species (Walkers et al., 2001; Jana et al., 2002; Khalil et al., 2003; Beladid et al., 2004). RAPD markers have been previously used to study inter- and intraspecific variation of 12 species isolated from cotton growing areas in Egypt (Abd-Elsalam et al., 2003). This analysis has also been used to identify *Fusarium* species in Kenyan Maize (Macdonald and Chapman, 1997).
Chapter 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study demonstrates that quantitative differences exist in *Fusarium* species under different LUTs. Differences attributable to land use practices are clear. The current trend in agriculture towards intensive use of land may explain the high abundance of soil *Fusarium* populations. Populations from native areas are likely to be found in fewer locations than are agricultural pathogens of major crops and probably are not dispersed as often since the environment will not be disturbed in a regular manner such as found in production agriculture fields with routine tillage and crop rotation practices. Agricultural ecosystems are unlikely to be in equilibrium, and strong selection for pathogenically specialized and aggressive populations of plant parasites normally is expected.

From the results obtained, it is clear that abundance of *Fusarium* in the soil is favoured by increased agricultural intensification. An increase in dominance of these potentially pathogenic fungi may have significant consequences for the susceptibility of plant communities to fungal
diseases. An understanding in agricultural soils of the ecological importance of soil *Fusarium* species in soil nutrient cycling, food web and trophic interactions, and plant disease expression and suppression, is vital. This study has extended knowledge on the distribution and diversity of *Fusarium* populations in relation to LUTs and soil depth. The study identified the plant species cover as the main factor determining the distribution of soil *Fusarium* species. Differences attributable to land use practices are clear. Understanding on how human activities encourage development of *Fusarium* diversity can aid in the management of this potentially pathogenic fungus. Differences in kind of plants exert a selective effect on species of *Fusarium*. These selective pressures may have led to the prominence of *F. oxysporum* and *F. solani*. In addition, it is possible that *Fusarium* species populations fluctuate within and between cropping seasons as reported by Bateman and Murray (2001). Differences in *Fusarium* abundance between the top soil layer and the lower soil level was significant (*P*=0.039). It is presumed that, increased resources created by root growth in the upper soil layer would create environmental conditions in which a greater number of *Fusarium* isolates could be supported. It is thus concluded that *Fusarium* distribution and diversity in the soil is a product of the influence of soil-endaphic and biotic factors.

This study is a first step in elucidating the genetic relatedness in *Fusarium* species isolated from Taita Taveta, using RAPD markers. Further studies using species representing broader geographic distribution may provide a better understanding of genetic relatedness among members of this genus. This study has also demonstrated the usefulness of RAPD analysis in determining the level of genetic relatedness among *Fusarium* species. RAPD analysis is extremely powerful and can separate individuals with interspecific variability. It gives more comprehensive information regarding the genetic variability among the *Fusarium* community as it is based on the entire genome of an organism. This assay has many advantages as a means of characterizing genetic
variability such as speed, low cost, minimal requirement for DNA and lack of radioactivity. It is thus concluded that, used correctly, RAPD analysis is a rapid, simple and reliable tool in estimating the genetic relatedness among *Fusarium* species.

### 5.2 Recommendations

The following recommendations are suggested based on the results obtained in this study:

1. Further experimental work is required to more precisely define the effects of temperature and water availability on the activity of each of the *Fusarium* species identified. There is also need for further research on seasonal variations of soil *Fusarium* species in terms of distribution and diversity.

2. The effects of genetically modified organisms need to be adequately assessed for their impact on the environmental. It is difficult to predict how genetically modified organisms will behave once in the agricultural ecosystems. Results show that soil *Fusarium* may be sensitive to the use of genetically engineered plants and the effects are unpredictable. Increased and frequent use of “roundup” ready soybean has produced changes in the populations of soil *Fusarium* (Kremer *et al.*, 2000).

3. It is also recommended that land management practices that decrease levels of *Fusarium* populations in the soil should be encouraged. This may include application of acid based fertilizers as abundance, richness and diversity of *Fusarium* isolates in the soil seem to reduce at high levels of soil acidity. Addition of N in the soil is recommended as a way of reducing potentially pathogenic *Fusarium* populations in the soil. Crop rotation of unrelated crops is also recommended to minimize pathogenic populations of *Fusarium* in the soil.

4. This study also revealed that *Fusarium* could be used to assess the effect of ecological disturbance. As new management systems are developed, their effects on biological properties must be carefully assessed since the soil microflora plays such a vital role. *Fusarium* is a cosmopolitan fungus with a wide distribution and with ability to colonize many hosts. It is easily isolated from the soil and identified. Therefore, it can be used effectively to monitor
effects of anthropogenic disturbance in other parts of the world especially on the status of soil health.

5. This study has also revealed a high prevalence of *Fusarium* in the soil of the area under study. It is therefore recommended that a survey to assess mycotoxicological risks facing humans and animals in this region be carried out.

6. This study has demonstrated the usefulness of RAPD-PCR analysis in determining the level of genetic relatedness among *Fusarium* species. The sequencing of interesting RAPD markers obtained is recommended, in order to create longer, locus-specific oligonucleotide primers that can be used in quick identification of unknown *Fusarium* species.

REFERENCES


Booth, C. (1971). The genus Fusarium. Commonwealth Mycological Institute,


Migheli, Q., Briatore, E. and Garibaldi, A. (1998). Use of random amplified polymorphic DNA (RAPD) to identify races 1, 2, 4 and 8 of Fusarium oxysporum f.sp. dianthi in Italy. European Journal of Plant Pathology 104: 49-57


APPENDICES

Appendix I. The protocol used to prepare PPA Fusarium selective media

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>15 g</td>
</tr>
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</table>
KH$_2$PO$_4$ | 1g  
MgSO$_4$.7H$_2$O | 0.5g  
Terrachlor | 1g (containing PCNB 75%w/w)  
Agar | 20g  
Distilled Water | 1L

### Appendix IIa. Analysis of variance of difference in abundance of *Fusarium* in relation to land use type

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
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<tbody>
<tr>
<td>Land use</td>
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<td>91.894</td>
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<tr>
<td>Residual</td>
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### Appendix IIb. Analysis of variance of difference in abundance of *Fusarium* isolates in relation to soil depth

<table>
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<th>Source of variation</th>
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<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
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### Appendix IIc. Analysis of variance of difference in richness of *Fusarium* species in relation to land use type

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### Appendix IIId. Analysis of variance of difference in richness of *Fusarium* species in relation to soil depth

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<th>Source of variation</th>
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Appendix IIe. Analysis of variance of difference in diversity of *Fusarium* species in relation to land use type

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Appendix IIf. Analysis of variance of difference in diversity of *Fusarium* species in relation to soil depth

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Appendix IIIa: The relationship between abundance of Fusaria and soil chemical characteristics ($P<0.05$).

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* Values are significant
**Appendix IIIb:** The relationship between richness of Fusaria and soil chemical characteristics ($P < 0.05$).

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* Values are significant
Appendix IIIc: The relationship between the diversity of *Fusaria* and soil chemical characteristics (*P* < 0.05).

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* Values are significant