DISTRIBUTION AND DIVERSITY OF *PYTHIUM SPP.* IN INDIGENOUS FORESTS AND ADJACENT FARM LANDS IN TAITA AND EMBU DISTRICTS IN KENYA

BY

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DECLARATION

I, David Ndung'u Mukundi, hereby declare that this thesis is my original work and has not been presented in any other University.

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DEDICATION

I dedicate this thesis to my dear wife Pauline, my daughter Ivy Wanjiru and my son David Mukundi jnr. for their love, care and patience during these trying moments of research work and also to my parents Mrs. Mary Wanjiru Mukundi, a peasant farmer who saw me through the education cycle with a full stomach and Mr. James Mwaura, a driver at the University of Nairobi till retirement for their unwavering support and encouragement.

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Abstract

Pythium is a water mould fungus which survive as a parasite, saprophyte or both. *Pythium spp.* play important ecological services like recycling of both Carbon and mineral nutrients for continued plant growth. Members of this genus are ubiquitous and found in soil, water, plants and animal substrate. Spatial distribution and species diversity of *Pythium* were studied with aim of determining the effects of intensive agricultural practices on distribution and diversity. Agricultural intensification interferes with the natural processes occurring within the soil and destroys useful bacteria and fungi, and other organisms leading to increase in parasitic species like *Pythium*. Application of agrochemicals like fertilizers, pesticides and fungicides as well as continuous cropping interferes with natural processes in the soil ecosystem leading to change in the number of *Pythium* species in the soil (Burge, 1988). The possibility of using *Pythium* as environmental quality indicator is considered as one important aspect of the study since their distribution is associated with a particular environmental condition, that the presence or absence of such condition means its presence or absence (Paoletti *et al.*,1991).

Land use practices in the two benchmark sites form a gradient of land use intensities drawn from the inputs which is perfect for testing the significance on the difference in distribution and diversity.

Each benchmark site was divided into windows, sampling plots and sampling points where soil samples were collected. Using the soil samples collected from Embu in Irangi forest and Taita in Ngangao forest, the presence of *Pythium* species propagules were tested by attempting to isolate species of the genera using baiting techniques. The growing mycelium were verified directly by water mounts in a Microscope or transferred from the bait to the isolation media CMA (Corn Meal Agar) amended with antibiotic Chloramphenicol (20mg/L) and Benomyl (10mg/L). To get data on distribution of *Pythium* at each sampling point, soil was collected at different depths. The isolates were characterized using morphological features in order to identify them tospecies level as guided by 'Monograph of genus *Pythium*' (Van Der Plaats – Niterink 1983).

Eighteen *Pythium* species were collected in Embu and ten species in Taita. This shows that Embu has a richer diversity than Taita. Over 20 morphospecies whose identification was not certain were isolated also in both Embu and Taita. ANOVA analysis of deviance table shows that dispersion of *Pythium* in Taita based on land use system is significant at $p \leq 0.04927$ at $ά=0.05$. Also, it was found that Chances of encountering *Pythium* increases significantly with increase in carbon in Embu ($p \leq 0.01830$) (fig.7), and nitrogen concentration ($p \leq 0.0003518$).

CHAPTER ONE

1.0 INTRODUCTION

1.1 DISTRIBUTION OF *PYTHIUM* SPECIES

Pythium is a parasitic water-mold fungus which persists in the soil, roots and crop residue as thick-walled spores called Oospores. Like most soil-borne pathogens of wheat, *Pythium* is largely confined to the top 4 to 6 inches (first 15 cm of soil) (Deacon, 1983). *Pythium* species are spread all over the world and occur as saprophytes or parasites in soil, water or on plant or animal substrates. Members of the genus *Pythium* are oftenly referred to as water moulds implying that their distribution is mainly dependent on the availability of water. Soil moisture is important in saprophytic growth of *Pythium spp*. and their survival by resistant structures is more important than saprophytic persistence.They are therefore expected to be more diverse in areas where moisture conservation is high and less diverse in areas where moisture is limiting especially in bare soil. It thrives in cool, wet conditions. They occur most abundantly in cultivated soils near the root region in superficial soil layers, less commonly in non-cultivated or acid soils where *Trichoderma* is made responsible for their absence due to allelopathy (Burge, 1988).

Pythium debaryanum is the most commonly known member and causes damping-off disease of seedlings (Edena, 2000). A good number of *Pythium* species are necrotrophic parasites in that they invade their hosts aggressively and feed on dead or decaying tissues as is the case in damping - off. They are generally referred to as weak parasites and more so opportunistic or facultative parasites. Other scientists refer to them as a low grade parasites on fibrous roots. Their parasitic role often depends on external factors. When conditions are favourable for the fungus but less for the host, *Pythium* species can become very pathogenic and cause rot of fruit, roots or stems, pre- or post-emergence damping-off of seeds and seedlings. Young or watery tissue is preferentially attacked. Infection takes place when zoospores produce germ tubes or hyphal elements, form appressoria and then penetrate the plant by means of infection pegs. Most species of *Pythium* affect mainly the juvenile or succulent tissues. This restricts their parasitism to seedlings, the feeder roots or root tips of older plants and to watery fruits or stem tissues. This is very critical pathology since they attack the seeds causing their decay and the seedlings which is nipping the crop growth by the bud. They also cause fruit rots of crops such as beans, squash and watermelons. At a later stage, when the cells of stems and main roots have developed secondary thickenings, infection is restricted to feeder roots. This causes them to become stunted or chlorotic. Peach decline, which kills thousands of trees each year, has been shown to be a feeder root problem caused by a combination of factors including *Pythium spp* (Hendrix *et al*., 1966).

Several pathogenic species commonly encountered are *P. debaryanum*, *P. aphanidermatum*, *P. ultimum* and *P. arrhenomanes*. Damping off of seedling is very common all over the world. It occurs in agricultural and forest soils, in tropical and temperate climates and in almost every greenhouse. The disease is most noticeable in nursery beds, greenhouse flats and row crops. According to Hickman (1958), *Pythium* have a high degree of competitive saprophytic ability in contrast with *Phytophthora*.

Modern methods of agriculture including; agricultural intensification application of chemical fertilizers, pesticides, and fungicides interferes with the natural processes occurring within the soil and destroys useful bacteria and fungi, and other organisms leading to increase in parasitic species like *Pythium* (Burgess 1981). According to Mitchel, (1974), the microbial community in natural ecosystems is brought under severe pressure by the products of human activities. At high concentration, chemical pollutants entering soil or water can be toxic to some components of the community and not to the others. In any ecosystem there is great diversity. Large numbers of species are present in the absence of stress. As the system becomes more perturbed, less adaptable species disappear and more resilient species take over the niche and this can lead to outbreaks of plant diseases. This is factored in as many pesticides are introduced and ends up accumulating in the soil. These chemicals upset the biological balance and lead to severe outbreaks of diseases.

In modern agriculture, a lot has been said about deteriorating soil fertility, increasing toxication of soil due to agrochemicals (Burgess 1981). Chemical control of plant diseases and predators has several demerits even though it seems to have rapid effectiveness. Some of the fertilisers have a detrimental effects to soil's physical, chemical and biological characteristics. For example, nitrogenous fertilisers like Ammonium Sulphate are acid forming fertilisers, have a scorching effect due to ammonium content and are highly corrosive. Soil acidification is a major aspect of soil degradation. This may result to the disruption of the equilibrium leading to certain disease attacks particularly the soil borne ones. The low pH favours Fungi like the *Pythium* species and discourages bacteria. In many countries, misuse of land has or is resulting in stressed lands which are degraded and whose production potential is considerably reduced. Stressed ecosystems are those that are degraded or have reached a stage of degradation at which they cannot support their original biotic communities or cannot support agriculture in the absence of relatively high inputs. In most instances, loss of species diversity has been the most obvious casualty accompanying the evolution of agroecosystems. Monoculture, cultivation, artificial fertilizers and agrochemicals all contribute to a reduction in species diversity and biomass (Paoletti *et al.*,1991, 1992).

Soil moisture, temperature, *p*H, cation composition (metallic ions like Na^{+}, K^{+} and Ca^{2+}), light intensity, high organic matter (supports saprophytic phase causing the disease when a suitable host is available) and presence of members of other microorganisms influence disease development and eventually distribution and diversity. In unperturbed systems like forest, the number of species or species diversity is high and the number of individuals in each species is relatively low and outbreaks of diseases are minimal. The inverse is true in highly perturbed systems (Mitchel, 1974).

1.2 JUSTIFICATION

Agriculture employs over 80% of the population in Kenya either directly or indirectly. More than 50% of export earnings are attributed to agricultural products like coffee, tea, horticultural products like flowers, fruits, vegetables, Chrysanthemums and also forestly products. Moreover, Kenya's total arable land is estimated at 8% while permanent pastures and forest and woodland respectively occupied 37% and 30% of the land leaving very little available land for agriculture. Several environmental problems currently threatens agricultural productivity mainly the losses caused by diseases of *Pythium*. The diseases primarily damping-off, root rot and seed rot poses such a great threat in productivity mainly since the pathogens attack seeds and seedlings causing them to rot thus killing large numbers of seedlings in local areas. They also cause fruit rots of crops such as beans, squash and watermelons. These are very critical phase in food production for it can some times lead to 100% crop failure leading to famine and eventual deaths.

A large number of plant diseases and destruction they have caused in the past is documented and there is a clear prove that diseases can entirely change the course of history and the economy of a country, and they have been and are still a limiting factor in crop production (Mehrotra 1997). The distribution of genus *Pythium* in the country and the factors influencing their occurrence is of utmost importance if the diseases have to be controlled. Intensification of agriculture has been the order of the day and Kenya has not been left out. This study explored the distribution of *Pythium* along a land use intensification gradient in order to show the effects of land management on the distribution of this Fungi.

1.3 STUDY SITE

The study was done in Irangi forest in Embu and Ngangao forest in Taita. These two benchmark sites were selected owing to their unique characteristics and locations. Taita hill forests are the only part of the Eastern Arc forests found in Kenya. The Eastern Arc forests run from southeastern Kenya to the Usambara region of Tanzania. As such they are important reservoirs of endemic flora and fauna. While the same hills have a mixture of exotic plantations established by the government and indigenous natural forest; the traces of original forest contain species that have been geographically and ecologically isolated for significant periods of time. These include 13 taxa of plants and 9 taxa of animals that are endemic to the region, as well as at least 22 plant species and 3 animal species that represent the Eastern Arc flora and fauna found only in Kenya and Tanzania. In addition, 37 species of plants are rare both nationally and globally. The weather pattern in the Taita Hills is also distinct from that experienced around Mount Kilimanjaro (which creates its own weather systems), being more influenced by the coastal climate. In addition to lower temperatures, the hills form the first barrier to clouds from the Indian Ocean, providing frequent morning fogs and high amounts of rainfall, averaging 1332-1910mm per year. The favorable climatic regime led to forest covering much of the hills, which in combination with the ecological isolation of the Taita Hills from other forests, permitted the development of various endemic species of wildlife, most famously birds, of which both the unique Taita Olive Thrush and Taita White-eye are endangered. African violets, too, are said to have had their origins in these hills, and were brought to Europe by the early missionaries; there are some twenty species in all. A 1984 study by the East African Wild Life Society and the National Museums of Kenya established the existence of thirteen taxa of plants and nine of animals which were endemic to these forests.

The Central point of the study area (Mt. Kenya Forest near Irangi Market and bordering Agricultural Lands) has Forests on lower slopes, the Bamboo vegetation in the upper middle slopes, the Afro-alpine grasslands (moorland) in the upper slopes and snow cap on top of the mountain. The area below the Forest belt is utilized for has intensive cultivation of Tea on the upper slopes and Coffee on the lower slopes. The rest of the area is under intensive subsistence agriculture with agroforestry being widely practiced in many parts as a means of soil and Water conservation. This gives a clear gradient of different land use intensities needed for comparative analysis on the impact of intensive cultivation on distribution and diversity of *Pythium* species.

1.4 OBJECTIVES OF THIS STUDY

1.4.1 Broad objectives

To determine the effect of land-use practices on distribution and diversity of *Pythium* species in indigenous forests and adjacent farm lands in Taita and Embu district.

1.4.2 Specific objectives

1. To determine the distribution and diversity of *Pythium* species in indigenous forests and adjacent farm lands in Taita and Embu district.

2. To establish the determinants of *Pythium* distribution and diversity.

Null (H_0) and alternative (H_A) hypothesis of the research are that agricultural intensification does not result in change of biodiversity of *Pythium* leading to loss of ecosystem services detrimental to sustainable productivity and that agricultural intensification results in change of *Pythium* biodiversity, leading to loss of ecosystem services detrimental to sustainable productivity. $\alpha = 0.05$

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *PYTHIUM* AS BIOINDICATOR OF SOIL DEGRADATION

The use of animals, plants and microorganisms as bioindicators of environmental impact is a well established concept (Paoletti *et al.*, 1991). Below ground biodiversity loss indicators are also the environmental quality indicators. An environmental quality indicator is defined as a plant or soil organism that is so associated with a particular environmental condition that the presence or absence of such condition means its presence or absence. The species of *Pythium* that infect plants are ubiquitous in soil making their absence, high or low diversity a good indicator of soil degradation. *Pythium* being a member of Oomycetes (water moulds) makes it a perfect choice as a study organism since it thrive very well in permanently saturated soils as is the almost the case in both Embu and Taita. Soil with large populations of higher Fungi and other organisms suppress both the saprophytic and pathogenic activity of *Pythium* species. The addition of organic matter contributes to soil microbial populations and results have shown that fertile soils like in natural forest record low *Pythium* while cultivated soil have recorded high levels. Owing to *Pythium* specificity and sensitivity it is a low grade parasite and their saprophytic activities greatly restricted. The species high number leads to indicate the reduced soil fertility and easily gives information on soil degradation (Atlas, 1992).

2.2 Land use history

From the late 1950's in Embu, colonial government started to subdivide and distribute the then indigenous Mt. Kenya forest and in the late 1961-1962 clearance of forests commenced to pave way for settlement and farming activities. The first crops were planted in 1962 including maize, arrowroots, bananas, yams, beans, sorghum, cassava and sweet potatoes. They were all planted without application of any farm inputs but harvests were impressive. Coffee which was introduced in 1962 as a cash crop was initiated with the use of chemical fertilizers and other agro-chemicals. However, from 1966 harvests of food crops drastically declined and in respect agriculture extension officers recommended to farmers the use of fertilizers and manure to improve production. The farmers interpreted declined harvests to loss of soil fertility. Therefore the year 1966 marked a turning point which is of significance to below ground biodiversity and its mandate which is to understand and explain below ground factors/processes leading to declining soil fertility and how to bolster and conserve soils for higher productivity and improved livelihoods (Mutsotso *et a.,l*).

Tea crop was first introduced in 1962 on small scale trial basis but in the period 1972-74 there was massive and rapid expansion involving all farmers. Its introduction started with the use of chemical fertilizers and this has continued to the present.

Since the onset of cash crops (tea and coffee) most farmers (over 90%) devoted over 80% of their total land to coffee and/or tea. This finding is according to the report of the Embu District Development Plan for 2002-2006 which shows that the total district acreage under food and cash crops is 12,600 and 18,354 hectares respectively. The apparent overemphasis on increased land under cash crops was initially the government's official policy to bolster production for exports.

While it was good in intention and practice, the unintended consequence is that it introduced massive use of chemical fertilizers and subsequently made many families food insecure.

In Taita, land use history is rather different but equally presents a similar consequence as that of Embu. By 1967 all of Taita Hills was a densely forested area with only intermittent and uncoordinated slash and burn agriculture practiced by a few "daring" families. The rivers were big and wildlife teeming. However between 1967-1968 there was land adjudication and consolidation in which different families were randomly allocated forest land for settlement without considering family background. However, by 1983 all the hills were already cleared of vegetation, the rivers were a pale shadow of their former status, soil erosion was high and food production had dwindled considerably. This situation prompted the Ministry of Agriculture to start an intensive agroforestry campaign as a way of greening the farmlands. Consequently in 1983 gravelia trees were introduced. All farmers planted them, a situation which today explains the existence of gravelia trees on all the farmlands.

The crops initially planted were maize, beans, sweet potatoes, cassava, arrow roots, bananas, fruit trees and horticulture crops like tomato, kale, cabbage, lettuce etc. These crops have been planted since then to the present and farm inputs remained the same. Therefore the farm and landscape history in both benchmark sites have remained the same since they were first settled.

The average land size is 1.2 and 5.4 acres per family in Embu and Taita respectively. However, this average may be much lower since a typical family consists of as many as four inter-dependent families living in one homestead but sharing the land. Land pressure is evidently very high especially in Embu hence more degraded a situation which has been attributed to higher diversity of *Pythium* species compared to Taita.

In Embu tea is monocropped but again in Embu and Taita, horticulture crops are monocropped. In both benchmarks sites again there is intercropping of coffee and other food crops especially between maize, beans, yams, cassava, sweet potatoes, passion fruit, sugarcane and macadamia nuts. Embu District Development Plan 2002-2006 shows that production in the coffee subsector dropped from 40,000 tonnes in 1997 to 29,500 tonnes in 2000. Coffee yield is still 3kg per stem against the ideal of 6kgs." On ascendancy now are macadamia nuts and passion fruit which have the most attractive prices but which again have the lowest inputs. In both benchmark sites macadamia nuts have minimal inputs if any and therefore a very attractive crop compared to others which require many inputs, intense labour requirements but low prices.

2.2.1 Farm Inputs

Depending with the inputs, land use intensities have been drawn where the inputs used are both organic and inorganic. As is shown in Figure 1, land use intensity increases from forest to maize based systems. As agricultural intensity increases, aboveground biodiversity is reduced to monoculture with an intention of increasing economic efficiency. These impacts negatively on belowground biodiversity including *Pythium* by lowering the biological capacity of the ecosystem for self-regulation. This leads to further need for use of agro-chemical to sustain the production that meets market demands for example tea.

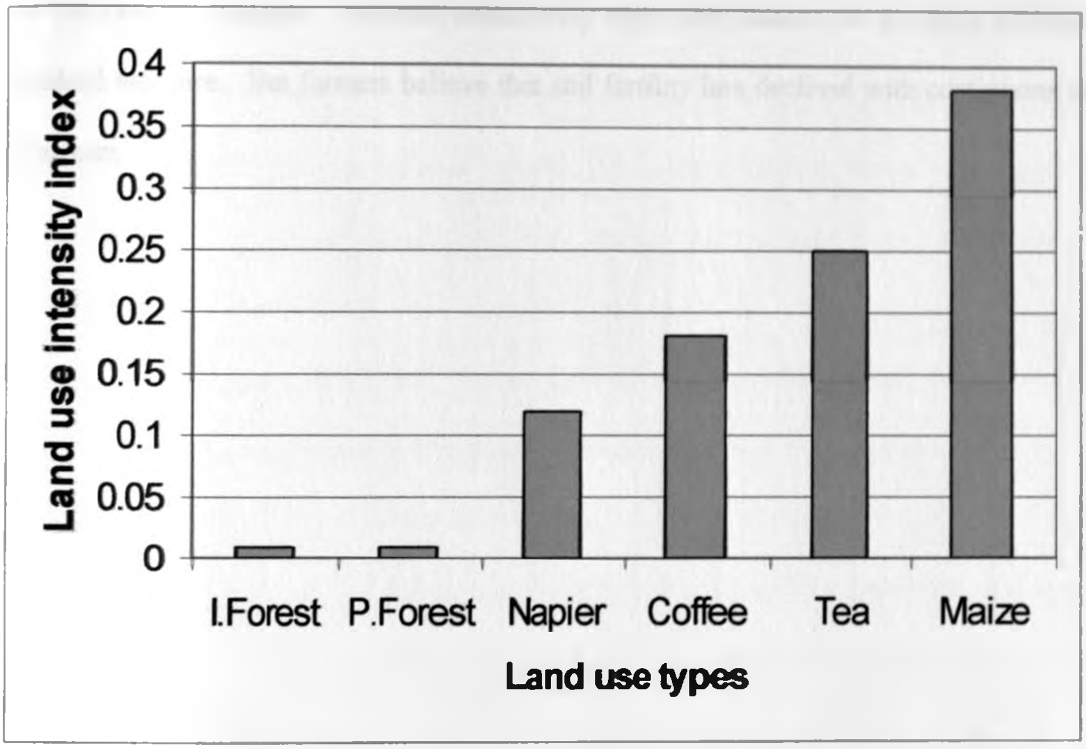


Figure 1: Land use intensity index across different land use types in Embu

The main inputs applied in tea, coffee, maize, beans and horticultural crops include fertilizers, fungicides, insecticides and manure. The commonly used fertilizers in both benchmark sites are: NPK, CAN, UREA, ASN, DAP, TSP, SSP and DSP. The fungicides are copper cobox, green coppers, mealraz, reval meal and cupro cuffallo. The insecticides used are: dimethoite, dusbarn, karate, brigade and thruricide. In Taita especially spraying of insecticides is so intensive at once per week for the three month horticulture season. The fertilizer is applied once per season in the case of tea and coffee. On tea, each fertilizer bag is applied to 500 stems and an acre carries 3000 stems. Fertilizer use on food crop farming is done without standard measure since the farms are tiny, but on an eighth of an acre of maize is applied 20 kg of fertilizer. For coffee, each acre is applied three bags of NPK fertilizer besides application of livestock manure. While the levels of inputs are known in the application of fertilizers, it is not the case for manure. Farmers haphazardly apply the manure on the farm without any standard measure. But farmers believe that soil fertility has declined with continuous use of fertilizers.

2.3 TAXONOMY OF THE GENUS *PYTHIUM*

The genus *Pythium* is recognized by their mycelium that consist of rather slender, coenocytic hyphae with cellulose walls, lives in the soil saprobically on dead organic matter or parasitically on the young seedlings of a great many susceptible species of the seed plants. The hyphae are both intracellular and intercellular. No haustoria's are produced. The sporangia of the organism, constituting the asexual stage, are globose to oval and are either terminal or intercalary on the somatic hyphae. Zoospores are released through bubble like vesicle where they flow from sporangia through tube. Sexual reproduction is by oogonia and antheridia. Upon gametangial contact, a fertilization tube develops and penetrates the oogonial wall and the periplasm and nuclear fusion results in formation of a zygote. The oospore develops and germinates after undergoing a rest period (Alexopoulos, 1983).

The taxonomic classification is primarily based on the comparison of morphological characteristics, including calculations of proportions of certain structures treatments of *Pythium* dealt with limited numbers of species. Middleton's work (1943) compiled all the species known at that time. Waterhouse (1967, 1968a) compiled the diagnoses and descriptions of more than 180 species of *Pythium* and provided a key to 89 recognized species. As several new species were subsequently described, a new monograph was required to cover all present-day knowledge of the genus and to evaluate the available taxonomic criteria. Most of the recognized species are represented by living strains preserved at the Centraalbureau voor Schimmelcultures (CBS).

Pythium is classified according to the hierachy below.

Kingdom *Stramenopila*, Phylum *Heterokonta*, Class *Oomycetes*, Order *Pythiales*

Family *Pythiaceae*, *Genus Pythium*.

Type species: *Pythium monospermum* Pringsheim (lectotype).

The genus differ, among other things in the number of flagella on their spores and in their wall compositions, the Oomycetes being unusual amongst fungi in having cellulose or cellulose- like polymer in their walls, instead of chitin. The genus is composed of 120 described species, which vary in their pathogenic ability and crop host. Hyphal diameter, hyphal swellings, oogonia, antheridia, sporangia and clamydospores are used to describe the specimen. The following are genera synonyms and include *Pythium* Pring, *Artotrogus* Mont, *Cystosiphon* Roze & Cornu, *Lucidium*Lohde,

Nematosporangium Fischer, *Rheosporangium* Edson and *Sphaerosporangium* Sparrow.

2.5.1 Morphological characteristics

Pythium has a well developed mycelium often with appressoria, hyphal swellings, rarely with chlamydospores. *Zoosporangia* either filamentous, not differentiated from the vegetative hyphae, or consisting of lobate or toruloid inflated elements, or made up of well-defined (sub) globose structures, sometimes internally proliferous. Sporangia terminal, intercalary or lateraly sessile, forming a discharge tube of varying length through which the sporangial contents move and form a vesicle at the tip with an undifferentiated mass of protoplasm; this mass then differentiates into a number of biflagellate zoospores. Oogonia (sub) globose, lemon-shaped or ellipsoidal, terminal or intercalary, with a smooth or ornamented wall. *Antheridia* none to several per oogonium, monoclinous, diclinous or hypogynous, stalked or sessile, of various shapes. *Oospores* usually single, rarely 2-4 in an oogonium, plerotic or aplerotic with a thin or thick (inspissate) wall. The genus *Pythium* in the present sense was introduced by Pringsheim (1858) but was antedated by *Pythium* Nees (1823). It was therefore conserved against the latter in 1974, as proposed by Waterhouse (1968b). Montagne (1845) described the congeneric *Artotrogus hydnosporus* (= *Pythium* *hydnosporum* (Mont.) Schröter), so that *Artotrogus* also has priority over *Pythium*. Pringsheim based his genus on two Fungi which he named *P. monospermum* and *P. entophytum*. Zopf (1890) transferred the latter to *Lagenidium*, so that *P. monospermum* remained as lectotype species. *Pythium* was placed in the Saprolegniaceae by Pringsheim (1858), Cornu (1872), and Berlese and de Toni (1888), and in the Peronosporaceae by de Bary (1881) and Fischer (1892). It is now regarded as type genus of the Pythiaceae Schröter in the Peronosporales.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Sampling site

Soil samples were collected from Embu and Taita benchmark study sites in Kenya with an aim of isolating *Pythium spp.* across the land-use gradient. Embu study site is located in the northern part of Embu town and bounded by Latitudes 37° 18' East and Latitudes 0° S and 0° 28'S. Three windows, I, II and III in Embu were selected each measuring 1x2 Km in order to capture as many land use types as possible. Windows I was mainly coffee; window II had tea and coffee as dominant land uses while window III was mainly tea and forest. These windows were then marked using Global positioning system (GPS). The sampling plots were established at fixed intervals of 200 meters in each window (Groupe, 1984). In Embu land uses are mainly coffee, tea, maize, beans, bananas, potatoes ,yams but tea and coffee are the dominant cash crops on which all farmers devote over 90% of their land. Tea is monocropped whereas there is intercropping of coffee and other food crops especially between maize, beans, yams, cassava, sweet potatoes, passion fruit, sugarcane and macadamia nuts. At random, sampling points were picked from each window basing on land use, where each land use type was replicated at least eight times due to intercropping: Tea farming 8 points, Coffee farming 10 points, Maize 9 points, Fallow 8 points, Napier farms 8 points, Plantation forests 9 points based on Chatfied (1988). These land uses were used to compare the abundance and distribution of *Pythium*.

The Taita Benchmark site is located in southeast Kenya, 25km west of Voi in the Taita Taveta District, at approximately 03^{0}20'S, 38^{0}15'E. Land use types in Taita are fallow, coffee, maize and beans, horticulture, natural forest and napier grass. The main land uses are horticulture, maize and beans. In Taita a smaller proportion of the farm is devoted to horticulture which is both the food and cash crop and is limited to valley bottoms. There is intercropping of coffee and other food crops especially between maize, beans, yams, cassava, sweet potatoes, passion fruit, sugarcane and macadamia nuts. Two windows were selected in Taita each measuring 1x3 km. These windows were then marked using GPS. The sampling plots were established at fixed intervals of 200 meters in each window (Groupe, 1984). At random, sampling points were picked from each window basing on land use, where each land use type was replicated at least eight times.

3.2 Sampling design

At each sampling point, twelve sub-sampling points were marked, four from the inner core at loci of 3m radius and eight sub samples from the outer core at loci of 6m radius as illustrated in the figure 2 below. The soil samples were collected at 3 levels; 0-10, 10-20 and 20-30cm.

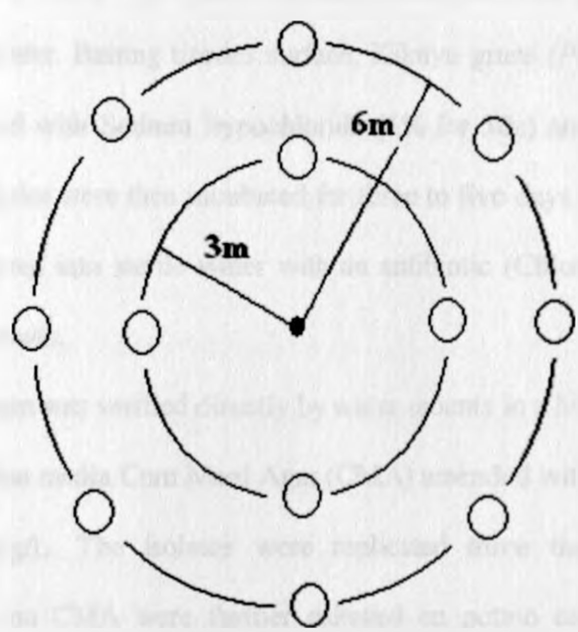


Figure 2: Soil sampling points

From each depth, all the collected twelve sub-samples of soil were mixed to form a homogenous mixture of each depth. Thus from each sampling point three soil composite samples from each depth were collected. About 500g of soil was sampled from this composite and packed in plastic bags, labeled and stored in a cool box at room temperature.

3.3 Isolation of *Pythium* species

In the laboratory, 50g of soil sub- samples were saturated with sterilized water in a 50 ml beaker and incubated at room temperature for three days. A further sub-sample of 0.25g of the pre-moistened soil was transferred in test tubes: three replicates per sample point per depth in baiting *Pythium*.

Baiting technique was used to recover *Pythium* species from soil. Sub-samples of 0.25g of pre-moistened soil were scooped with a sterilized spatula and placed in sterilized glass test tubes with 10ml of sterilized water. Baiting tissues surface, Kikuyu grass (*Pennisetum clandestinum*) leaf blades were sterilized with Sodium hypochloride (1% for 30s) and added to the test tubes for incubation. The samples were then incubated for three to five days at 25^0 C in the dark. Infected tissues were transferred into sterile water with an antibiotic (Chloramphenicol) for 12 hours to suppress bacterial growth.

The growing mycelium was verified directly by water mounts in a Microscope or transferred from the bait to the isolation media Corn Meal Agar (CMA) amended with Chloramphenicol (20mg/L) and Benomyl (10mg/L. The isolates were replicated three times. Isolates that produced submerged cultures on CMA were further cultured on potato carrot agar (PCA) for further identification of species.

In isolates where sporangia did not form, or sporangia were few, spore induction was carried out. This was done by a small piece of culture on CMA in a Petri dish with shallow layer of water (sterilized pond water). Grass leaf boiled for 10 min was added to the water. After 12hours to a three days, the *Pythium* would colonize the grass leaf and develop zoosporangia along its margin.

In order to key-out the species isolated from the study site samples, key morphological characteristics were used to categorise the isolates into various morphological groups, which were later screened to species level. This helps in the establishment the species that grow below the media (divers species) present under each land use category (See appendix). After successful isolation, morphological features used in identification were based on *Monograph of genus Pythium* by Van Der Plaats – Niterink (1983). Characters of *Genus Pythium* considered for classification were Oogonia, Sporangia, Antheridia, Clamydospores, Hyphae and Mycelia.

3.4 Microscopy

After the baiting, the inoculation was done and the mycelia grew rapidly and filled some plates in the in the incubator. The summary of the plate cultures obtained after culturing the isolated propagules from different sampling points and mycelia were mounted on a slide and observed under a microscope.

Microscopic examination was carried out by mounting the culture in acid fuchsin. In order to measure the dimensions of the fungal sporangia, spores, Oogonia, Oospores, hyphae, antheridia and other descriptive structures, both stage micrometer and eyepiece micrometer were used. Using a high power objective (x40), it was found that the disc scale where 40 small divisions of the latter are exactly included in 1/100 small divisons on the stage scale, hence 40 small divisions of the disc scale=0.001 mm. and one small division=0.000025mm. The formulae X=2.5Y was used and units used were micrometers (µm) (Sharma, 1991).

40 units=0.001mm

1 unit=0.001mm/40=0.000025mm=2.5^{-4}cm

1μm=10^{-6}m. 1 unit=2.5^{-4}cm/10^{-6}cm =2.5μm

NB therefore that all the dimensions were observed at magnification x400

3.5 Data Analysis

Pythium was isolated using screw capped test tubes in triplicates. The test tubes where mycelium was baited, it was tallied as **+** and where nothing was baited, it was tallied as **—**. The total number of the positively baited mycelium at different depths was tallied and quantitative assays on distribution was reached by the relative colonization frequency of the bait tissues among replicates in each soil sample was compared and significance tested using a two-tailed test (Zar, 1996). Statistical analysis was done using done using principal component analysis approach (PCA) and Biodiversity.r software computer package. Biodiversity.R software computer package was used to key in the raw data collected from the experiments and also plot graphs thereof. Each experimental data was subjected to several analyses including Analysis of variance (ANOVA). Where there was no significant differences in repeated experiments, pooled data analysis was carried out for instance species accumulation curve which potray the average pooled species richness when all sites were combined together. In PCA approach, species analysis dictates marking of LUTs where they were separated on the basis of species counts. This gives two dimensional plot correlation circle with fewer and manageable factors. Trends in the two sites in Embu and Taita were compared. A PCA on correlation matrix was done on the data consisting *Pythium* species groups and land-use types and the correlation circle is given showing an assemblage pattern of *Pythium* within the landscape

Diversity index is a mathematical measure which describes the species richness and apportionment within the community (evenness). It provides a summary of richness and evenness by combining these two facets of diversity into a single statistic. Shannon weaver measure assumes that a random sample is taken from an infinitely large population, and that all the species in the community population are represented in the sample.

CHAPTER FOUR

4.0 RESULTS

4.1 DIVERSITY OF PYTHIUM BETWEEN EMBU LAND USE TYPES

A total of eighteen species were recovered. *Pythium ostracodes* and *P. sylvaticum* are the most abundant species in Embu followed by *P. ultimum*, *P. macrosporum*, *P. spinosum*, *P. splendens*, *P. heterothallicum*, *P. hypogynum*, *P. flovoense*, *P. anadrum*, *P. buismania*, *P .papillatum*, *P. coloratum*, *P. orthogonon*, *P. graminicola*, *P. grandisporangium*, *P.palchrum* and *P aphanidermatum* in that order (Table2). The species frequencies also elucidate the species proportions in the study site.

Diversity was not significantly affected by land use systems in Embu ($p \leq 0.810$) (table 1). Napier had the highest mean diversity of 0.085 followed by fallow (0.080), plantation forest (0.066), Tea (0.060), Maize (0.046) and Natural forest (0.032) in that order. The order of species richness from the highest to the lowest in Embu where fallow and pasture having the highest mean species richness of 0.833 followed by plantation forest, natural forest, tea, nappier, maize and coffee in that order. Richness was not affected by land use systems in Embu ($p \leq 0.297$). Fallow/pasture has the highest mean species richness (0.833) followed by, plantation forest, natural forest, tea, nappier, maize and coffee in that order. Total richness in Embu was 18 while the jacknife estimate of richness was 27.9 far higher than the encountered species (Table1).

Table 1: Frequency of isolation of *Pythium spp* in Embu

SPECIES	FREQUENCY	PERCENTAGE
P. ostracodes	4	9.09 %
P. ultimum	2	4.55 %
P. sylvaticum	4	9.09 %
P. flovoense	1	2.27 %
P. anadrum	1	2.27 %
P. buismania	1	2.27 %
P. papillatum	1	2.27%
P. macrosporum	2	4.55 %
P. coloratum	1	2.27 %
P. spinosum	2	4.55 %
P. splendens	2	4.55 %
P. heterothallicum	2	4.55 %
P. orthogonon	1	2.27 %
P. graminicola	1	2.27 %
P. hypogynum	2	4.55 %
P. grandiosporangium	1	2.27 %
P. palchrum	1	2.27 %
P. aphanidermatum	1	2.27 %
3C	3	6.82 %
2B1	1	2.27 %
Sub	10	22.73 %

Key: 3C, 2B1 are species that were isolated but could not be identified to species level but grouped to a given morphological group. Sub. are species that were isolated but formed submerged cultures hence could not be identified to species level (appendix)

Table 2: Diversity of *Pythium* with land use types in Embu.

Land use	Mean diversity	Mean richness	Mean evenness
Coffee	0.026	0.444	0.025
Fall/Past	0.080	0.833	0.018
Maize	0.046	0.500	0.025
Napier	0.085	0.619	0.039
Natu Fore	0.032	0.750	0.018
Plan Fore	0.066	0.778	0.019
Tea	0.060	0.727	0.020
P value	**0.810**	**0.297**	

Jacknife estimates on richness shows that far higher species could be baited than the encountered species especially in fallow/ plantations land uses confirming the limitations in the baiting method in isolation of *Pythium*. Tea estimates however, indicates that all possible species were encountered. The highest expected total richness is in Fallow LUT followed by nappier and the least is natural forest and tea with a Jackknife estimate of three species. (Figure 3).

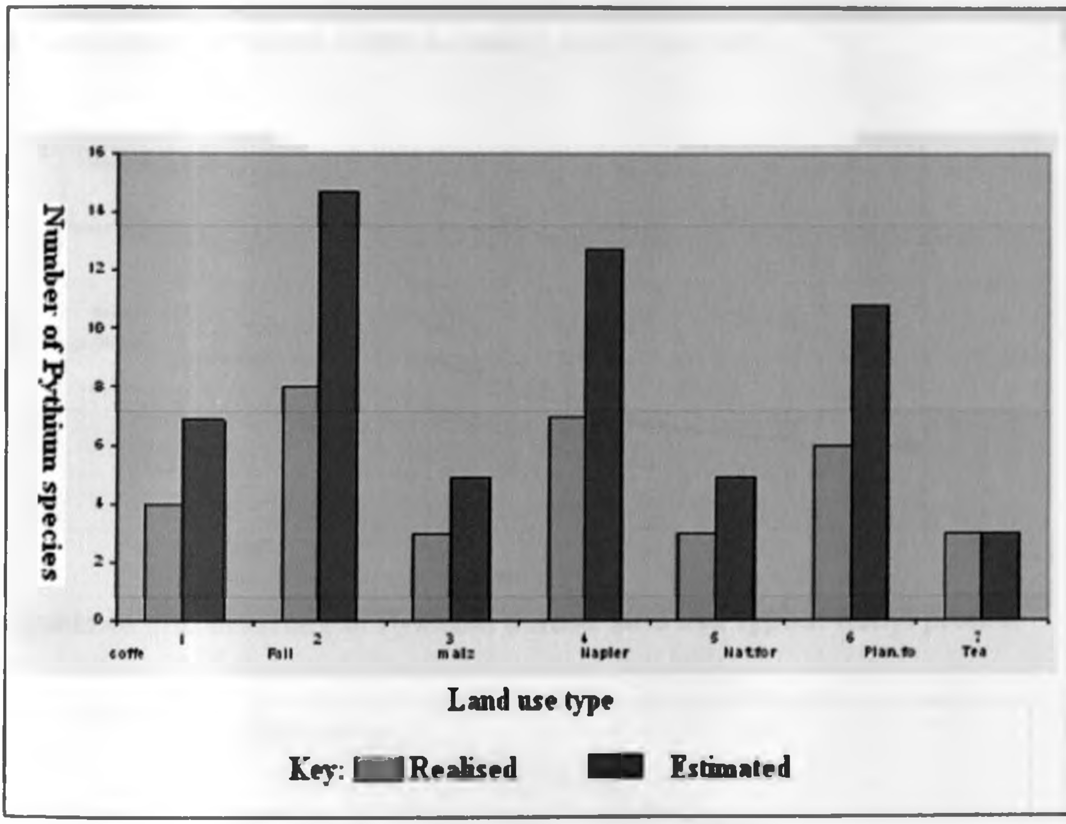


Figure 3: Realized species vs Jacknife estimates in Embu.

Rényi profiles in Embu indicates that napier is the most diverse land use type while natural forest is the least. Quasi- binomial Generalised linear model in Embu gave a dispersion parameter of $p \leq 0.4326$ indicating that it is not significant. Rényi evenness Profiles below indicates that fallow/

pasture is the least even/ least relative abundance LUT while tea, coffee and maize are the most even/ highest relative abundance LUTS.

In Embu, nappier is the most diverse land use at point 0 giving richness of 2.0, followed by fallow, plantation forest, coffee, maize, natural forest and tea in decreasing order as shown in figure (4a). Natural forest is the most even land use followed by maize, tea, coffee, nappier, plantation forest and fallow in that decreasing order. Figure (4b).

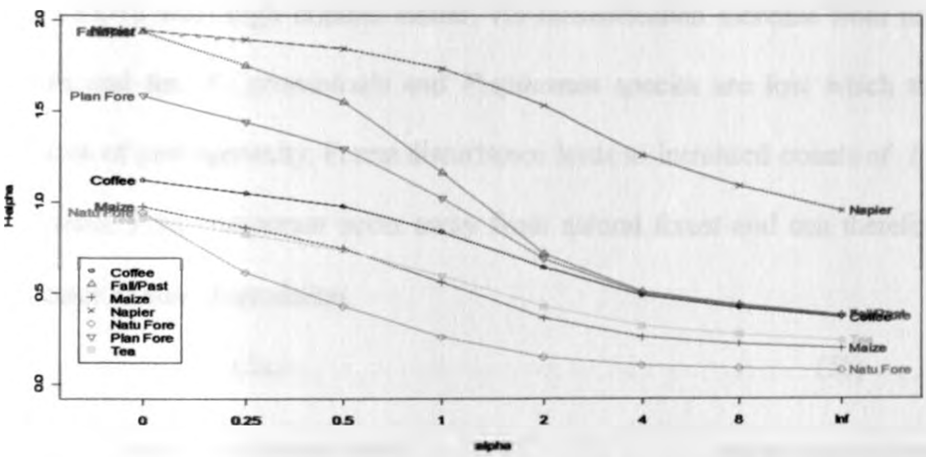


Figure: 4a The diversity of *Pythium* across land use types: Renyi profile.

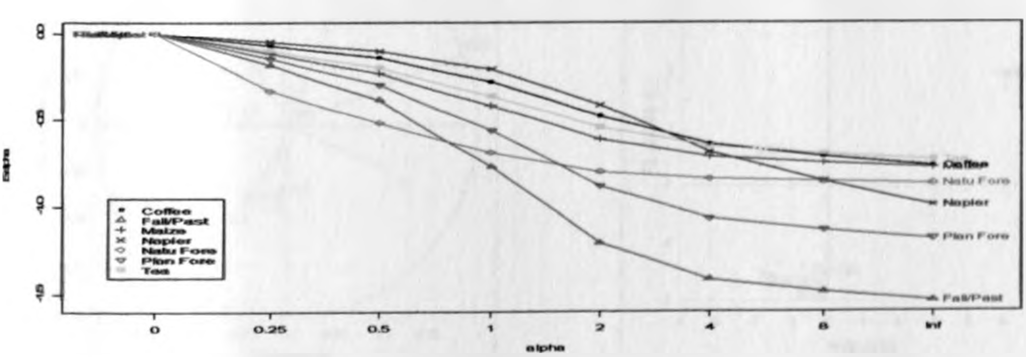


Figure: 4b Influence of land use on *Pythium* richness: Renyi profile.

Principle component analysis in Embu as shown in figure 5a, first factor which accounted for 33.94 % of the total variation, indicated clear differences in *Pythium* composition since the variables were either positively or negatively correlated to that axis. Land-use types in Embu as shown were separated on the basis of species counts (figure 5b). The ordination of land-use types across the landscape revealed the effects of two main land-use factors. The first factor separates LUTs that encourage high host diversity with LUTs with low host diversity (fallow/Pasteur with the rest of the land-use types). The second axis separates LUTs with low organic matter with those LUTs with high organic matter. As intensification increase from napier towards fallow, coffee and tea, *P. graminicola* and *P.spinosum* species are lost which translates to reducing chances of pathogenecity. Forest disturbance leads to increased counts of *P. graminicola* and *P. spinosum*. *P.macrosporum* occur away from natural forest and can therefore be used as a bio-indicator of land degradation.

(5a) (5b)

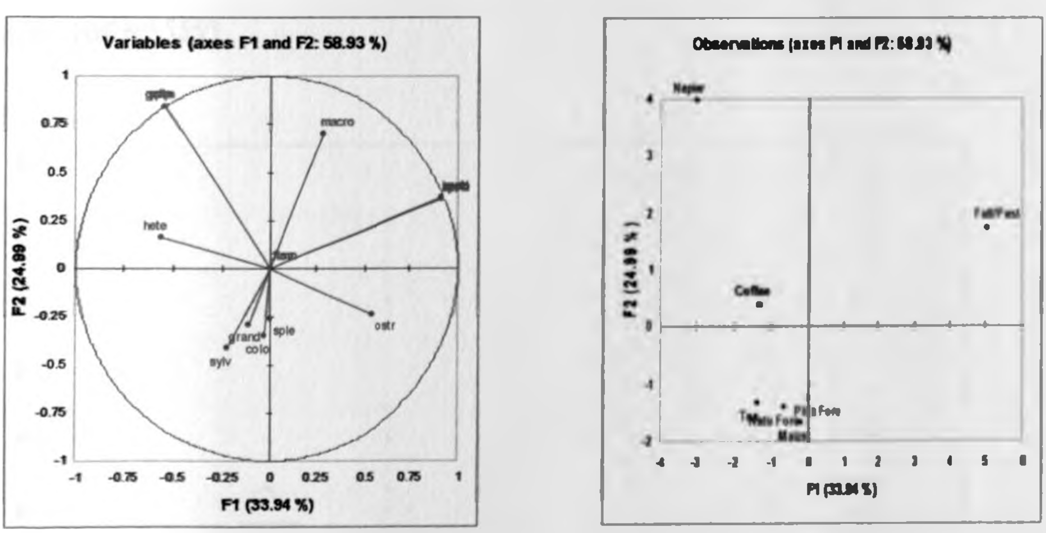


Figure 5: Component analysis of *Pythium* species in Embu. (a) *Pythium* species (b) Land use types

In Embu, there are two major groups as evident from the correlation circle and the LUTs are separated into three distinct clusters as well. The first two factors, which account for 58.93 % of the total observation, separates species into two major groups as evident in the correlation circle below. The first group consists of *P.macrospurmum*, *P. graminicola*, *P. heterothalicum* and *P. spinosum*. with high negative correlation and the second group is for the *P. grandiosporangium*, *P. splendens*, *P. coloratum* and *P. sylvaticum* with positive correlation.

Dispersion of *Pythium* in Embu based on LUTS is not significant (fig.6). Chances of encountering *Pythium* increases significantly with increase in carbon in Embu ($p \leq 0.01830$) (fig.7), and nitrogen concentration ($p \leq 0.0003518$). This also increases insignificantly with increase in Zinc ($p \leq 0.5469$), Phosphorous ($p \leq 0.1543$), Sodium ($p \leq 0.9545$), Potassium ($p \leq 0.1068$), and pH ($p \leq 0.6574$). Chances of encountering *Pythium* does not change insignificantly with increase in calcium concentration in Embu ($p \leq 0.9573$). Chances of encountering *Pythium* decreases insignificantly with increase in concentration of Manganese ($p \leq 0.3425$), Copper ($p \leq 0.6013$), Acidity ($p \leq 0.5559$).

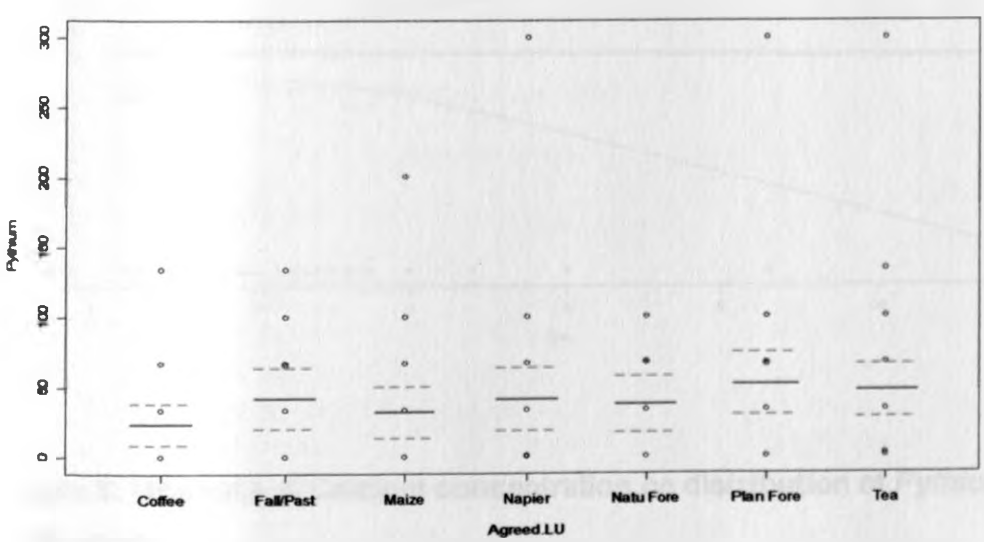


Figure 6: Dispersion of *Pythium* in Embu based on Land use types in Embu: GLM model.

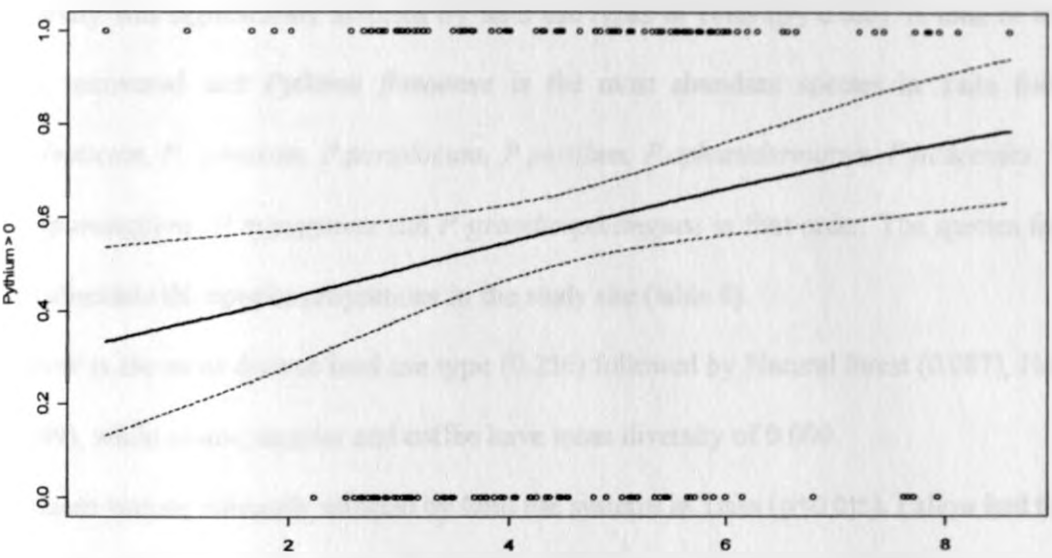


Figure 7: Influence of Carbon concentration on distribution of *Pythium* in Embu: GLM model.

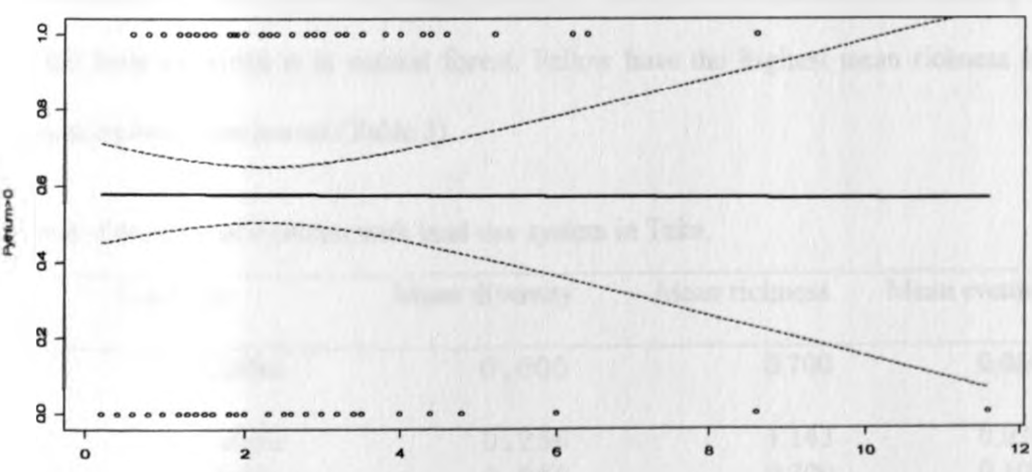


Figure 8: Influence of Calcium concentration on distribution of *Pythium* in Embu: GLM model.

4.2 SPECIES ABUNDANCE AND DIVERSITY IN TAITA

Diversity was significantly affected by land use types in Taita ($p \leq 0.030$). A total of ten species were recovered and *Pythium flovoense* is the most abundant species in Taita followed by *P.sylvaticum, P.spinosum, P.periplocum, P.perilum, P.aphanidermatum, P.helicoides, P.ultimun var.sporangifera, P hypogynum* and *P grandiosporangium* in that order. The species frequencies also elucidate the species proportions in the study site (table 4).

Fallow is the most diverse land use type (0.256) followed by Natural forest (0.087), Horticulture (0.069), while maize, nappier and coffee have mean diversity of 0.000.

Richness was significantly affected by land use systems in Taita ($p \leq 0.015$). Fallow had the highest richness while indigenous forest and coffee with the least .Total richness richness in Taita was 10 species whie the jacknife estimates of richness was 10.9875 with standard error 0f 0.9875 indicating that all species present were encountered. This indicates that there were no possibilities of encountering more species in Taita (Figure 9). The highest expected total richness is in Fallow and the least expected is in natural forest. Fallow have the highest mean richness index while indigenous forest the lowest (Table 3)

Table 3: Diversity of *Pythium* with land use system in Taita.

Land use	Mean diversity	Mean richness	Mean evenness
Coffee	0.000	0.700	0.0667
Fallow	0.256	1.143	0.0254
Hortic	0.069	0.700	0.1000
Nat.fore	0.087	0.250	0.1250
Maize	0.000	0.375	0.0612
Nappier	0.000	0.625	0.0983
P value	0.030	0.015	

Table 4: Frequency of isolation of *Pythium spp* in Taita

SPECIES	FREQUENCY	PERCENTAGE
P. spinosum	3	7.9 %
P. periilum	1	2.6 %
P. aphanidermatum	1	2.6 %
P. periplocum	2	5.26%
P. helicoides	1	2.6 %
P. ultimun var sporangifera	1	2.6 %
P. flovoense	6	15.8 %
P. sylvaticum	3	7.9 %
P. hypogynum	1	2.6 %
P grandiosporangium	1	2.6 %
2B2	9	23.7 %
3B	1	2.6 %
3C	4	10.53 %
Group G	1	2.6 %
Sub.	3	7.9 %

Key: 3B, 2B2, 3C and group G are species that were isolated but could not be identified to species level but grouped to a given morphological group. Sub. are species that were isolated but formed submerged cultures hence could not be identified to species level.

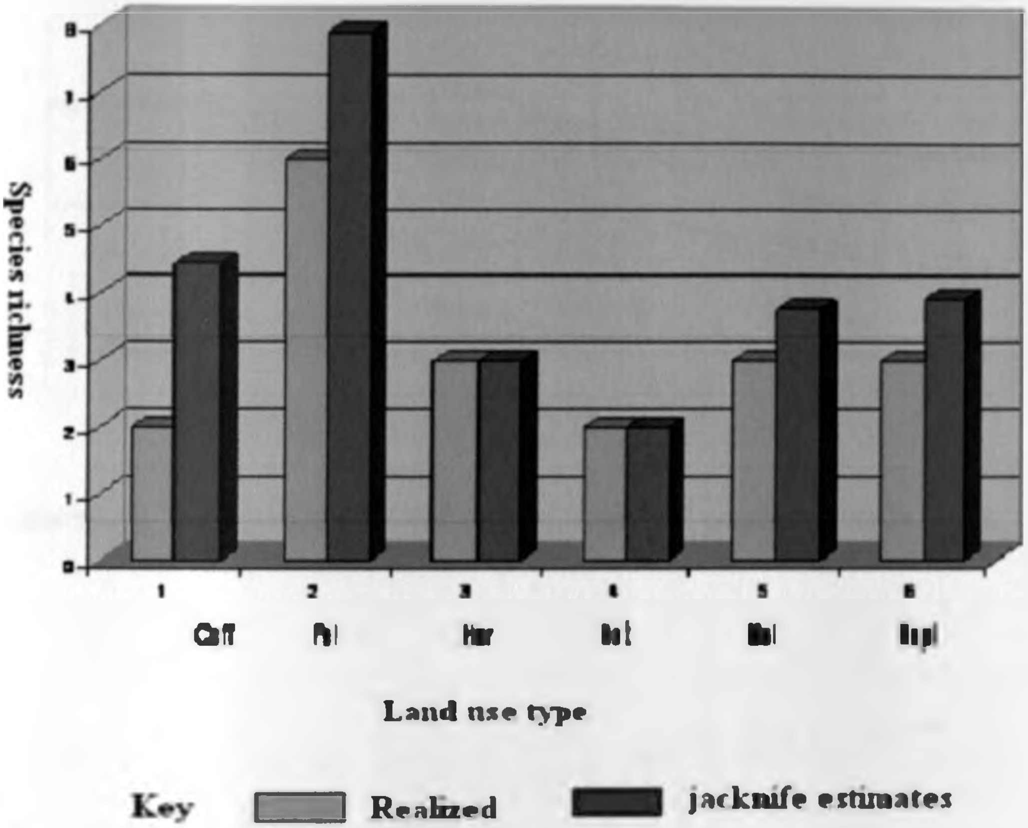


Figure 9: Realized species vs Jacknife estimates in Taita

The Taita study shows that fallow was the most diverse land use at point 0 with 14 species followed by nappier, horticulture, maize natural forest and coffee in decreasing order (figure 10a). Quasi- binomial Generalised linear model in Taita gave a dispersion parameter of $p \leq 0.04927$ at $\acute{\alpha}=0.05$ indicating that it is significant. Rényi evenness profiles indicates that natural forest was the most even land use followed by horticulture, coffee, nappier and fallow in decreasing order. (figure 10b)

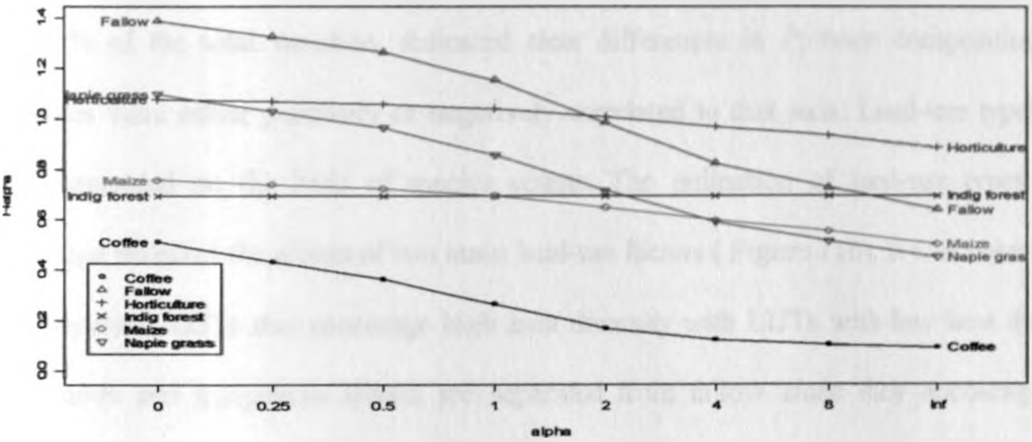


Figure 10 (a): The diversity of *Pythium* across land use systems in Taita.

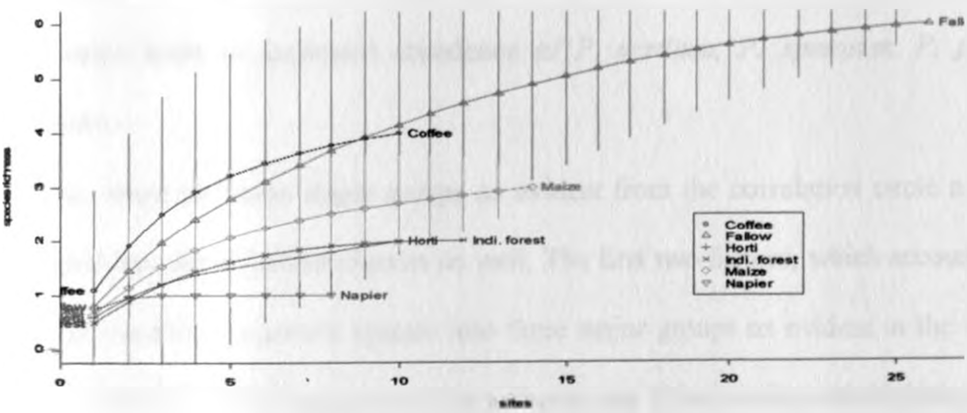


Figure 10b: Effects of land use on Evenness of *Pythium* in Taita

Principle component analysis in Taita shows the first axis, (Figure 11a) which accounted for 56.45 % of the total variation, indicated clear differences in *Pythium* composition since the variables were either positively or negatively correlated to that axis. Land-use types as shown were separated on the basis of species counts. The ordination of land-use types across the landscape revealed the effects of two main land-use factors (Figure 11b). It was observe that first axis separates LUTs that encourage high host diversity with LUTs with low host diversity. All crop lands and indigenous forests are separated from fallow since they encourage low host diversity. The second axis separates LUTs with low organic matter with those LUTs with high organic matter. As intensification reduce from horticulture to maize, fallow, indigeneous forest *Pythium flovoescence*, and *P. helicoides* are lost while *ultimum* and sylvaticum species increases. *P. ultimum* is known to cause damping off of and root rot (Mehrotra, 1997). Therefore, Forest disturbance leads to increased abundance of *P. perilium*, *P. spinosum*, *P. flovoense* and *P. helicoides*.

In Taita, there are three major groups as evident from the correlation circle and the LUTs are separated into three distinct clusters as well. The first two factors, which account for 77.44 % of the total variation, separates species into three major groups as evident in the correlation circle below. The first group consists of *P.flovoecense* and *P.helicoides* with high negative correlation and the second group is for the *P. periplocum*, *P. sylvaticum* and *P. hypogynum* with low positive correlation, and the while the third group comprises of *P.ultimum* and *P. sylvaticum* with moderate negative correlation for both factor 1 and 2.

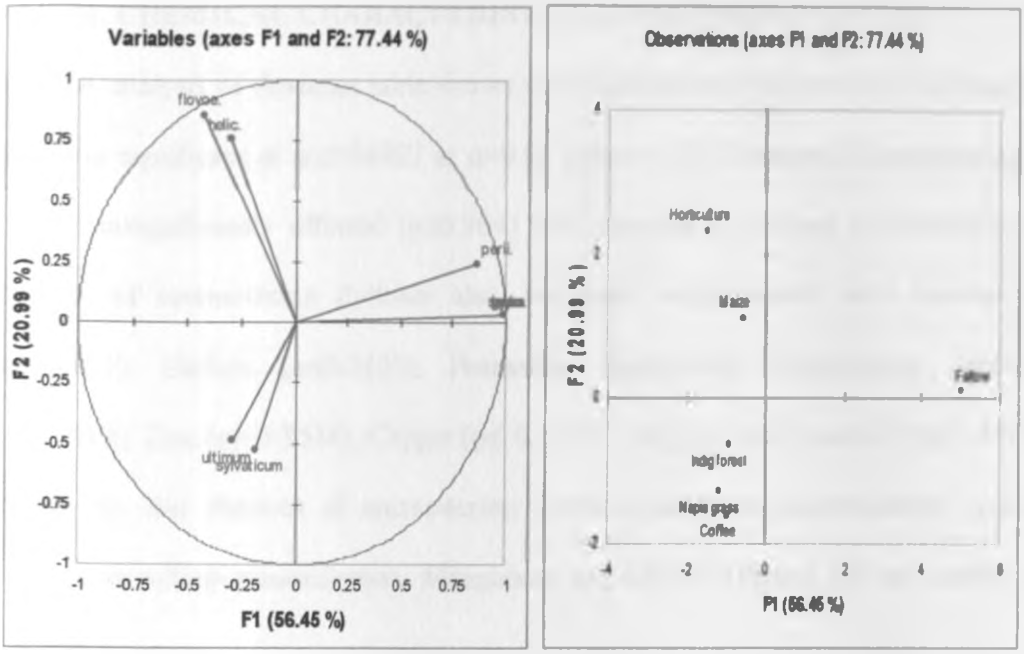


Figure 11a: Principle component analysis of *Pythium* species in Taita.
Figure 11b: Principle component analysis with respect to land use type in Taita.11(a), 11(b)

ANOVA analysis of deviance shows that dispersion of *Pythium* in Embu based on LUTS is not significant While in Taita it is significant at P value = 0.04927 at ά=0.05.

This indicates that there were high possibilities of encountering more species in Embu. This can be explained by presence of large number of morphospecies or unidentified species.

4.3 SOIL CHEMICAL CHARACTERISTICS EFFECTS ON *PYTHIUM*

ANOVA analysis of deviance table shows that dispersion of *Pythium* in Taita based on land use system is significant at $p \leq 0.04927$ at $\alpha = 0.05$ (Figure 12). Chances of encountering *Pythium* in Taita is insignificantly affected ($p \leq 0.964$) with increase in calcium concentration (Figure 14). Chances of encountering *Pythium* also decreases insignificantly with increase in nitrogen ($p \leq 0.1477$), Carbon ($p \leq 0.3467$), Potassium ($p \leq 0.9524$), Phosphorous ($p \leq 0.12000$), pH ($p \leq 0.3519$), Zinc ($p \leq 0.8514$), Copper ($p \leq 0.479$), Iron ($p \leq 0.7462$) and pH ($p \leq 0.3519$) (Table 5). But chances of encountering *Pythium* increases insignificantly ($p \leq 0.1987$) with increase in sodium concentration, Manganese ($p \leq 0.8189$) (Figure 15) and acidity ($p \leq 0.0534$) (Table 5).

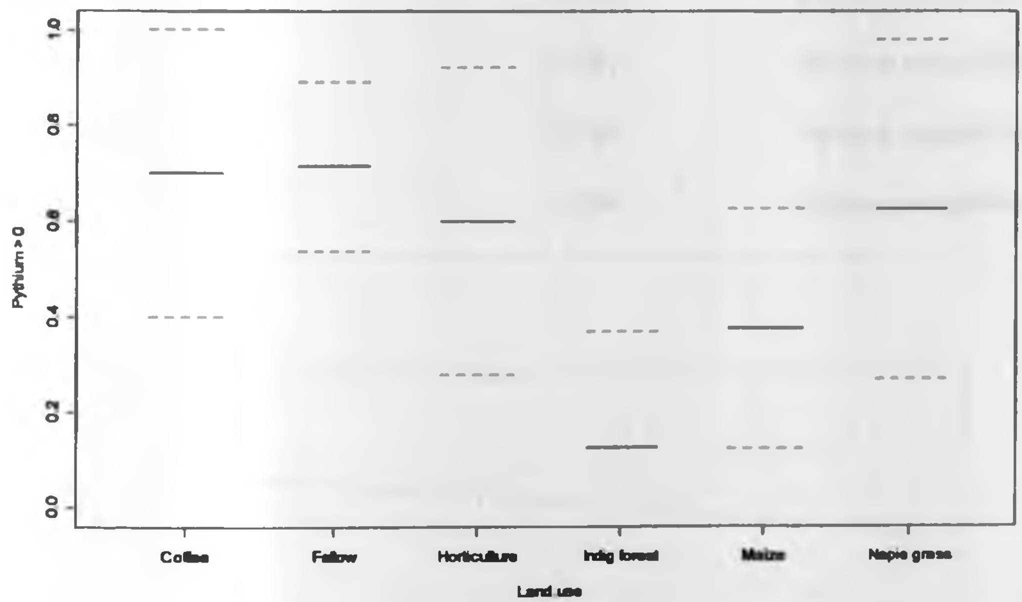


Figure 12: Dispersion of *Pythium* in Taita based on Land use types: GLM model.

Table 5: Summary of influence of soil chemical characteristics on distribution of *Pythium*.

Soil chemical	P-value	Significance
Calcium	0.964	Decrease insignificant
Nitrogen	0.1477	Decrease insignificant
Carbon	0.3467	Decrease insignificant
Potassium	0.9524	Decrease insignificant
Phosphorous	0.1200	Decrease insignificant
pH	0.3519	Decrease insignificant
Zinc	0.8514	Decrease insignificant
Copper	0.479	Decrease insignificant
Iron	0.7462	Decrease insignificant
Sodium	0.1987	Increase insignificant
Manganese	0.8189	Increase insignificant
Acidity	0.0534	Increase insignificant

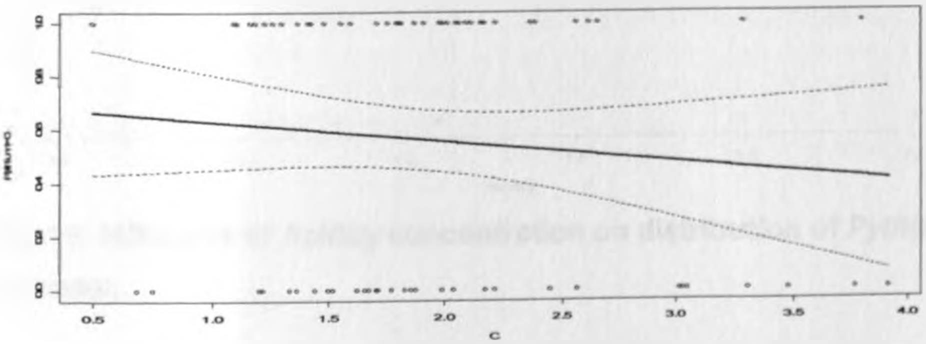


Figure 13: Influence of Carbon concentration on distribution of *Pythium* in Taita: GLM model.

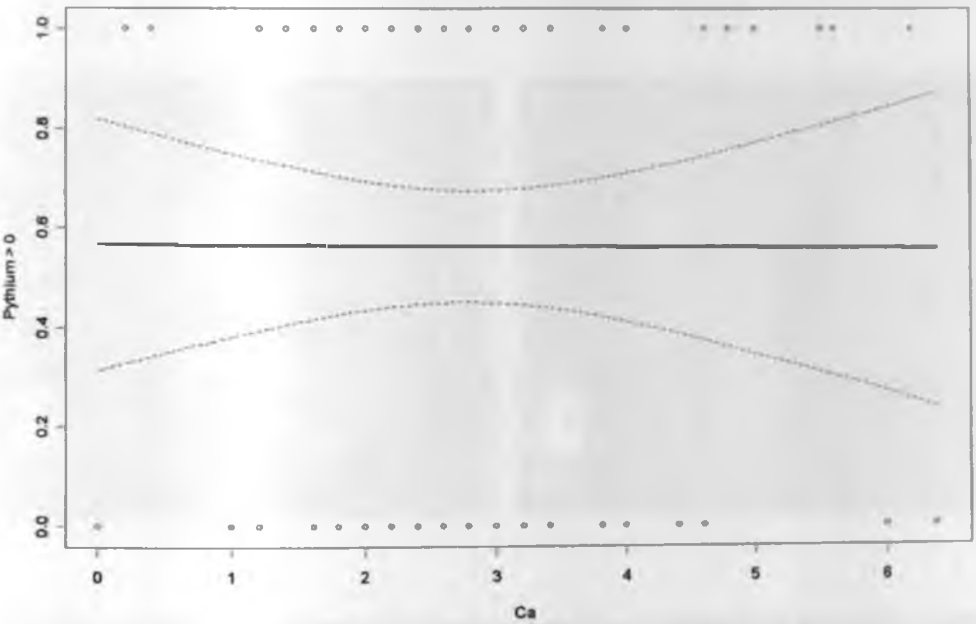


Figure 14: Influence of Calcium concentration on distribution of *Pythium* in Taita: GLM model.

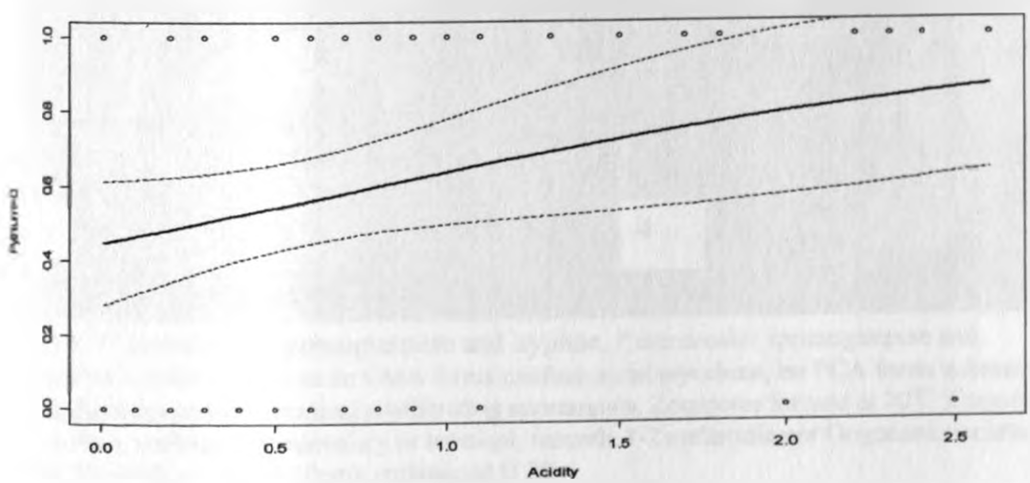


Figure 15: Influence of Acidity concentration on distribution of *Pythium* in Taita: GLM model.

Some of the *Pythium* isolates obtained from the two study sites are shown and described in the plates below.

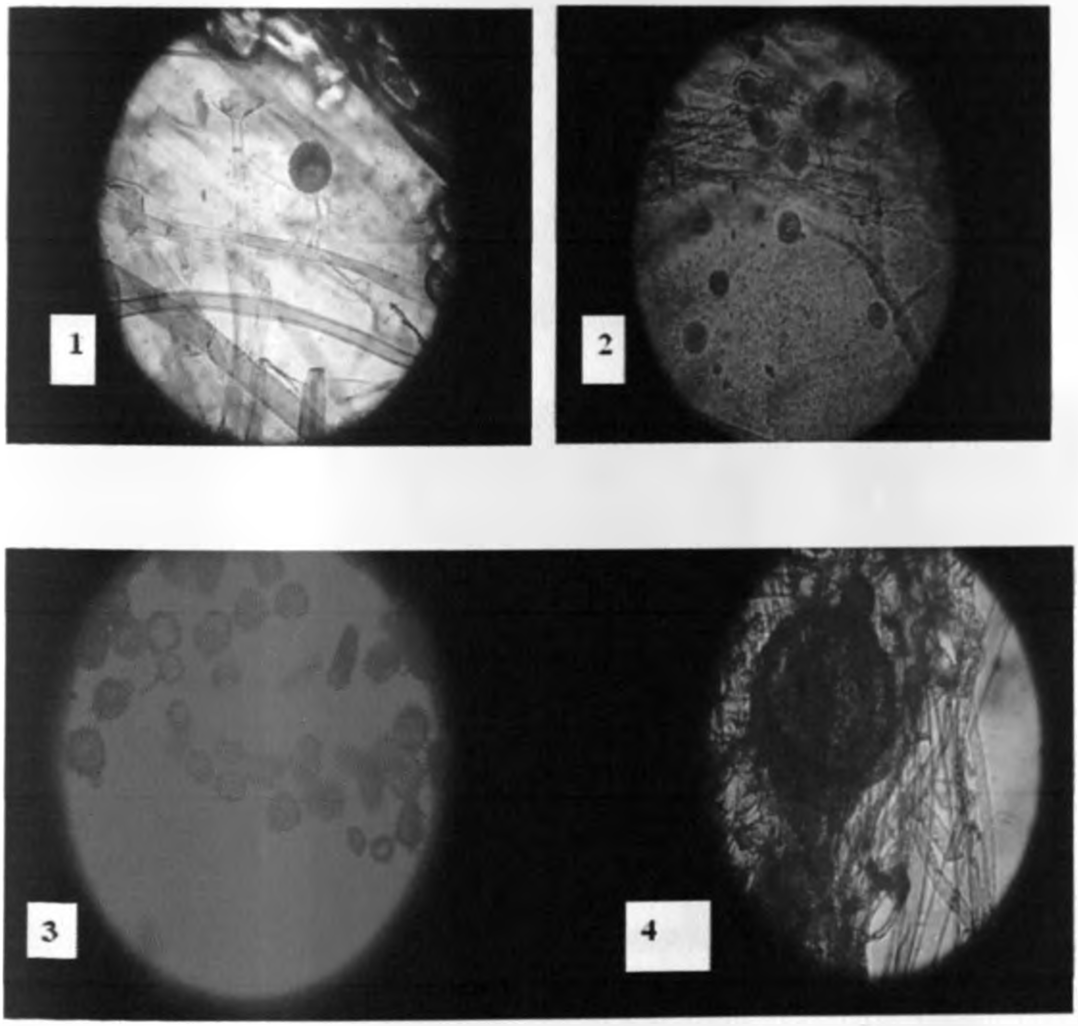
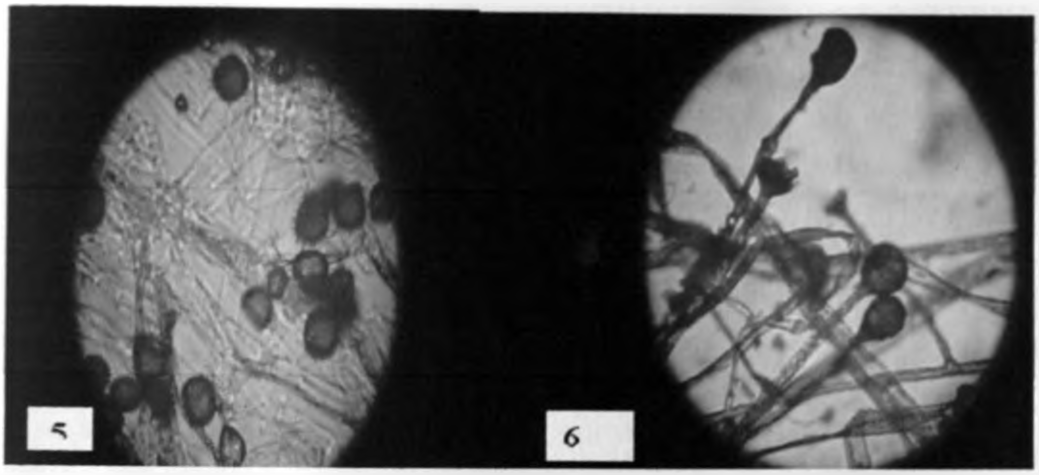


Plate 1. *P ostracodes*; sporangiaspore and hyphae, *P ostracodes*, sporangiaspore and hyphae.MG=X400, -colonies on CMA forms profuse aerial mycelium, on PCA forms submerged Crysanthemum pattern. Terminal proliferating sporangium. Zoospores formed at 20^{0}C. Oogonia intercarlary, unilaterally intercalary or terminal, smooth. 1-2 antheridia per Oogonium. Isolated in Embu at points 20 (0-10cm), 44 (0-10cm), endemic at E 20.
Plate 2 *P ultimum*; sporangiaspore and hyphae,
Plate 3. *P spinosum*; spiny Oospores and
Plate 4. *P grandiosporangium*



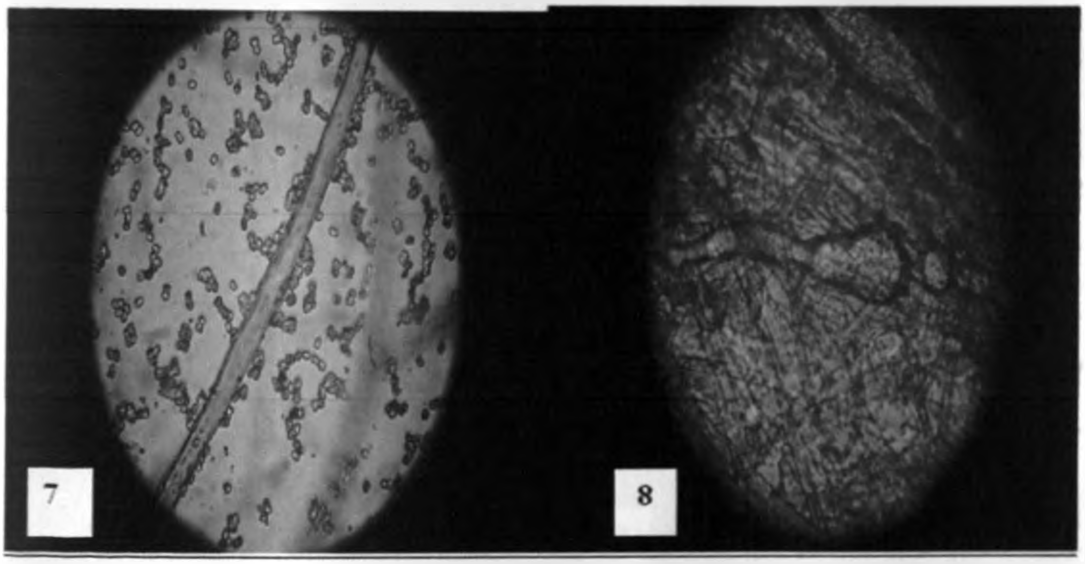


Plate 5. MG=X400. *P spinosum* ; spiny Ooogonia. -isolated from Embu points 30 (20-30cm), 5(0-10cm) and Taita points 29 (10-20cm).

Plate 6. *P .hypogynum*. MG=X400. forms radiate patterns colonies on CMA. ; sporangium. Oospore plerotic and plerotic antheridia. - Isolated from Embu points 54 (20-30cm), Taita point 34 (0-10cm).

Plate 7.Zoospores . MG=X400.

Plate 8. MG=X400. *P aphanidermatum* ; globose hyphal swellings. -Filamentous catenulate hyphae and no sporangia. Toruloid hyphal swellings -isolated in Taita at point 34 (0-10cm). No isolates in Embu.

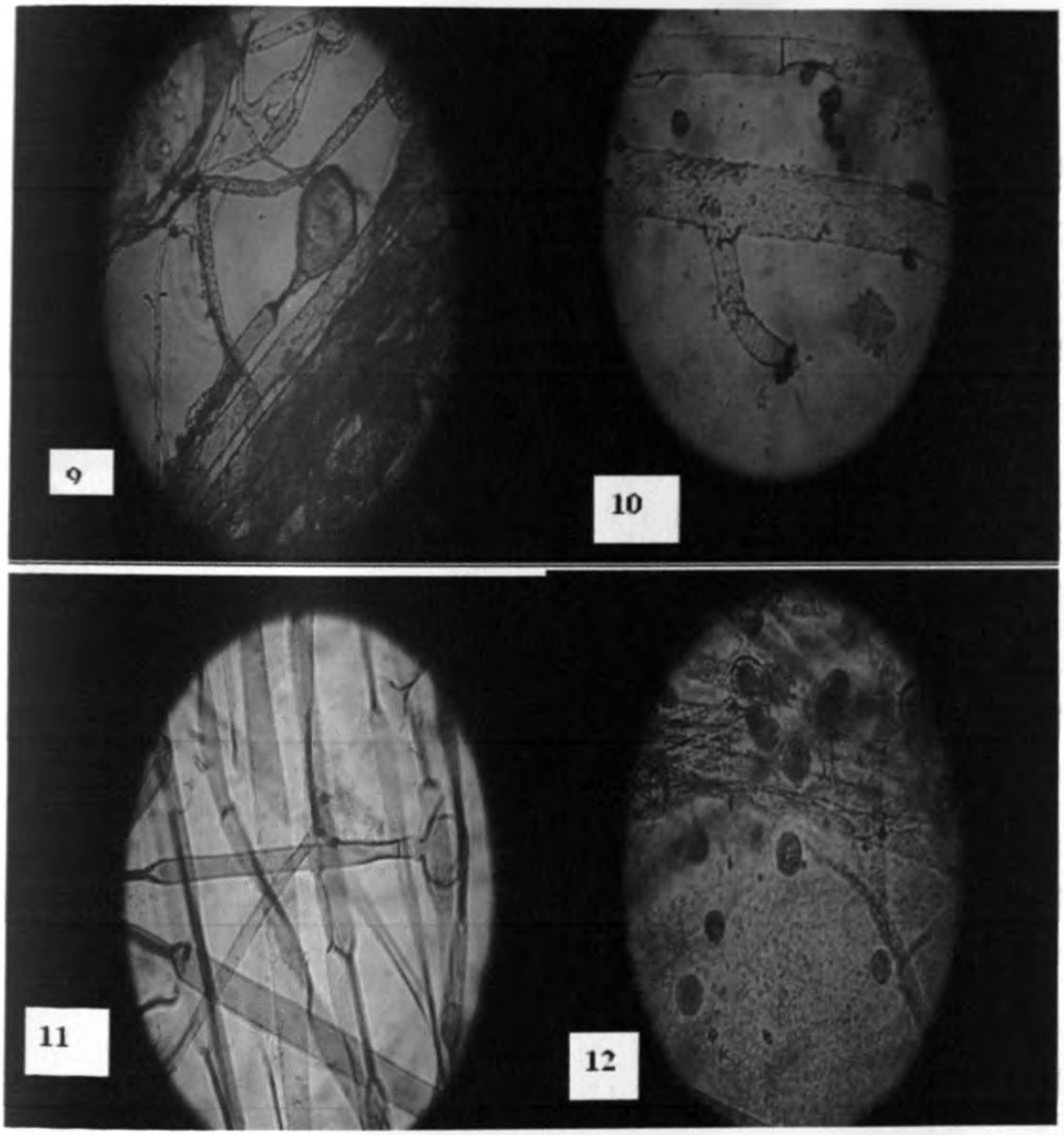


Plate 9. *P grandiosporangium*; sporangiophore and hyphae
Plate 10. MG=X400. *P ostrocodes*; showing hyphae Oogonia smooth globose, perfect monoclinous. Oospore plerotic Forms yellow mycelia with no aerial growth
Plate 11. MG=X400. *P macrosporum*; showing ornamented hyphae smooth, globose, terminal sporangia Forms submerged cultures on CMA with some aerial mycelium at the margin at first coarsely radiate, later with a more or less *Chrysanthemum*-like pattern.
Plate 12. MG=X400 . *P ultimum* zoospores -cottony aerial mycelia on CMA on PCA with a radiate pattern. Sporangia mostly not formed and Zoospores very rarely produced through short discharge tubes at 5°C. isolated in Embu at points 5 (20-30) and Taita point 25 (10-20 cm), 40 (0-10cm)

Plate 13. MG=X400. *P papillatum* Spiny Oogonia

INTERACTION RELATIONSHIPS LIKE ANTAGONISM WERE OBSERVED IN SOME PLATES CONTAINING ACTINOMYCETES SP.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSSION

5.1 DISCUSSION

This study has demonstrated that *Pythium* species are widely spread in Embu and Taita benchmark sites as influenced by land uses in different densities and diversities. Of all *Pythium* isolates recovered in both study sites, 80% are parasitic to common crops and some to forest vegetation while the rest 20% are saprophytes. The species have been reported in a study in Japan, where quarter of Okra grown was affected by *Pythium ultimum* every winter. The study also revealed that increased land use intensity in form of intensive subsistence agriculture resulted in increased occurrence and diversity of *Pythium* species. These findings are consistent with previous reports indicating that *Pythium* species are ubiquitous but at different densities and diversities (Van Der Plaats, 1983). As hypothesised, pathogenic microorganisms increase with increased intensity with land use which is supported by Paoletti *et al.*1991,. The diversity of *Pythium* species was higher in nappier and maize based land uses than in natural forest ecosystem.

In the more intact and stable land uses (forest and shrub land), only a few isolates of *Pythium* were recovered at lower frequencies. This study has revealed that forest disturbance leads to increased abundance of *P. perilium*, *P. spinosum*, *P. flovoense* and *P. helicoids* in Taita while in Embu *P .graminicola*, *P.spinosum* and *P. hypogynum* increase in their abundance. High organic matter especially in the unperturbed land uses like forests supports mainly saprophytic microorganisms like bacteria, fungi and actinomycetes (climax community) while low organic matter where intensification is high like the maize/beans supports parasitic microorganisms like *Pythium*. This is in consistence with Van Der Plaats (1983) who depicts that 80% of all the isolates in both sites are parasitic. *Pythium* species are reported to be opportunistic parasites and saprophytic, are acidophilic, hydrophilic and have a wide host range which are factors attributed to a typical intensive agricultural cam disturbed ecosystem. Diverse number of indigenous microorganisms are present in the absence of stress and as the system becomes more perturbed, less adaptable species disappear and more resilient species take over the niche (Mitchel, 1974). Monoculture, cultivation, artificial fertilizers and agrochemicals all contribute to a reduction in species diversity and biomass (Paoletti *et al.*1991, 1992). *P.flovoense* was the most abundant species in the both study areas and more so in Taita. It was isolated from all the land use types with an overall occurrence frequency of 18.07%. *P.ostracodes* and *P. sylvaticum* are the most abundant species in Embu. Regional difference in richness, species occurrence and abundance was observed. The Fungus was richer in Embu than in Taita with jack knife estimates of 28 and 11 respectively. Primarily this could be due to the differences in ecological parameters especially rainfall between the two sites which occupy different geographical locations. The rainfall pattern is bimodal in Embu with two rain seasons with average annual rainfall of about 1495- 2000 mm and Taita 1332- 1910 mm. Other factors could be due to the fact that land pressure is greater in Embu than in Taita. The average land size is 1.2 and 5.4 acres per family in Embu and Taita respectively hence more degraded, a situation which has been attributed to higher diversity of *Pythium* species compared to Taita. Tea crop in Embu was first introduced in 1962 and introduction started with the use of chemical fertilizers and this has continued to the present (Mutsotso, 2006). As a result, the combination of soil characteristics like organic matter, moisture, temperature, pH, soil texture, structure cation composition and the presence of members of other microorganisms has profoundly been affected at different degree and in turn the habitable niches in soil. Organic components like carbon, nitrogen and phosphorous level seems to affect

Pythium positively in Embu but not in Taita. From this peculiar observation, Embu could be rich in more facultative species than Taita. These are species that are primarily saprophytes but when conditions allow, they can become parasitic. Different seasons vis-à-vis wet and dry season is also a very important determinant of diversity and distribution of *Pythium* with respect to availability of water in the soil. Alexopoulos, 1989 described members of class Oomycetes as aquatic Fungi where their reproduction primarily depends on water due production of zoospores whose motility depends on water. Dry sandy places and dry forests are generally poor in *Pythium* species. Equilibrium relative humidity is term used to define the availability of water to an organism and 70 % is the lowest RH limit for *Pythium* growth.

From the study, napier had the highest mean diversity followed by coffee, plantation forest, fallow, natural forest, tea maize and pasture in that order. A number of explanations can be used to account for the higher diversity of *Pythium* species in land uses that are subjected to regular disturbance compared to stable ecosystems like indigenous forest. Intensive cultivation is mostly accompanied by application of agrochemicals like inorganic fertilizers and pesticides which accumulate to toxic levels upsetting the ecological balance of the soil microflora. These interact profoundly with other biological factors to produce surprising effects on *Pythium* distribution and diversity in both Embu and Taita. Intensive cultivation is also characterized by increased movement of soil which may result in increased spread of microorganisms in the field. Soil disturbance, coupled with frequent changes in crop cover, subjects the soil biota to stress making it difficult for a particular species to establish itself in the soil to out-compete the others. In contrast, soils under forest and shrub are less disturbed meaning that certain species of bacteria, actinomycetes or Fungi are able to establish and suppress *Pythium* species which are sensitive and their saprophytic activities greatly restricted thus poorly suited to compete effectively. Members of the micro flora rely on one another for certain growth substances but at the same time they exert detrimental influence so that both beneficial and harmful effects are evident. This is in consistence with the study carried by Muiru (2000), where by from the forty-one actinomycetes he recovered, thirty-five isolates produced antibiotics anatagonistic to *Pythium spp* (JTM, Oct. 2004) and Van Der Plaats (1983). Other cases of antagonism with *Trichoderma*, *Streptomyces*, *Penicillium* and *Actinomycetes* against *Pythium* are reported by Van Der Plaats (1983). This could be another factor influencing their diversity and distribution in less distrubed ecosystems. Napier had the largest mean diversity in Embu, natural forest, tea, maize and pasture in that order. Rényi profiles in Embu also indicates that napier is the most diverse land use type while in Taita Fallow is the most diverse land use type while indigenous forest and coffee with the least. This could be as result of difference in land cover vegetation and rooting system which in turn influence distribution of *Pythium*. In consistence with that, Penning L. (1997) reported that the structure of the plant root system contributes to the establishment of the rhizosphere microbial population. The interactions of plant roots and rhizosphere microorganisms are based largely on interactive modification of the soil environment by the processes such as water uptake by the plant system, release of organic chemicals to the soil by the plant roots, microbial production of plant growth factors, and microbially mediated availability of mineral nutrients. Within the rhizosphere, plant roots have a direct influence on the composition and density of the soil microbial community, known as the rhizosphere effect. The rhizosphere effect can be seen by looking at the ratio of the number of microorganisms in the rhizosphere soil to the number of corresponding microorganisms in soil remote from roots (the R/S ratio). Land use has an important role in the formation and maintenance of soil structure. The soil structure under napier, tea and fallow are relatively well formed with relatively stable aggregates, while those under maize and coffee are weak and massive, breaking into dust. When wet, these soils become puddled with reduced water permeability. This is due to intensive and high frequency of cultivation, particularly in maize-based systems.

The study also show that evenness of *Pythium* species was higher in least disturbed land use types like forest and lower in the highly disturbed land use types like maize and horticultural areas. Apart from the negative effects from the agrochemicals, human activities may also impose selective pressure on naturally-present microorganisms. This may account for higher evenness of *Pythium* in the forest, which is more stable ecosystem as compared to horticulture, nappier and maize which are subjected to diverse management practices adapted by farmers in a given area. In conclusion, it is clear that forest disturbance affects mostly *P .graminicola*, *P.spinosum* and *P. hypogynum* increasing their abundance and diversity. Widespread occurrence and abundance of *Pythium* could be an indicator of great potential that can be exploited to benefit crop production in as far as disease mitigation is concerned.

5.2 CONCLUSSION AND RECOMMENDATIONS

Agricultural intensification has been found to accompany changes in distribution and diversity of genus *Pythium*. The following factors have been found to contribute to these finding which are: Land use types which affect the organic matter content, crop cover, host diversity, rooting system, frequency of cultivation, soil structure, antagonism and water availability

This sets the justification for continued work to establish other determinants of distribution of *Pythium* species in soil. Agricultural experts should design how to enhance soil organic matter content by addition of animal manures, green manures or compost, improve soil structure, increase soil biodiversity, minimize losses due to erosion and degradation, through, Conservation tillage (minimum tillage), Mulch farming or cover cropping, Cover crop and planted fallows, Agroforestry (eco-farming), Lay farming, Balanced fertilizer and Improved crops and cultivars and new crop. Crop pathogen control by cultural methods should be adopted. These measures involve avoidance of the pathogen, proper selection of Geographic area, selection of Field, choice of time of sowing, disease escaping Varieties and selection of seed and planting stock.

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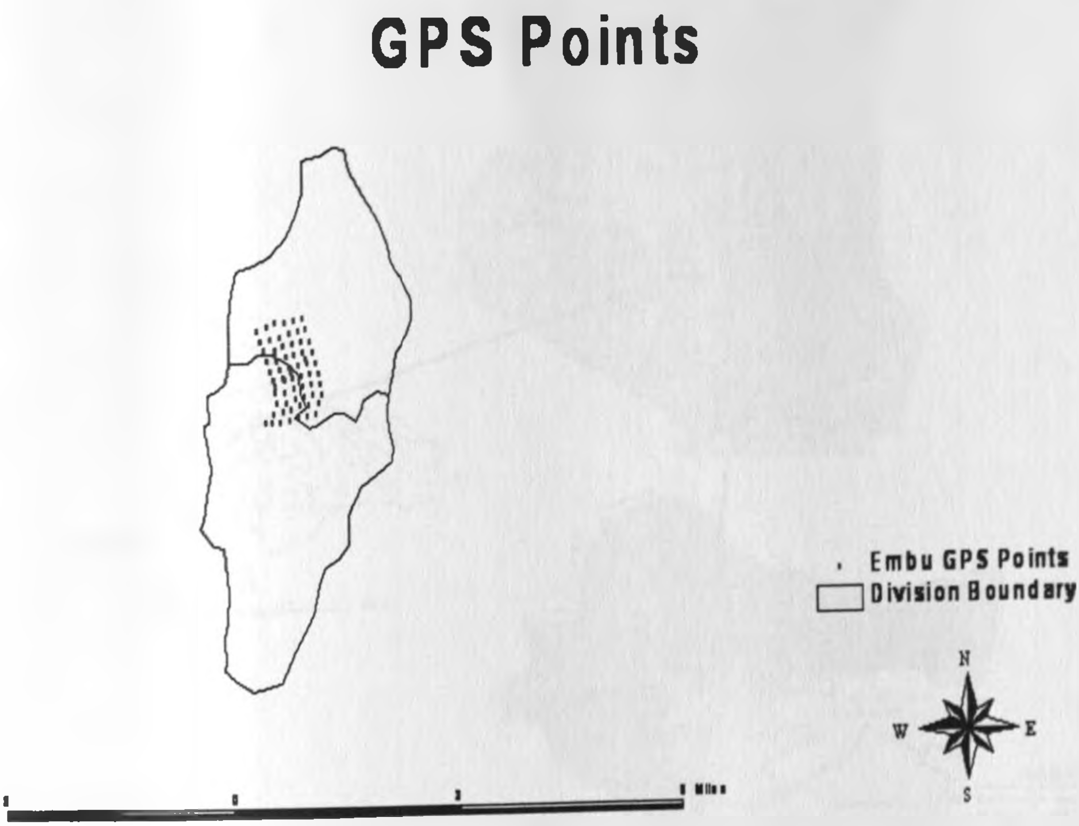
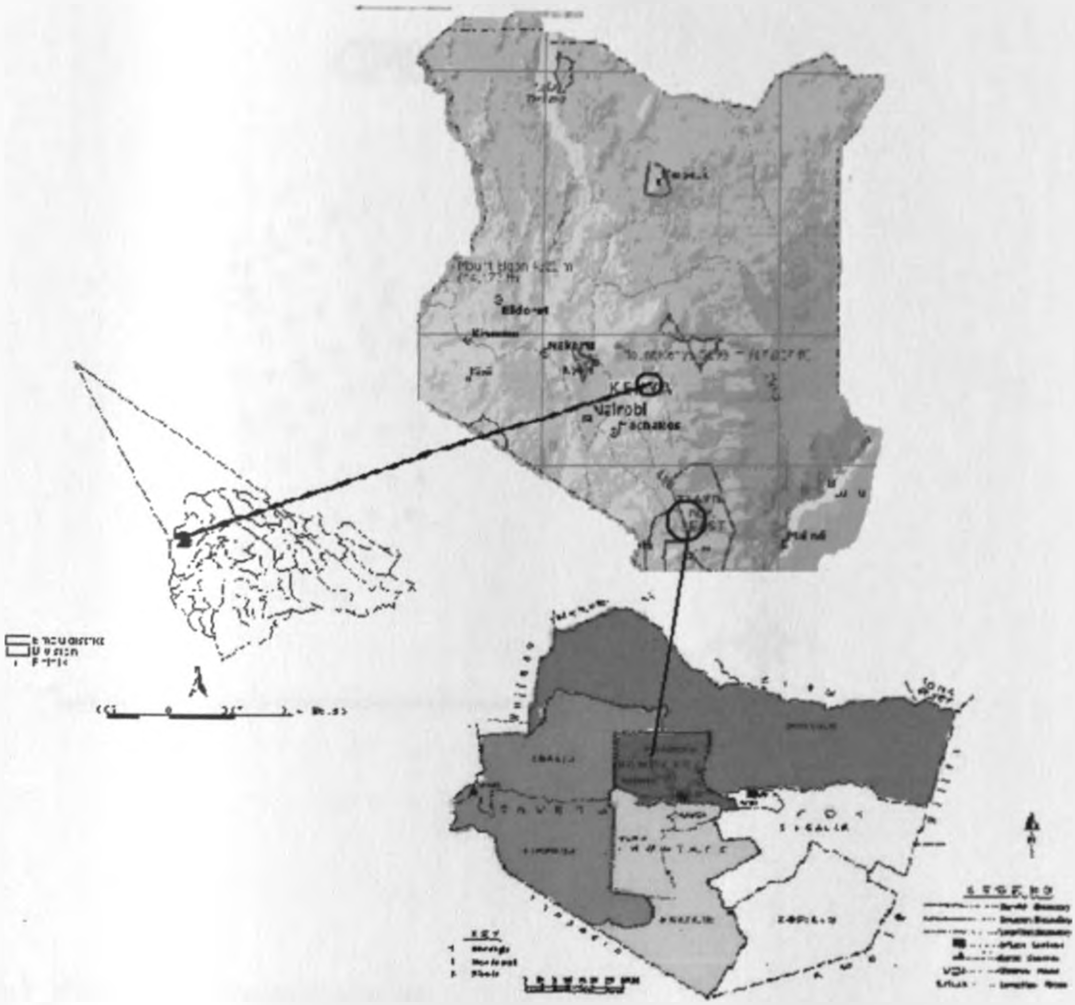
APPENDICES

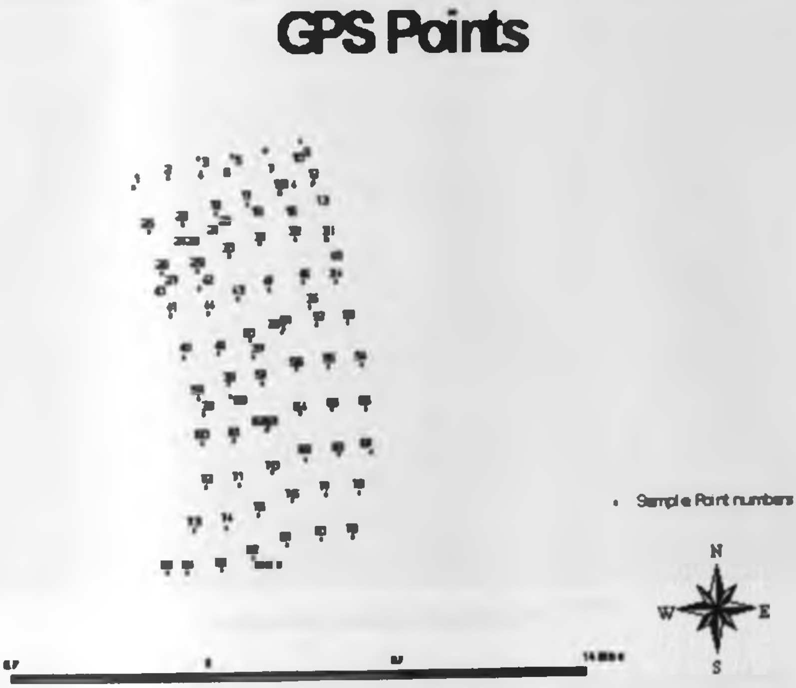
APPENDIX PAGE



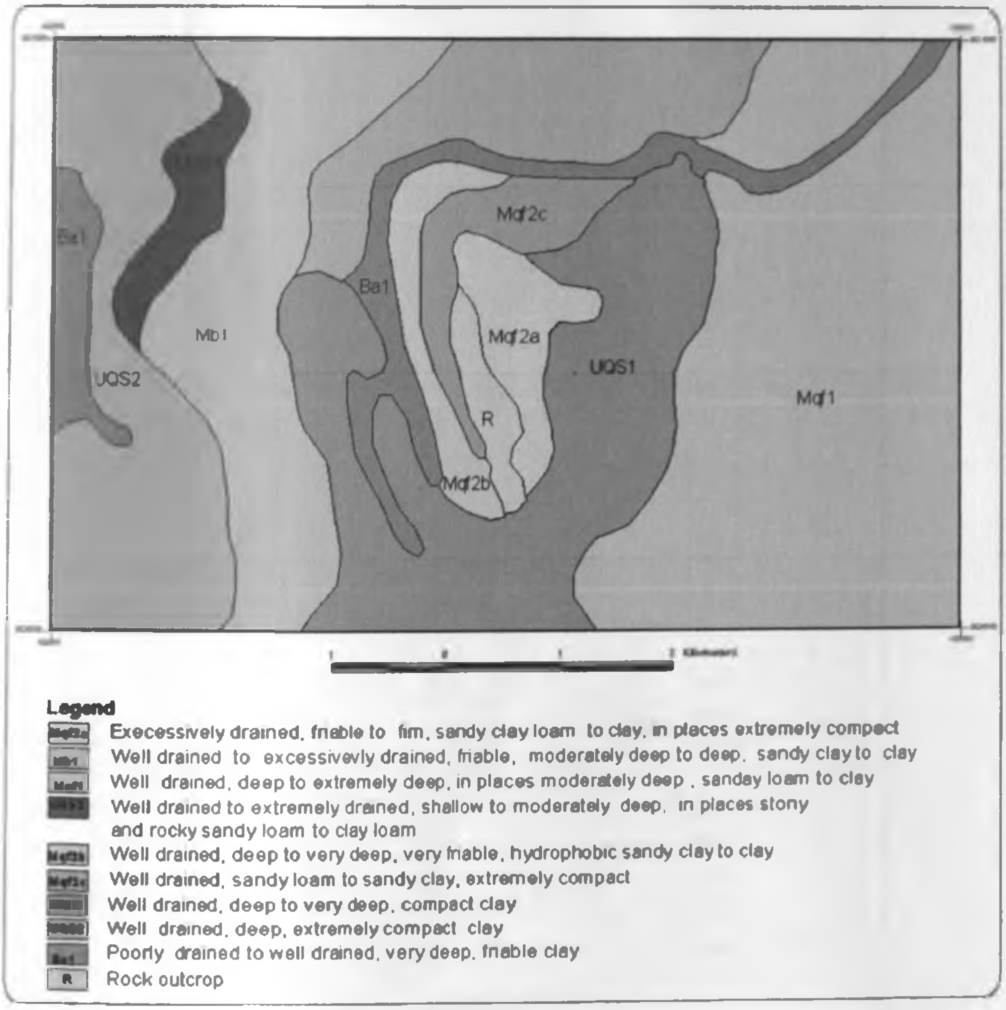
Appeendix 1: Embu Global positioning system points



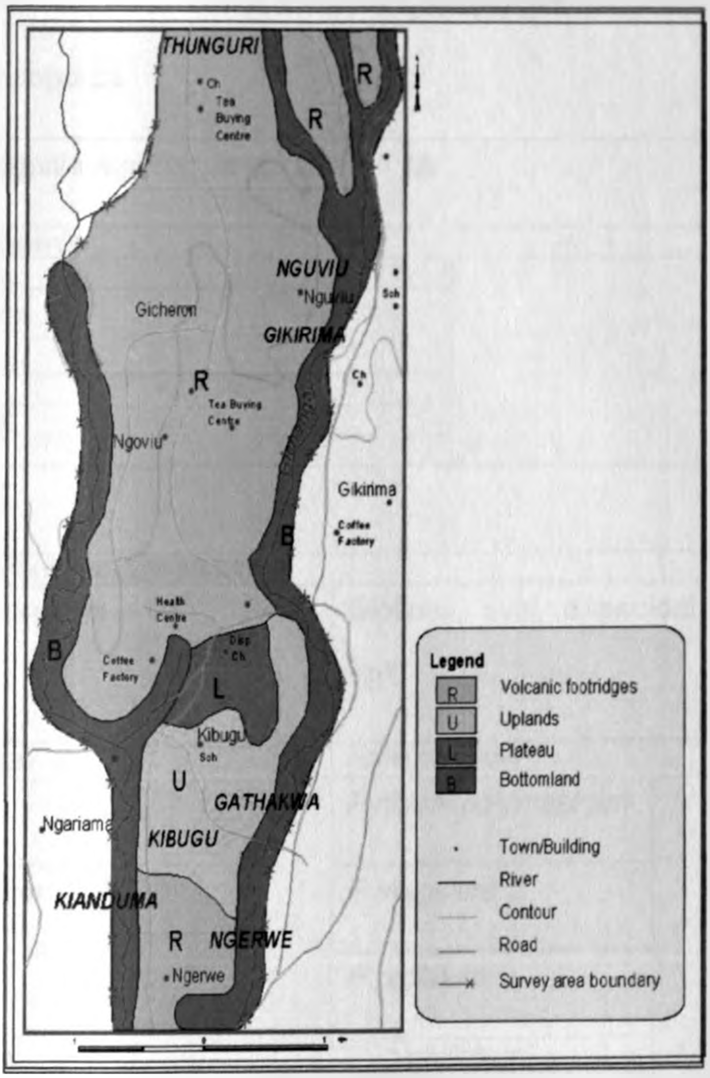
Appendix 2: Study areas



Appendix 3: Taita global positioning points



Appendix 4: Soils of Taita Taveta benchmark site



Appendix 5: Geomorphic map of Window 1 and 2, Embu benchmark

THE 9 MORPHOLOGICAL GROUPS SIMPLIFIED KEY.

1. Ornamented oogonia.

Ornamented oogonia /sporangia unknown – **1A**	
Culture with aerial mycelia	
Pythium buismaniae	
P acanthophoron	
P spinosum	
Submerged cultures	
P hydnosporum	

Ornamented oogonia /Sporangia present			
Filamentous sporangia – **1B**[1]		Globose, oval, ellipsoidal elongated – **1B**[2]	
Aerial mycelium		Aerial mycelium	
P periplocum		*Pythium polymastrum*	
P megalacanthum		*P irregulare*	
P papillatum		*P prolatum*	
Submerged cultures		*P mamillatum*	
Pythium dimorphum		*P anandrum*	
		P polypapilatum	
		P paddicum	
		P stellata	
		Submerged mycelium	

		P acanthicum	
		P oligandrum	
		P helicandrum	
		P cerinaceous	
		P mastophoron	
		P uncinulatum	
		P echinulatum	

Smooth or few projections oogonia wall			
Sporangia not or slightly inflated – **2A**[1]		Sporangia inflated, forming lobules, diverticles or toruloid structures – **2A**[2]	
Aerial mycelium		**Aerial mycelium**	
Pythium papillatum		*P inflatum*	
P marinum		*P deliense*	
P monospermum		*P aphanidermatum*	
P poryphyrae		*P pyrilobum*	

P dictyosporum		*P vanterpoolii*	
P fluminum var flavum		*P catenulatum*	
P aquatile		*P myriotylum*	
P adhaerens		*P graminicola*	
P apleroticum		**Submerged cultures**	
P perniciosum		*P volutum*	
P tenue		*P periilum*	
Submerged cultures		*P indigoferae*	
P dissotocum		*Pythium dissimile*	
P coloratum		*P arrhenomanes*	
P sulcatum		*P torulosum*	
P angustratum		*P aristosporum*	

Smooth or few projections oogonia wall

Sporangia (sub) globose proliferating or not – **2B**[1]		Sporangia never observed – **2B**[2]	
Aerial cultures		**Aerial cultures**	
Pythium pythioides		*Pythium uladhum*	
P grandisporangium		P ultimum	
P hypogynum		*P hemmianum*	
P salinum		*P violae*	

P perplexum		*P cannatum*	
P vexans		*P plerotum*	
P pulchrum		*P tardicrescens*	
P jirovecii		*P splendense*	
P chamaehypton		*P sylvaticum*	
P irregulare		*P heterothallicum*	
P ultimum var sporongiiferum		**Submerged culture**	
P ultimum var ultimum		*P scleroteichum*	
P ostracodes			
P helicoids			
P oedochilum			
P palingenes			
P polytylum			
P salpingophorum			
P nagaii			
Submerged cultures			
P pyrilobum			
P rostratum			
P orthogonon			
P salpingophorum			

P tracheiphilum			
P iwayamai			
P paroecandrum			
P macrosporum			
P okanoganense			
P multisporum			
P marsipium			
P middletoni			

Heterothalic species and species without oogonia		
Sporangia present Filametous inflated or not Globose proliferating or not		Sporangia not formed, hyphal swellings present
Pythium 'group F'	Pythium 'group G'	Pythium 'group HS'
P catenulatum	*Pythium 'group P'*	P splendens
P 'group T'	P macrosporum	P Intermedium
P flevoense	P undulatum	P sylvaticum
	P carolinianum	*P heterothallicum*

Chemical characteristics at the given depths in cm	Napier grass (soil profile No 1)				Tea (soil profile No 2)			Fallow (profile 3)		Maize (profile No 4)		Coffee (profile 5)	
	0-5	**5-17**	**17-34**	**34-130**	**0-20**	**20-77**	**77-137**	**0-45**	**45-117**	**0-30**	**30-47**	**0-73**	**73-160**
% Sand	26	16	8	8	12	4	6	20	14	40	28	12	10
% Silt	8	18	16	12	12	12	6	14	18	34	26	20	10
% Clay	66	66	76	80	76	84	88	66	68	26	46	68	80
Texture class	C	C	C	C	C	C	C	C	C	C	C	C	C
pH–Water 1:2.5 suspension	5.2	5.3	5.6	5.7	3.3	3.5	3.7	3.3	3.4	3.9	4.0	3.4	3.5
EC Ms/cm	0.09	0.06	0.06	0.07	0.05	0.02	0.01	0.06	0.05	0.03	0.05	0.05	0.04
C%	1.83	2.49	1.58	1.06	2.13	1.24	0.86	3.01	2.38	3.07	3.54	2.48	1.65
Cation Exchange Capacity (CEC)	13.19	14.72	11.41	11.78	13.29	11.32	14.41	15.42	12.48	17.05	17.22	13.85	13.55
Exchangeable cations	Me/100g												
Ca	8.12	10.25	5.27	3.96	0.80	1.16	1.11	0.27	0.17	1.59	0.83	0.83	0.39
Mg	0.87	1.14	0.83	1.24	0.14	0.18	0.26	0.09	0.08	0.11	0.15	0.17	0.10
K	1.08	1.02	1.08	1.08	0.56	0.20	0.20	0.14	0.22	0.28	0.44	0.50	0.46
Na	1.20	1.10	1.10	1.20	0.65	1.40	1.40	0.55	0.65	0.90	1.40	0.75	0.85
Sum	11.27	13.51	8.28	7.48	2.15	2.94	2.97	1.05	1.12	2.88	2.82	2.25	1.80
Base saturation %	85.4	91.8	72.6	63.5	16.2	26.0	20.6	6.81	8.97	16.9	16.4	16.37	13.3

Chemical characteristics of the soils for different soil profiles

GLOSSARY OF ABBREVIATIONS

Abbreviations	Expanded form
%	Percent
°C	Degrees Celsius
-ve	Negative
+ve	Positive
ANOVA	Analysis of variance
ASN	
BGBD	Below ground biodiversity
CAN	Calcium ammonium nitrate
CMS	Conservation and sustainable management
CO_2	Carbon (IV) dioxide
Cm	centimeters
DAP	Diammonium phosphate
DSP	disuperphasphate
E	East
GEF	Global environment fund
g	grams
GLASOD	Global assessment on soil degradation
GLM	Generalised linear model
GPS	Global positioning system
Kg	kilograms
LUTs	Land use types
mm	milimeters
m	meters
min	minutes
NPK	Nitrogen phosphorus and potassium
PDA	Potato dextrose agar
pH	Hydrogen ion concentration
SSP	Single super phosphate
S	South
TSP	Trisuper phospate

UNEP United Nations Environmental Program