DIVERSITY OF NEMATODE DESTROYING FUNGI AND NEMATODE COMMUNITY IN SELECTED VEGETABLE GROWING AREAS IN KENYA

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DECLARATION

This is my original work and has not been presented for a degree in this or any other university.

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DEDICATION

I dedicate this study to my parents; Mr. Jackson Muindi and Mrs. Florence Kanini who have been a source of inspiration and for their love, moral and financial support throughout my study. May God always bless you.

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ABBREVIATIONS

ANOVA-Analysis of variance

PDA–Potato dextrose agar

SAR–Systematic acquired resistance

SPSS-Statistical package for the social sciences

TWA-Tap water agar

USDI – Under secretary of defense for intelligence

FAO – Food and agriculture organization

ABSTRACT

Nematodes are a diverse group of microscopic worm like creatures. They provide essential ecological services that keep soil healthy. They are also parasitic to plants and cause economic damage to many plant crops. Chemical control of plant parasitic nematodes essentially involves the use of synthetic nematicides. However, apart from its very high cost; increased concern for the environment contamination has necessitated a reduction in the amount of nematicides used for nematode control. There has been an increase in the intensity for the search of efficient ecologically sound plant parasitic nematode management strategies. An environmental friendly management strategy that utilizes natural enemies to lower the population of pest has been employed on other agricultural pests. Likewise natural enemies of plant parasitic nematode can be used to control nematode pests. Nematode destroying fungi have received a lot of attention for development as biological control agent of plant parasitic nematodes. Unfortunately, there exist multidimensional drawbacks to the realization of the full potential of the nematode destroying fungi in the contest of plant parasitic nematodes. Unavailability of reliable methods to visualize the fungi and demonstrate their activity in their natural habitat is a major impediment. Consequently activity of the fungi in the soil has been inferential through the reduction in numbers of nematodes or reduction of their damage to plants. Unfortunately all factors affecting these groups of fungi have not been documented; some of the reported factors include soil condition, nematode species, rate of development and host plant. The objective of this study was to investigate the effect of temperature on the population of nematode and nematode destroying fungi in selected vegetable growing areas in Kenya. Soil samples were collected from five areas

with different temperature ranges, these areas were Kinale, Kabete, Athiriver, Machakos and Kibwezi. A total of 171 nematode destroying Fungi isolates were identified. Kabete had the highest frequency of occurrence at 33.92%, Followed by Machakos, Kibwezi, Athiriver and Kinale at frequencies of 24.56, 22.81, 11.70 and 7.02% respectively. The identified fungi belonged to the genera *Athrobotrys, Monacrosporium* and *Stylopage. Arthrobotrys oligospora* was the most diverse fungi and had the highest frequency of occurrence, followed by *A.dactyloides, Monacrosporium cionopagium, Stylopage grandis* and the least was *Arthrobotrys longispora* with frequencies of occurrence of 46.20, 45.61, 5.85, 1.17 and 1.17 % in that decreasing order. A total of 11,050 nematodes were collected from the five areas. Kinale had the highest nematode population followed by Athiriver, Kabete, then Machakos and the least was recorded at Kibwezi with population of 5,070, 2,080, 1,625, 1,235 and 1,040 in that decreasing order. From this study, it was evident that fungal population was low in soils with high fertilizer application. While nematode population was high in areas with low temperature. From this study, it can be concluded that agricultural activities affected the diversity and occurrence of nematode destroying fungi. The study shed some light on effect of agricultural activities and temperature changes on population, occurrence and diversity of nematode destroying fungi and nematodes.

KEY WORDS:

Biological control, *A.oligospora*, Agricultural practices, Vegetable field, Plant parasitic nematode.

CHAPTER ONE

1.0 INTRODUCTION

Nematodes are non-segmented, bilaterally symmetric worm-like invertebrates that possess a body cavity and a complete digestive system but lacks respiratory and circulatory systems (Chitwood, 2002). More than 15,000 species and 2,200 genera of nematodes had been described by the mid-1980s. Soil-inhabiting nematodes can be classified according to their feeding habits (Mcsorley *et al.*, 2009). They can be divided into five categories, namely: Plant-feeding nematodes, Fungal-feeding nematodes, Bacterial-feeding nematodes, Predatory nematodes and omnivorous nematodes (Norton, 1991; Niblack, 1991; Ingham, 1996; Zunke,1997; Perry, 1997). The feeding modes of plant parasitic nematodes differ according to the plant parts they feed upon. One group can feed from the outside of the roots on outer cortical cell layers, while the other group can penetrate the roots or outer cortical layers. Generally they are referred to as ecto and endoparasitic respectively (Buchinski, 2013).

Nematodes are thought to play various roles in the soil, for example nutrient cycling. Nutrients such as ammonium (NH4+), stored in the bodies of bacteria and fungi, are released when nematodes eat them. The bacteria and fungi contain more nitrogen than the nematodes need, so the excess is released into the soil in a more stable form where it can be used by plants or other soil organisms. Nematodes also physically break down organic matter which increases its surface area, making it easier for other organisms to break it down further (Ingham and Moidenke, 2000). Dispersal of microbes is another role played by nematodes in the soil, bacteria and fungi cannot move around in the soil without ‘hitching a ride’ inside or on the back of nematodes.

Nematodes are parasitized by some bacteria and fungi, which help their dispersal through the soil. Beneficial nematodes are also important in disease and pest control, they attack and kill a range of pests such as borers, grubs, thrips and beetles with negligible effects on non-target species (Ingham and Moidenke, 2000).

Nematodes are common economic pests of agricultural crops in the world; they cause huge yield losses globally. Plant parasitic nematodes cause annual losses estimated at USDI 25 billion worldwide (Chitwood, 2003). All crops are susceptible to nematodes. Total crop failures frequently occur when crops are planted in areas with high nematode population levels (Noling, 2012). Plant symptoms which develop in response to nematode parasitism are generally those associated with root dysfunction (Noling, 2012). Development of small, stunted and chlorotic plants generally reflects reduced water and nutrient uptake caused by injury to the root system. Correspondingly, root damage generally increases with nematode infestation level, particularly where plants are grown on fine to coarse textured, sandy soils with low water holding capacity (Noling, 2012). Direct damage to plant tissues by shoot-feeding nematodes includes reduced vigor, distortion of plants parts and death of infected tissues depending upon the nematode species (Lambert and Bekel, 2002).

Severe vegetable damage by root knot nematode in Kenya has been reported with infected plants rendered unacceptable for export (Nchore *et al*., 2011). Plant parasitic nematodes cause losses of, up to 80%, on vegetables (Galip, 2007; Nchore *et al.,* 2011). Sasser (1990) reported the prevalence of root knot nematodes in tomatoes causing severe losses in Kenya. Nematodes increase wounding of the root system providing points of ingress of the pathogen. The nematode

may also modify the tissue in that it becomes more suitable for bacteria colonization (Hayward, 1991).

Some of the options for plant-parasitic nematode control practiced in Kenya include cover crop, green manure, organic or inorganic soil amendments, resistant cultivar, crop rotation and biological control (Barker and Koenning, 1998). Pesticides may cause heavy environmental pollution, for example water contamination and toxicity to animals and humans. These negative effects on the environment led to restrictions in nematicide use and are nowadays less widely applied than in the past. Newer methods of nematode suppression include organic matter addition (Akhtar and Malik, 2000; Widmer *et al.,* 2002) and biocontrol practices (Kerry and Gowen, 1995; Alabouvette *et al*., 2006). Plant-growth promoting rhizobacteria especially belonging to the genera *Pseudomonas* and *Bacillus* have demonstrated potential for disease suppression without negative effects on the user, consumer or the environment (Levit *et al.,* 1998).

Of all microorganisms that parasitize or prey on nematodes, fungi are more promising and some of them have shown great potential as bio-control agents (Stirling, 1991). Fungi that destroy nematodes belong to different ecological groups, including endoparasitic, predacious, opportunistic, plant pathogenic and mycorrhizal fungi (Siddiqui and Mahmood, 1996). They are natural enemies of nematodes; they have been shown to predate the root-knot nematode species that most frequently affects vegetable crops (Duponnois *et al.,* 1996; Kumar and Singh, 2006; Azad and Gitanjali, 2007).

Studies on abiotic factors affecting the population occurrence and diversity of nematode and nematode destroying fungi concurrently are limited (Kimenju *et al*., 2004; Kimenju *et al*., 2008). One of the factors is temperature which is suspected to be important in success of utilization of natural enemies. This study therefore investigated the effect of temperature on the population, occurrence and diversity of nematode and nematode destroying fungi in intensively cultivated vegetable farms. Due to the changing climatic conditions which has resulted in increase in temperature, it was prudent to investigate the effect of temperature on both nematode destroying fungi and nematodes community. The information formed basis for the biological management of plant parasitic nematodes.

1.1 PROBLEM STATEMENT

Plant parasitic nematodes have caused huge yield loss on all agricultural crops. Although chemical control of these pests has been efficient and fast acting, they are being reappraised due to their environmental effect and affordability by the farmers. They also cause a decline in the soil biodiversity due to nonspecific destruction of organisms. There is therefore need to develop sustainable and affordable plant parasitic nematodes management systems. Biological control options may offer this much needed alternative. The diverse numbers of nematode destroying fungi may have a considerable advantage for development as biological control organisms (Stirling, 1991). Although these fungi provide such opportunity for exploitation some factors that affect their growth have not been well understood, one of the factors is temperature. Therefore it is important to investigate the effect of temperature *insitu* on occurrence and diversity of both nematodes and nematode destroying fungi.

1.2 JUSTIFICATION

Studies have been carried out on nematode destroying fungi and how they vary in diversity with soil depth, season and soil fertility. None of the studies however, have been carried on the effect of temperature on population, occurrence and diversity of nematode and nematode destroying fungi in farms where vegetables have been planted. Studies also show that nematode destroying fungi are able to be used as bio-control against plant parasitic nematodes; therefore it was important to carry out an efficacy test to determine which of the isolated nematode destroying fungi was the most effective as a bio-control. The study demonstrated the importance of using organic amendments to increase nematode destroying fungi population to manage plant parasitic nematodes population. From the study, *Arthrobotrys* was recommended for development as a biological control against plant parasitic nematodes.

1.3 OBJECTIVES

1.3.1 Main objective

To promote sustainable management of plant parasitic nematodes in vegetable gardens.

1.3.2 Specific objectives

- To characterize nematode destroying fungi from vegetable farms in different temperature regimes.
- To compare the nematode population with the isolated nematode destroying fungi.
- To determine the efficacy of selected nematode destroying fungi isolates in the control of extracted plant parasitic nematodes.

1.4 HYPOTHESIS

- Temperature has no significant effect on occurrence and diversity of nematodes and nematode destroying fungi.
- There is no relationship between nematode population and nematode destroying fungi population.
- All the isolated nematode destroying fungi are equally effective in destroying plant parasitic nematode.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Problems associated with nematodes

Plant parasitic nematodes are tiny round worms that can cause tremendous economical damage to crop production but often overlooked because of their small size, hidden activities and often non-specific damage to their hosts (Becker, 2003). Plant parasitic nematodes, the majority of which are root feeders, completing their life cycles in the root zone are found in association with most plants (Khalil, 2013). A single endo-parasitic nematode can kill a plant or reduce its productivity, while several hundreds ecto-parasitic nematodes might feed on a plant without seriously affecting production (Ingham *et al*., 1996). A few species are highly host- specific such as *Heterodera glycines* on soy beans and *Globodera rostochiensis* on potatoes. Endo-parasitic root feeders include such economically important pests as the root-knot nematodes (*Meloidogyne* species), the cyst nematodes (*Heterodera* species) and the (*Pratylenchus* species) root lesion nematodes (Ingham *et al*., 1996).

2.2 Common plant parasitic nematodes

2.2.1 Root-knot nematodes

Root-knot nematodes belong to the genus *Meloidogyne.* The two most common species in the tropics are *Meloidogyne incognita* (Southern root-knot) and *Meloidogyne javanica*. Other species are present but occur less frequent (Sipes and Schmitt, 2000). Root knot nematodes are the most damaging species in the home garden. These nematodes have a very wide host range, affecting more than 2000 plant species worldwide. Root knot nematodes enter the roots and establish feeding sites in susceptible hosts (Becker, 2003). They undergo three molts during which the

roots become swollen and attain a characteristic knotty appearance. Each female can produce several hundred of eggs that under favorable conditions continue to develop into the next generation (Becker, 2003). Water and nutrient uptake as well as transport are severely restricted by the root galling (Becker, 2003). Increase of plant metabolites from those galls attracts fungi and bacteria that enter and weaken the plant root tissues and accelerate decay. Limitation of normal root function is typically expressed with symptoms of malnutrition, chlorosis and stunting. Consequently vigor and production capacity of the diseased crop is noticeably reduced (Becker, 2003). Root knot nematodes are diagnosed primarily by the presence of root galls (Sipes and Schmitt, 2000).

2.2.2 Reni form nematode

The reniform nematode, *Rotylenchulus reniformis*, has a wide range on cultivated and non cultivated plants. The juvenile stages and male live in the soil and do not feed. The adult female is swollen and is the only parasitic stage of this nematode life cycle. The female inserts her head and neck into the root. The reniform nematode survives in the soil as eggs and coiled juveniles. Reniform nematodes cause root rotting and reduced uptake of water and soil nutrients. The symptoms are generally lack of vigor, discoloration of foliage and stunted plants. The reniform nematode can be accurately diagnosed only through laboratory assay of soil and root samples (Sipes and Schmitt, 2000).

2.2.3 Root-lesion and Burrowing nematodes

Adult burrowing nematodes *Radopholussimilis* and lesion nematodes *Pratylenchus* species cause root rot (Sipes and Schmitt, 2000). Root lesion and burrowing nematodes are more damaging to

broad-acre crops such as cereals. They use the stylet to puncture roots and enter the cells. They move through the root, piercing cells, extracting cell contents and leaving behind a trail of both cell-killing metabolites and eggs (Sipes and Schmitt, 2000). Root cell death results in browning and lessoning of the roots. Root lesion nematodes also damage feeder roots and root hairs, further reducing a plants effective extraction of water, shallow root system with many dead or dying areas. When the soil dries out, root lesions nematodes become inactive and survive in a dry form in the soil or in root tissue of old crops. As the soil moistens, the nematodes become active again and reinfect the fresh roots of the new crops (Sipes and Schmitt, 2000). It is necessary to have the soil and roots assayed to determine the numbers and kinds of nematodes species present: root assays are the most reliable (Sipes and Schmitt, 2000).

2.2.4 Foliar nematodes

Aphelenchoides besseyi, *A.ritzema-bosi*, and *A.Fragariae* feed inside leaf tissue. The entire nematode life cycle is completed in the leaves. Plants can be stunted with deformed, discolored, or dying leaf tissue. Accurate identification requires laboratory assay of leaf-tissue sample (Sipes and Schmitt, 2000).

2.2.5 Sugar-beet cyst nematode

The sugar-beet cyst nematode *Heterodera schachtii*, penetrates the root and the female enlarges as it matures to become a white, lemon –shaped structure that breaks through the root surface at maturity. When the female dies her body turns brown. Egg survives inside the dead females body direct observation of the organism with a magnifying glass is helpful (Sipes and Schmitt, 2000).

2.3 Control of nematodes

Plant parasitic nematodes need to be managed to maintain the quality and abundance of food and fiber produced by growers around the world (Pal and Mcspadden, 2006). Eliminating nematodes is not possible; the goal is to manage their population, reducing their numbers below damaging levels. Common management methods used include planting resistant crop varieties, rotating crops, land fallowing, flooding, ploughing, rougueing, incorporating soil amendments, time of planting, nematode suppressive plants and applying pesticides. In some cases, soil solarization also may be practical (Sipes and Schmitt, 2000).

2.3.1 Soil solarization and hot water treatment of planting materials

High temperatures will kill nematodes, therefore steam sterilization or other forms of heat treatment are often used for sterilizing soil used in greenhouses or nurseries. Soil solarization is receiving increased attention for the management of nematodes and other soil borne pests. It involves covering raised and moist beds with 14 clear plastics for two-to-four months during the hottest part of the year, allowing the sun to heat the uppermost layers of soil (Elmore *et al.,* 1997). Performance has been variable, depending on application technique and season (McSorley and Gallaher, 1991). This increase in soil temperature helps to kill many soil borne pests and pathogens including root-knot nematodes. According to Elmore *et al.* (1997), plant material infected with nematodes can be treated in hot water, provided that a suitable temperature range can be found which is high enough to kill nematodes but not lethal to the plant.

The method disinfects soils without leaving toxic residues, increases the levels of available mineral nutrients in soils by breaking down soluble organic matter and making it more bio-

available, and changes the soil micro flora to favor beneficial organisms (Elmore *et al*., 1997).The main drawback of this strategy is that temperature must be controlled critically and is usually just below that which injures plant tissues. The challenge is that most small-scale farmers in developing countries do not have enough knowledge and equipment to detect the precise temperature necessary for killing nematodes and at the same time not fatal to the plant (Elmore *et al*., 1997).

2.3.2 The time of planting/harvesting

The time of planting or harvesting may be utilized to exploit differential environmental effects on nematode populations versus crop growth and maturity. For example, early planting of crops such as wheat, barley, rye, chickpea and potato has restricted associated nematode damage in some instances (Duncan, 1991). Because of the prevailing temperatures and the conditions required for optimum growth of most crops, this approach often is impractical (Duncan, 1991).

2.3.3 Crop rotation and cover crops

Crop rotation is a very effective means of managing plant-parasitic nematodes. Crop rotation with a non-host crop is often adequate by itself to prevent nematode populations from reaching economically damaging levels. Corn, onions, garlic and small grains are good rotation crops for reducing root knot nematode populations. Velvet bean and grasses such as rye are usually resistant to root-knot nematodes. However, it is necessary to positively identify the species of nematode in order to know what plants are its host(s) and non-hosts (Peet and Mary, 1996; McSorley *et al.,* 2004).Rotation crops and cover crops can be helpful in manipulating nematode populations during those times of the year when most susceptible crops cannot be successfully

grown (Elmore *et al.*, 1997). Due to the wide host range of root knot nematodes, care must be taken in selecting alternative crops for rotation. Since some cover crop species can become serious weeds if improperly selected or managed (Ingel, 1996). This practice is however impractible due to lack of knowledge by many small scale farmers.

2.3.4 Rogueing and burning diseased plants

Rogueing involves removing diseased plants from the nursery or field. This method is best for new farmers or when disease is detected early. It prevents or minimizes the spread of nematodes from diseased plants to healthy plants along rows or between farms and nurseries. If root-knot disease is already established and severe, rogueing will not help to stop the disease from spreading (Scot, 2005).

2.3.5 Land fallowing

A fallow period of two years with no susceptible plants in the field decreases nematode populations (Flint, 1999). Fallowing, in which all vegetation is kept off the infested area, is a cheap and effective way to reduce nematodes number. This however will not stop nematode eggs from hatching but without food the young nematode will die (Flint, 1999). Frequent fallowing will keep the soil dry and free of plant growth and expose soil-borne disease organisms, such as nematodes, to killing heat and excessive drying. Other benefits of fallowing are weed and insect control. Land scarcity in most countries has caused this control strategy to be unfeasible (Flint, 1999).

2.3.6 Flooding

Nematode densities can drop significantly when soils are flooded for prolonged periods of time (Bridge, 1996). Flooding the soil for seven to nine months kills nematodes by reducing the amount of oxygen available for respiration and increasing concentrations of naturally occurring substances such as organic acids, methane, and hydrogen sulphide which are toxic to nematodes (MacGuidwin, 1993). Flooding leaves no toxic residues, it also conserves carbon in organic matter by slowing decomposition, increases the availability of certain micronutrients such as magnesium and iodine to crop plants, and changes the soil micro flora to favor biological pest control. As an added benefit, instead of leaving flooded fields fallow, it may be possible to grow cash crops such as rice (Allen and Sotomayor, 1996). It may take two years to kill all the nematode egg masses. The duration of flooding for effective nematode control needs to be determined for each nematode species and it is a costly and uneconomic means (Allen and Sotomayor, 1996).

2.3.7 Nematode-suppressive plants

According to Widmer and Abawi (2000), certain plants are able to kill or repel pests including nematodes, disrupt their lifecycle, or discourage them from feeding. Some of these plants are marigolds, castor bean, and various brassicas (powerful nematode-suppressive cover crops). Plant extracts, such as those from marigold (*Tagetesspecies*), have also been effective in killing plant-parasitic nematodes. They are useful for reducing nematode populations as well as conserving soil and often improving soil texture. In localities where carefully selected cover

crops may serve as living mulches and provide multiple pest control (Sciences, 1991). Results of the effectiveness of nematode-suppressive plants refer mainly to *in vitro* or pot experiments and practical application of these extracts is yet to be profitable (Dover *et al* ., 2003).

2.3.8 Organic amendments

According to Widmer and Abawi (2000), certain plants are able to kill or repel pests including nematodes, disrupt their lifecycle, or discourage them from feeding. Some of these plants are marigolds, castor bean, and various brassicas (powerful nematode-suppressive cover crops). Plant extracts, such as those from marigold, have also been effective in killing plant-parasitic nematodes. They are useful for reducing nematode populations as well as conserving soil and often improving soil texture. In localities where carefully selected cover crops may serve as living mulches and provide multiple pest control (Sciences, 1991). Results of the effectiveness of nematode-suppressive plants refer mainly to *in vitro* or pot experiments and practical application of these extracts is yet to be profitable (Dover *et al* ., 2003).

2.3.9 Ploughing

Prasad and Chawla (1991) reported that summer ploughing in parts of India allowed land temperature to reach 40-42 degree celcious thereby reducing populations of *Heterodera avenea*, *Meloidogyne* species and *Rotylenchus reniformis* by 40%. The practice of deep tillage / ploughing has been shown to increase yields in fields where nematodes are present. Deep tillage opens up soil for early cotton root development which is presumed to allow the roots to escape invasion by Root-knot nematode (Nandini and Mukewar, 2006). The labor needed, the

difficulties of cultivating soil in the dry season and lack of immediate and tangible benefits to the farmers normally rule out this practice for nematode control (Nandini and Mukewar, 2006).

2.3.10 Host plant resistance

Resistant cultivars can produce the most dramatic increase in yields of many crops and appear to hold the solution to most nematode problems (Luc *et al*., 2005). It is the most cost-effective and sustainable management tactic for preventing root knot nematode damage and reducing growers' losses (Khan, 1994). Resistance is crucial to the reliable production of food, and it provides significant reductions in agricultural use of synthetic chemicals and other inputs. Resistant crop cultivars have comparatively better crop yield than susceptible crop cultivars (Luc *et al*., 2005).

Plant disease resistance derives both from pre-formed defenses and from infection-induced responses (Friedman and Baker, 2007).

Although obvious qualitative differences in disease resistance can be observed when some plants are compared after infection by the same nematode strain at similar inoculums levels, a gradation of quantitative differences in disease resistance is observed between plant lines or genotypes (Newton, 1999).A major limiting factor affecting the effectiveness of newly introduced resistance cultivars is the selection of pathotypes or races that are able to break down the resistance (Luc *et al*., 2005).

2.3.11 Chemicals

There are two types of nematicides, fumigants and non fumigants. Fumigants nematicides are usually more effective but non-fumigant nematicides can also be used effectively. Fumigant nematicides such as metam sodium and 1, 3-dichloropropene are applied before planting. Some

non-fumigant nematicides such as Nemacur, Mocap or Vydate are moderately effective and can be used both during pre-planting and post-planting (Sipes and Schmitt, 2000). However the environmental pollution caused by excessive use and misuse of these chemicals has led to considerable change in people's attitudes towards their use in agriculture (Pal and Mcspadden, 2006). Today there are strict regulations on use of chemical pesticide (Pal and Mcspadden, 2006).

2.3.12 Biological control

Biological control is considered to result from the action of soil microorganisms and the soil micro fauna and is mediated through mechanisms such as parasitism, predation, competition and antibiosis (Stirling, 1991). There are three major types of organisms that are antagonistic to nematodes, predators, parasites and antagonists. Predators seek out nematodes and then consume them, while parasites grow within the nematodes and obtain their nutrition. The antagonists influence nematodes abundance through mechanisms other than predation and parasitism (Stirling, 1991). These organisms include the bacteria and fungi.

The bacteria *Pasteuria* species have been used to control nematodes and are host specific (Sandeepa, 2011). Generally they are only efficient parasites of the nematode species from which they originated. There are four described species of *Pasteuria* and include *P. ramosa, P. penetrans, P. thornei and P. nishizawae* others are undescribed. Rhizobacteria are antagonistic to plant-parasitic nematodes. These bacteria inhibit nematode egg hatch and/or penetration of roots. The mechanism by which antagonistic bacteria inhibit plant-parasitic nematodes is not known purpose (Sandeepa, 2011). However, several hypotheses have been put forth, such as production of antibiotics that kill nematode eggs, degradation of the root exudates that the nematode relies

on for host location and to stimulate egg hatch and induction of systemic acquired resistance (SAR).Bacteria are easy to culture *in vitro* and they can be applied as seed treatments and reduce plant damage (Sikora, 1992; Stirling, 1991).Their disadvantage is that they are effective for a relatively short period; their activities are affected by crop cultivar and nematode species and have little effect on nematode multiplication(Sikora, 1991; Stirling, 1992).

Nematophagous fungi are carnivorous fungi that have developed methods and structures that enable them to successfully trap and consume nematodes. Nematode trapping fungi are responsible for keeping the nematode population in check and are in turn consumed by organisms on the next trophic level (Microbewiki, 2013). living stages of nematodes (eggs, juveniles, vermiform adults and feeding sedentary females) can be attacked, penetrated and digested by several types of nematophagous fungi (Jansson and Lopez-Llorca, 2001). Most nematophagous fungi are facultative parasites and exist in both saprophytic and parasitic stages induced by external and internal signals (Jansson *et al*., 1997).Nematode destroying fungi are divided into groups depending on their mode of infecting nematodes such as, nematode trapping fungi, endo-parasitic, egg and female parasitic and toxin producing fungi (Jansson *et al*., 1997). Endo-parasitic fungi use their spores to infect nematodes. The spores adhere to the nematode cuticle or, in some species, are ingested together with food. Nematode trapping fungi use hyphal trapping devices to capture nematodes. Nematode trapping fungi can produce various trapping devices to capture nematodes (Ahren *et al*., 2004). The three basic types of trapping devices are adhesive knobs, constricting rings and adhesive networks (Rubner, 1996). These traps can have either adhesive or mechanical function. In contrast to the endoparasites, the nematode trapping fungi can live as saprophytes in soil (Ahren *et al*., 2004).

Having nematophagous fungi in the soil confers many benefits both environmentally and economically. Nematophagous fungi are nonpolluting and environmentally safe and acceptable as bio-control agents against plant parasitic nematodes (Microbewiki, 2013). They are species specific to targeted pest and keep the nematode population under control; this allows a wide variety of plants to grow, even those that are susceptible to nematodes. The mass that is gained by the fungus by consuming the nematodes is also beneficial (Microbewiki, 2013). This mass provides a food source to other organisms that are higher up the food chain. Having this microbial interaction promotes the cycling of nutrients (Microbewiki, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study areas

Soil samples were collected from five vegetable growing areas characterized with different temperatures which were Kinale, Kabete, Athiriver, Machakos and Kibwezi. All the areas chosen were characterized with horticulture production but at different intensities. Kinale is largely influenced by altitude and predominated by two agro ecological zones the lower and upper highland zones with altitude varying from 1760 m and 2610 m. Rainfall varies from a minimum of 20 mm to 200mm, while the average temperatures vary from a minimum of 10 – 14° C. The dominant soils are and andasols (Gachene *et al*., 2012). The majority of the people depend on small scale farming with the average land being 0.8 hectares (Oberthur *et al*., 2009).Vegetables grown are cabbages, kales, tomatoes and spinach (figure 1). Kabete has an altitude of 1844m area has a rainfall amount of 21.9 mm and annual temperature of 16° C. The type of soil in the study area is humic nitisol (Tiptopglobe, 2013). Vegetables grown are cabbages, kales, tomatoes and spinach (Figure 1). Kibwezi area rises slightly below 600 m above sea level. The climate of this region is typically semi-arid, with a mean temperature range from 20.2 to 24.6° C but the ongoing drought period has recorded temperatures as high as 32 ° C. The annual rainfall ranges from 150 mm to 600 mm (friendsofamericafoundation, 2013). Soils at Kibwezi are feral-chromic luvisols and the mean farm size range from 12-14 acres Vegetables grown are Capsicum, kales, tomatoes, bringles and spinach (Figure 2). Athiriver has a temperature of 20° C, the annual rainfall falling between 450mm – 900 mm. The area has an altitude of between 600m-1600m.

Major soil types include afisols, lava, oxisols, vertisols and andasols (Dickens, 2010). Vegetables grown in the area include spinach, green bell pepper and Kales (Figure 3). Machakos rises from 700m to 1700m above sea level. The annual average rainfall ranges between 500 mm to 1300 mm, while the mean monthly temperature vary between 18 and 25° C. The area has five major soil types which include, afisols, acrisols, ferrasols , vertisols and andasols. Majority of the people depend on agriculture and farm holding range from 0.5 – 2 acres (Ayub, 2009). Vegetables grown are Capsicum, kales, tomatoes and spinach (Figure 3). The five vegetable production zones were climatically different (Table 1).

Table 1: Comparison of selected environmental factors in Kinale, Kabete, Athiriver, Machakos and Kibwezi.

Site	**County**	**Mean temperature range**	**Altitude**	**Rainfall**
Kinale	Kiambu	10 - 14 ° **C**	1760 – 2610m	20 – 200 mm
Kabete	Kiambu	16° C	1844 m	21.9 mm
Athiriver	Machakos	20 ° C	600 – 1600m	450 – 900 mm
Machakos	Machakos	18-25 °C	700 – 1700m	500 – 1300 mm
Kibwezi	Makueni	20.2-32° C	600 m	150 600 mm

(Courtesy of: friendsofamericafoundation, 2013; Tiptopglobe, 2013; Gachene *et al*., 2012; Dickens, 2010; Ayub, 2009; Oberthur *et al*., 2009).

Kinale area

Kabete area

Figure 1: Map of Kiambu County showing the sampled areas: Kinale and Kabete.

(Courtesy of Geography Department, University of Nairobi)

Plate of selected vegetables grown in Athiriver and Kibwezi study areas

Plate 1: Image of green bell pepper from Athiriver study area

Plate 2: Image of kales from Kibwezi study area.

Plate 3: Image of tomatoes from Kibwezi study area

Plate 4: Image of onion from Machakos study area

(Source: Kibwezi and Athiriver study areas)

3.2 Soil samples collection

From each of the area described above, five farms under intensive vegetable production were randomly selected for this study. From each of the farm, five soil samples were collected from five different vegetables, using a soil auger. The soil auger was sterilized using ethanol after every sampling point to avoid cross contamination. Five soil samples were collected from each vegetable farm and mixed in a bucket to make one composite sample. 1 kg of soil was then resampled from the composite sample, put in plastic bags, labeled and placed in a cool box. All the samples were later transported to the laboratory for extraction of nematodes and isolation of nematode destroying fungi. Information on soil management practices, such as application of fertilizer, organic manure and pesticides was recorded through interviews and observations from each farm.

3.3 Isolation of nematode destroying fungi from the soil samples

In the laboratory nematodes destroying fungi were isolated using the soil sprinkle technique as described by Jaffee *et al.,* (1996). Tap water agar (TWA) was prepared by dissolving 20 g agar in one litre of tap water. The medium was autoclaved and cooled to 45^0cbefore amending it with 0.1 grams per liter of streptomycin sulfate of water and poured in Petri dishes under a laminar flow. The media was used when cool. Approximately 1g of soil sample was sprinkled on the medium in the petridish. Suspension of *Meloidogyne* species of approximately 1000 nematodes was then added into the petridishes as baits (Jansson *et al*., 2000). The plates were then incubated at room temperature (20^0c)and observed daily from the third week up to sixth week under a microscope at low (40X) magnification. The examination was focused on trapped nematodes,

trapping organs and conidia of the nematodes destroying fungi that grew from the soil (Wachira *et al.,* 2008).

Taxonomic identification of the nematode destroying fungi was done using the slide culture technique where slides were observed under a microscope in order to identify fungal characteristics and size of each conidia (Orozco, 2005). Identification of the genus was done using identification keys described by Cooke and Godfrey, (1964).

3.4 Extraction of Nematodes

Nematodes were isolated using the modified Bearmann technique as described by Kleynhans, (1999). Soil lumps were broken, stones and plant debris removed. 100 grams of soil was spread evenly on a circle of double ply paper towel (serviette) supported on a coarse meshed plastic screen standing in a plastic container. Water was added to the container until the soil was thoroughly wet but not immersed. The container was covered with a large Petri dish to reduce evaporation of the water. The set up was left for at least 24 hours. Soil was then removed, discarded and the nematode suspension poured from the container for examination. The nematodes were identified using nematode identification key as described by Armen *et al*., (1977), they were then quantified and recorded in various trophic levels. Quantification of nematodes was done by drawing a fixed volume of 2ml of nematode suspension using a micropipette, the suspension was then placed into a counting dish. The nematodes were allowed to settle then they were counted under a microscope at magnification (40X) this was repeated two times. The number of nematodes present in the sample drawn was calculated in volume

3.5 Determining efficacy of selected nematode destroying fungi isolate

After identification of nematode destroying fungi, pure cultures of the fungi isolates were made. 5mm of a pure nematode destroying fungi was inoculated into PDA in a Petri dish and allowed to grow for five days. Pure cultures were made by inoculating the nematode destroying fungi in tap water agar, 50 plant parasitic nematodes were added in the plates with the pure cultures of nematode trapping fungi. The efficacy of the fungi isolate was monitored; this was done by leaving the experiment for a period of 3-6 weeks to enable the different fungi to capture the nematodes. Three weeks after inoculation, trapped nematodes by the nematode destroying fungi were counted everyday in each plate, for five days and the number noted until all nematodes were captured.

3.6 Data analysis

Data on the presence, absence, the different types and the numbers of nematodes and nematode destroying fungi was entered into Microsoft excel, arranged and cleaned. Comparison of means was done using ANOVA. All means in this study were compared at 0.05 level of significance. Data obtained from isolation of nematode destroying fungi and nematodes was used to explain their population and diversity in the five vegetable growing areas. These results were used to explain whether temperature had any effect on their frequency of occurrence, diversity and total in the five zones.

The information on the different agricultural activities recorded in the areas were, increased disturbance of soil through tillage, use of mineral fertilizers, application of manure, use of irrigation and pesticides. This information was used to explain whether these activities effected the population of nematodes and nematode destroying fungi from the area. The efficacy test was

used to determine which of the isolated nematode destroying fungi could be developed as bio-control against plant parasitic nematode.

CHAPTER FOUR

4.0 RESULTS

4.1 Soil fertility and pests' management

In Kinale, all the sampled farms recorded application of chemical fertilizers while only 3 out of the five selected farms recorded pesticides application. In Athiriver and Kibwezi, farmers in each of the selected 5 farms used both fertilizer and pesticides. In Machakos animal manure from cattle was used in 4 out of the 5 selected farms while on the other one farm use of fertilizer was recorded. In Kabete, the soil was collected from the university farm where animal manure is regularly applied (Table 2**).**

Table 2: Number of farms receiving fertilizer, animal manure and pesticide in the five vegetable production zones

Production Zone	**Fertilizer**	**Manure**	**Pesticide**
Kinale	5	0	3
Kabete	0	5	0
Athiriver	5	0	5
Machakos	1	4	0
Kibwezi	5	0	5

In Kinale, Athiriver and Kibwezi where vegetables were mainly grown for commercial purposes, fertilizer and pesticides were applied in the farms to increase the yields. In Kabete vegetables were mainly for home use and only animal manure was applied in the soil, while in Machakos

vegetables were grown for both home and commercial purpose. Application of manure was practiced with only a few of the farmers using fertilizer.

4.2 Fungal population

A total of 171 isolates of nematode destroying fungi were identified from all the five vegetable growing areas in different frequencies. Kabete had the highest frequency of 33.92 %. Machakos, Kibwezi, Athiriver and Kinale recorded frequencies of 24.6, 22.8, 11.7 and 7.0 % in that decreasing order (Figure 2). Kibwezi had the highest diversity index with a mean of 1.017, followed by Machakos with 0.652 then Kinale 0.471, Kabete 0.458 and least diversity index was recorded in Athiriver with a mean of 0.333. The renyi diversity profile, showed that Machakos was the most diverse zone, followed by Kibwezi, Kinale, Athiriver and finally Kabete (Figure 3). Kibwezi had the highest species richness, followed by Machakos and Kabete then Kinale and least was Athiriver with mean total records 3.4, 2.2, 2.2, 1.8 and 1.6 in that decreasing order. Kabete had the highest species abundance with a mean of 11.6, followed by Kibwezi with 7.6, then Machakos which recorded 7.4, Athiriver 4.0 and least was Kinale with a species mean abundance of 2.6 (Table 3).

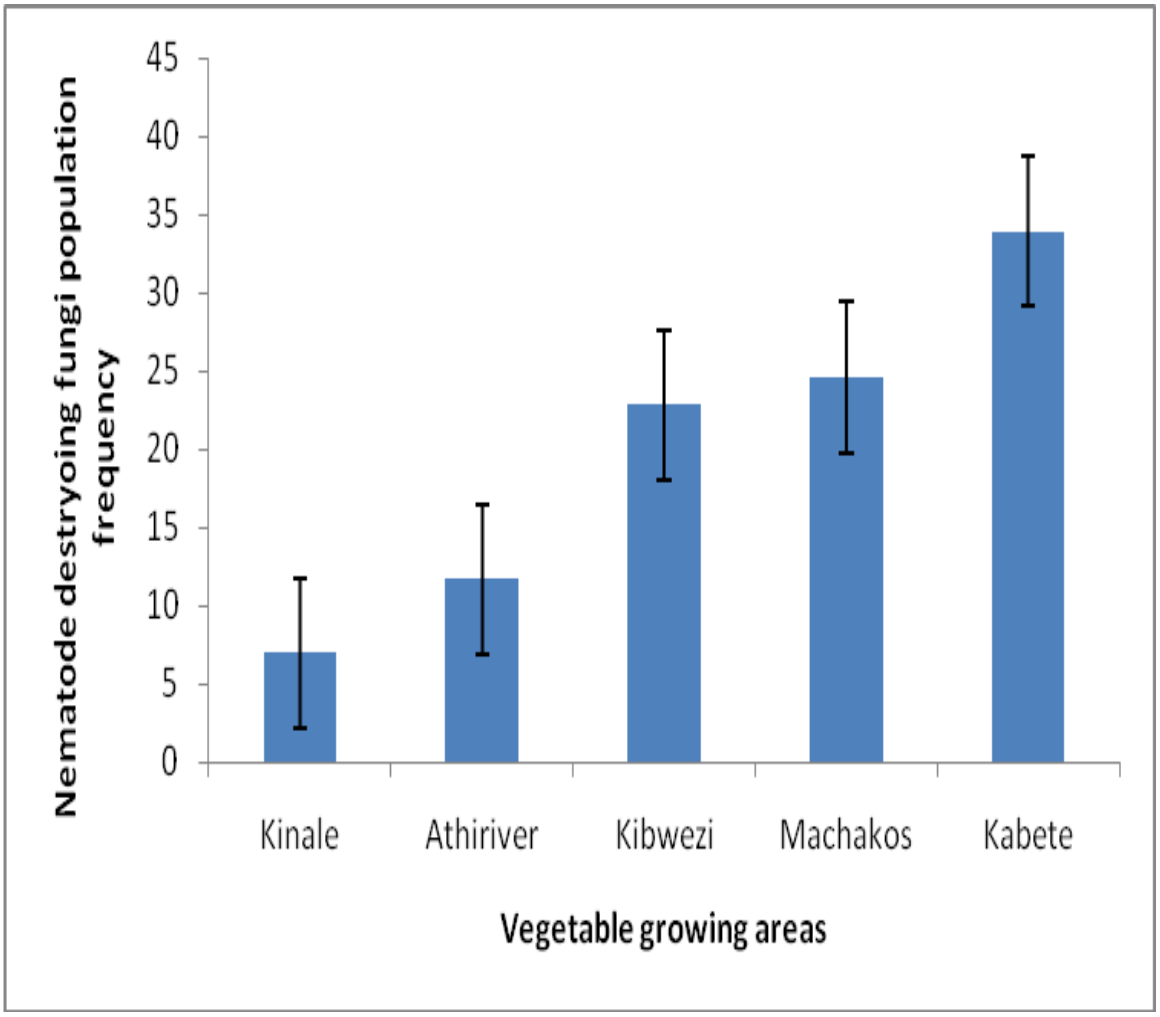

Figure 2: Percentage frequency of occurrence of nematode destroying fungi in the different vegetable growing areas

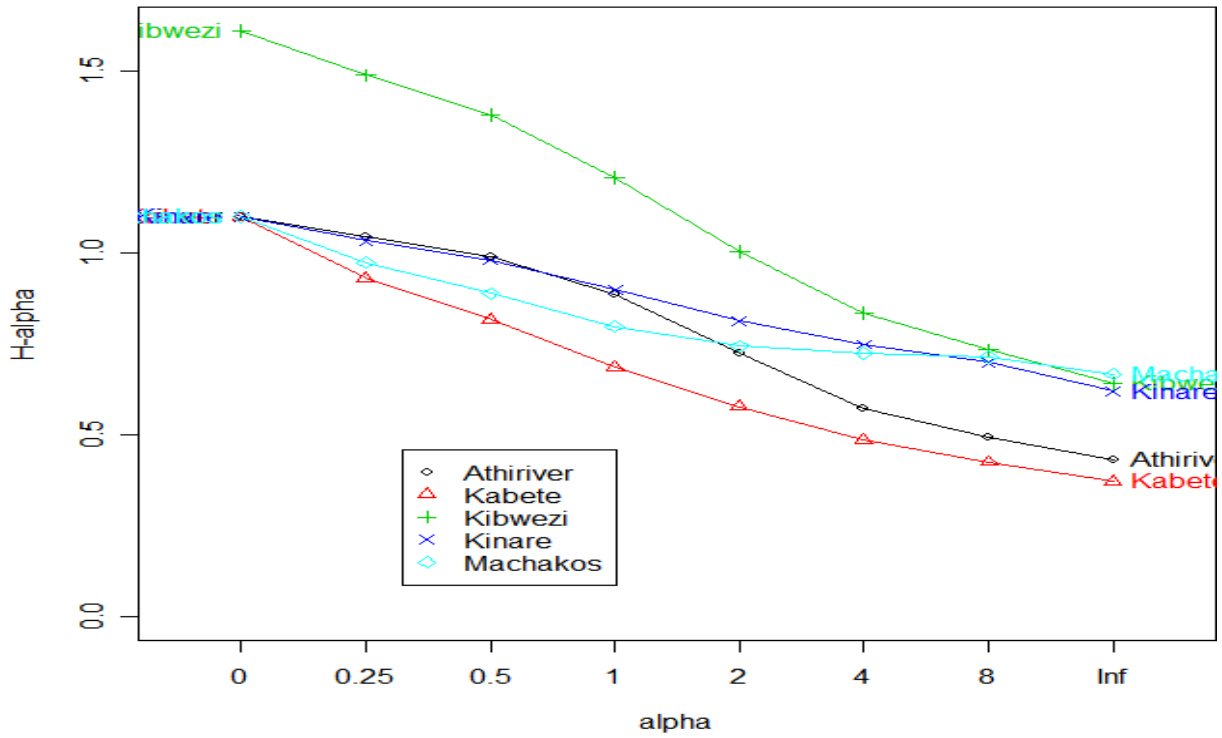

Figure 3: Renyi diversity profiles of the five vegetable growing areas

Table 3: Record of mean abundance, diversity and richness of nematode-destroying fungi in the different vegetable growing zones

Vegetable zone	Abundance	Diversity	Richness
Kinale	2.6	0.471	1.8
Kabete	11.6	0.458	2.2
Athiriver	4.0	0.333	1.6
Machakos	7.4	0.652	2.2
Kibwezi	7.6	1.017	3.4

All the isolates from this study were grouped into three genera and five taxa. These genera were *Arthrobotrys, Monacrosporium* and *Stylopage. Arthrobotrys* had the highest occurrence in all

ecological zones; followed by *Monacrosporium* and least were *Stylopage* with the following frequencies respectively 92.98, 5.85 and 1.16 % respectively. The genera *Arthrobotrys* was represented by, *A. oligospora, A. dactyloides* and *A. longispora*, the genera *Monacrosporium* was represented by *M. cionopagium* while genera *Stylopage* was represented by *Stylopage grandis. A. oligospora* had the highest frequency of occurrence followed by *A .dactyloides, M. cionopagium, S. grandis* and the least was *A. longispora* with occurrence frequencies of 46.20, 45.6, 5.9, 1.2 and 1.2 % in that decreasing order (Figure 4).

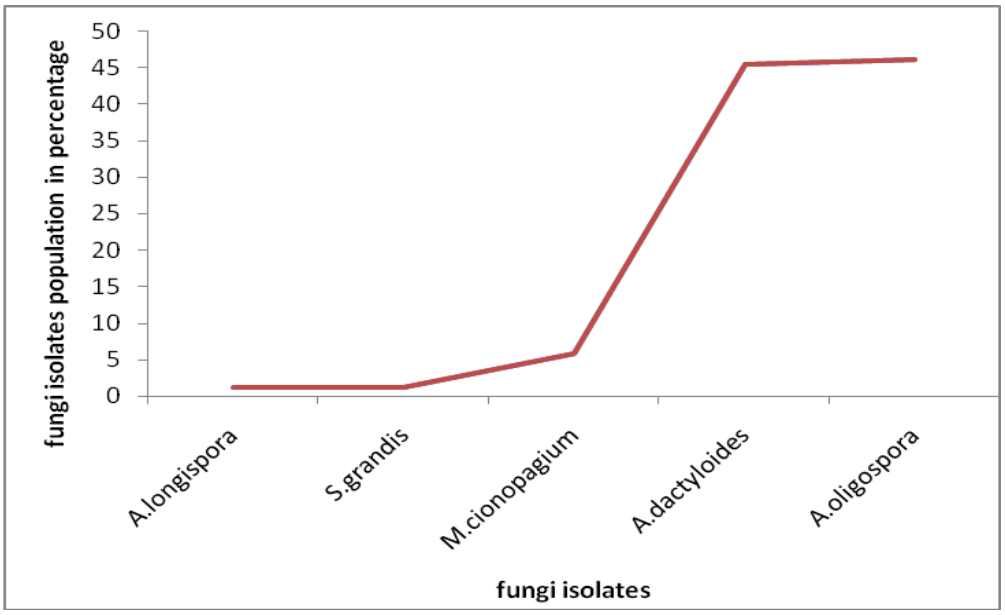

Figure 4: Percentage frequency of occurrence of nematode destroying fungi in the different vegetable growing areas.

Plates of images from the study showing nematode destroying fungi from genera *Stylopage* (Plate 5) and *Arthrobotrys* (Plate 6) with trapped nematodes viewed at magnification (40 X).

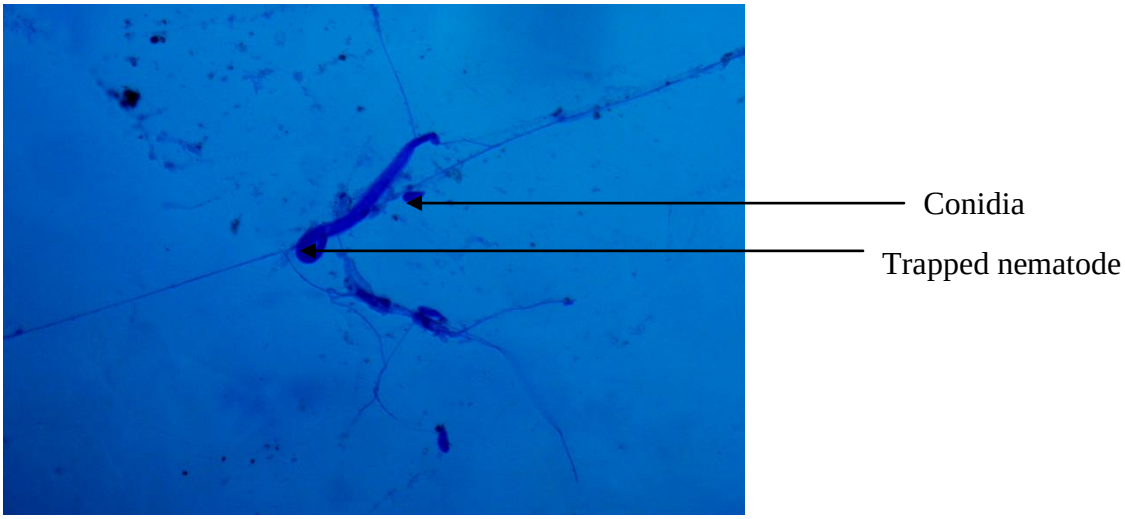

Plate 5: Conidia and trapped nematode by *Stylopage grandis* viewed at magnification (40X)

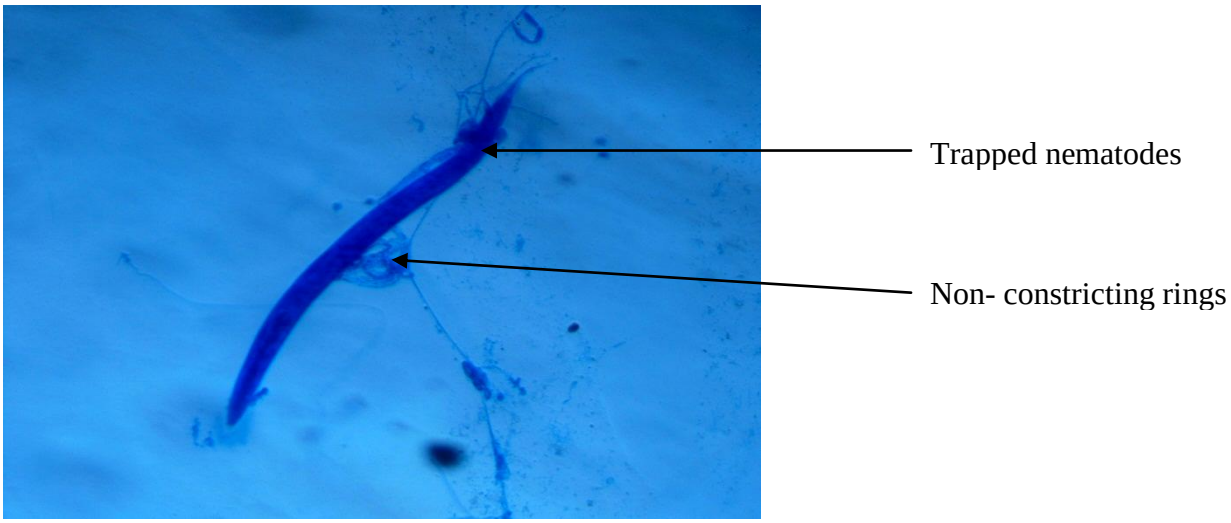

Plate 6: Trapped nematode and constricted rings of *Arthrobotrys dactyloides* viewed at magnification (40X)

A.dactyloides was significantly affected (P=0.002) by the vegetable growing areas with a mean of 15.6, while all the other fungi isolates were not. *A.oligospora* had a mean of 16.2, *M.cionopagium* 2.0, while *A.longispora* and *S.grandis* both had a mean of 0.4 (Table 4).

Table 4: Means of nematode destroying fungi isolated in the different vegetable growing areas and the p.values

Zone	*A.dactyloides*	*A.oligospora*	*A.longispora*	*M.cionopagium*	*S.grandis*
Kabete	40	17	0	1	0
Machakos	19	22	0	1	0
Kinale	5	7	0	0	1
Kibwezi	10	20	2	5	1
Athiriver	4	13	0	3	0
P. value	0.002	0.395	0.062	0.165	0.062

The species cumulative curve indicated that 25 soil samples were adequate to capture the majority of the species in the five vegetable growing zones (Figure 5).

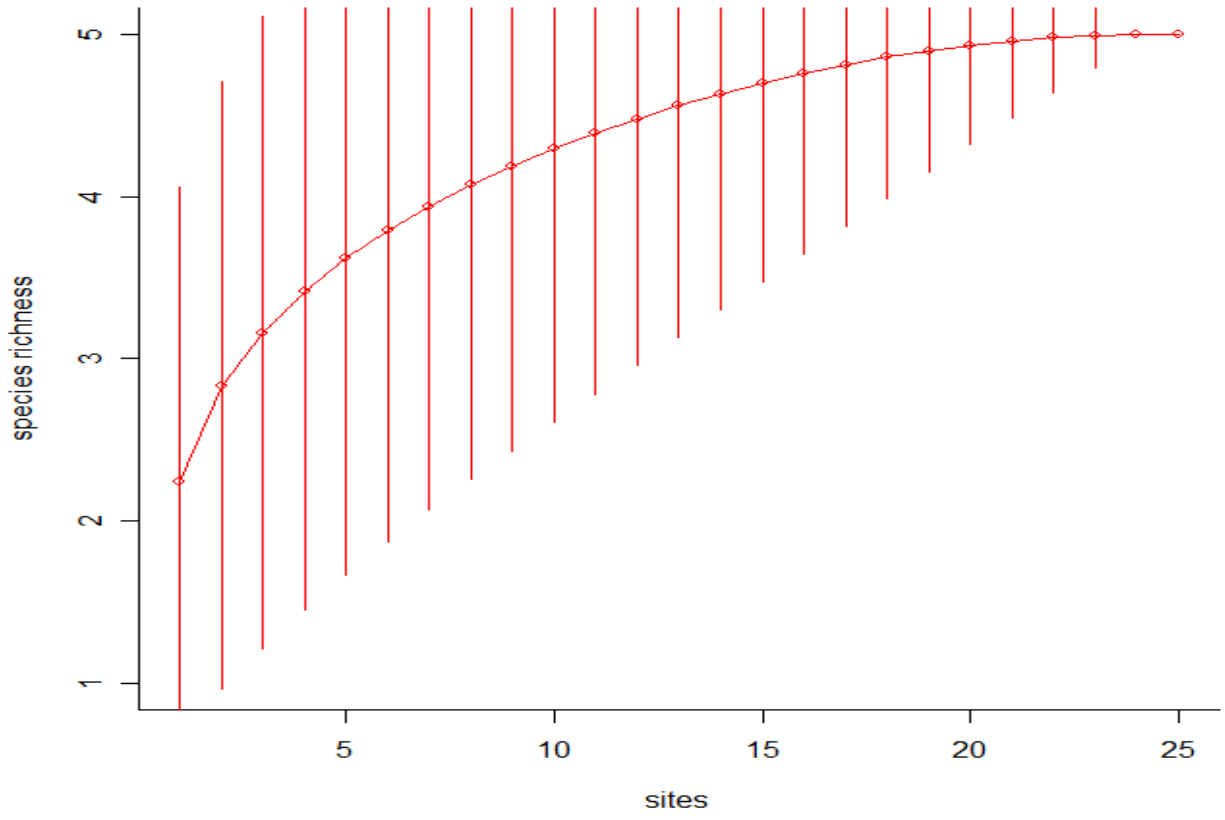

Figure 5: Nematode destroying fungi species cumulative curve in the five vegetable growing areas

4.3 Nematode population

A total population of 11,050 nematodes were identified in all the vegetable growing areas. The highets nematode population was highest in Kinale, followed by Athiriver then Kabete, Machakos and the least was recorded in Kibwezi. 5,070 nematodes were recorded in Kinale 2,080 recorded in Athiriver, 1,625 recorded in Kabete, 1,235 recorded in Machakos and 1,040 nematodes being recorded in Kibwezi in that decreasing order (Figure 6).

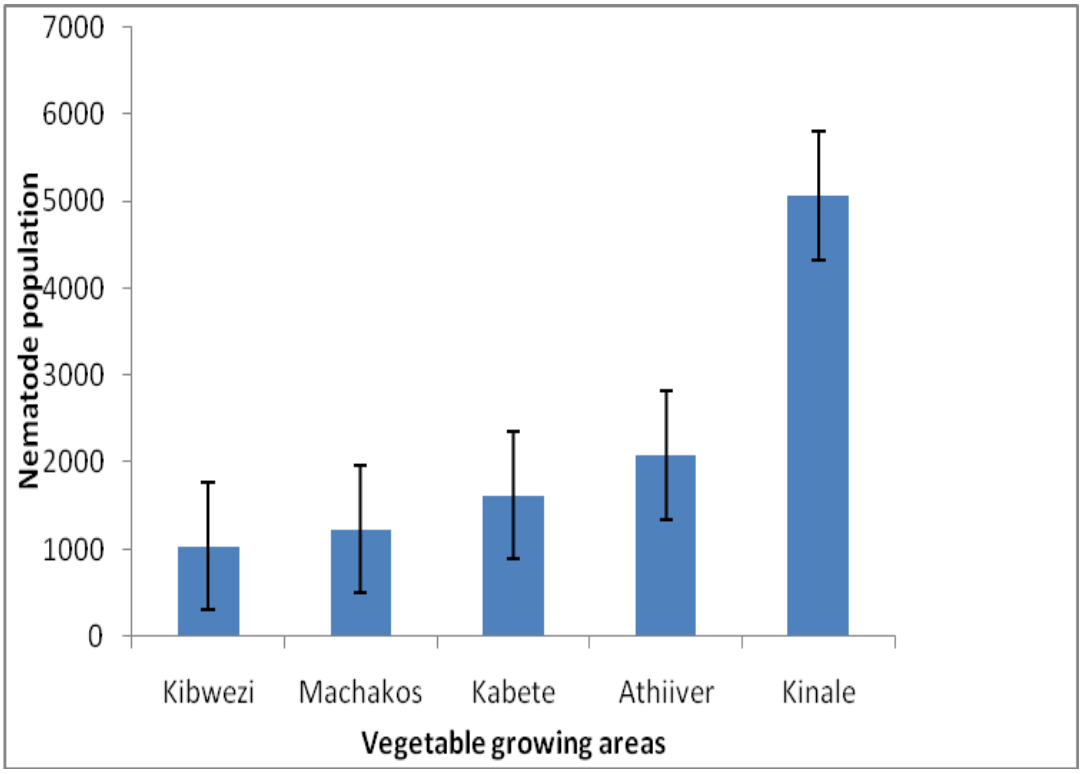

Figure 6: Total nematode population in the five vegetable growing areas

Thenematodes were grouped into three trophic levels including; predator nematodes, plant parasitic nematodes and bacterial feeder nematodes. Predator nematodes had the highest population with a total population of 468, followed by plant parasitic nematodes while the bacterial feeders were the least with 258 and 127 respectively (Table 5).

Table 5: Total counts of nematodes trophic levels in the different vegetable growing areas**.**

Zones	Plant parasitic	Bacterial	Predator
Kinale	39	47	304
Athiriver	109	29	24
Machakos	22	17	54
Kibwezi	52	16	14
Kabete	36	18	72
Total	258	127	468

From the total nematode population recorded at Kinale (5,070), the predator feeders were 77.95% while bacterial feeders were 12.05 %. while the least were the plant parasitic which was only 10 %. Kabete, with a total population of 1,625 recorded 57.14 % predator feeders 28.57 % plant parasitic feeders and 14.29 %. bacterial feeders. In Machakos recorded 58.06 % , of the total poulation was predator feeders, while plant parasitic were 23.66 % and bacterial feeders recorded only 18.28 % . In Kibwezi agroecological zone,with a populatiom of 1,040 nematodes, 63.41 %, of these were identified as plant parasitic feeders while bacterial feeders were 19.51 %, the predator feeders made the remaining 17.07 %. In Athiriver 2,080 nematodes were recorded ,out of which plant parasitic feeders constituted 67.28%, with bacterial feeders making 17.90 % and the predator feeders recording 14.81 % of the total . All the three nematode trophic levels

were present in all zones with Kinale having the highest population of nematodes and the highest population of predator nematodes (Table 6).

Table 6: Percentage frequency of nematode trophic levels in different vegetable growing areas

Zone	Plant parasitic feeders	Bacterial feeders	Predator feeders	Total
Kinale	10	12.05	77.95	100
Athiriver	67.28	17.9	14.81	99.99
Machakos	23.66	18.28	58.06	100
Kibwezi	63.41	19.51	17.07	99.99
Kabete	28.57	14.29	57.14	100

The occurrence of nematodes was significantly (P = 0.000) affected by the different vegetable growing areas . A mean of 13.33 was recorded for Kinale, 5.4 for Athiriver, 4.2 for Kabete,3.1 for Machakos and 3.067 for Kibwezi. The community structure of the nematodes differed in all ecological zones with plant parasitic nematodes being highest in Athiriver and least in Kabete and Machakos (Table 7).

Table 7: variation of nematodes with trophic level and p values of trophic levels

Zone	Plant parasitic	Bacterial	Predator
Kinale	39	47	304
Athiriver	63	19	17
Machakos	22	17	54
Kibwezi	52	16	14
Kabete	36	18	74
P. value	0.000	0.000	0.000

From this study, it was observed that nematode population was highest in areas with low temperatures and lowest in areas with high temperatures. Kinale had low temperature (10 -14 ° C) and high nematode population while Kibwezi had high temperature (20.2-32° C) and a low nematode population. In Kinale crops were mainly cultivated for commercial purposes and application of chemical fertilizer and a low population of soil fungi were recorded. In Kabete cow manure was applied in the farms and the population of nematode destroying fungi was the highest during this study, this means agricultural practices affected fungi population in the areas. Plant parasitic nematodes, bacterial and predator feeders' nematodes were recorded in all the vegetable production zones.

In comparison of the plant parasitic nematodes and the nematode destroying fungi, a total of 212 plant parasitic nematodes and 171 nematode destroying fungi were recorded. It is evident that in areas where there was high population of nematode destroying fungi there was low population of

plant parasitic nematode. Machakos and Kabete recorded high populations of nematode destroying fungi and low population of plant parasitic nematode (Table 8).

Table 8: Plant parasitic nematode population and nematode destroying fungi in the five vegetable growing areas

Zones	**Plant parasitic nematode**	**Nematode destroying fungi**
Kinale	39	13
Kabete	36	58
Athiriver	63	20
Machakos	22	42
Kibwezi	52	38
Total population	212	171

4.4 Efficacy test

At 8 hours the genera *Monacrosporium* and *Arthrobotrys* had trapped some of the inoculated nematodes, while *Stylopage* had not. The genera *Arthrobotrys* had the highest number of trapped nematodes within a period of 104 hours, with a total population of 57 trapped nematodes, followed by *Monacrosporium* and least in number of trapped nematodes were recorded in the genera *Stylopage* with a total of 45 and 36 respectively (Figure 7).

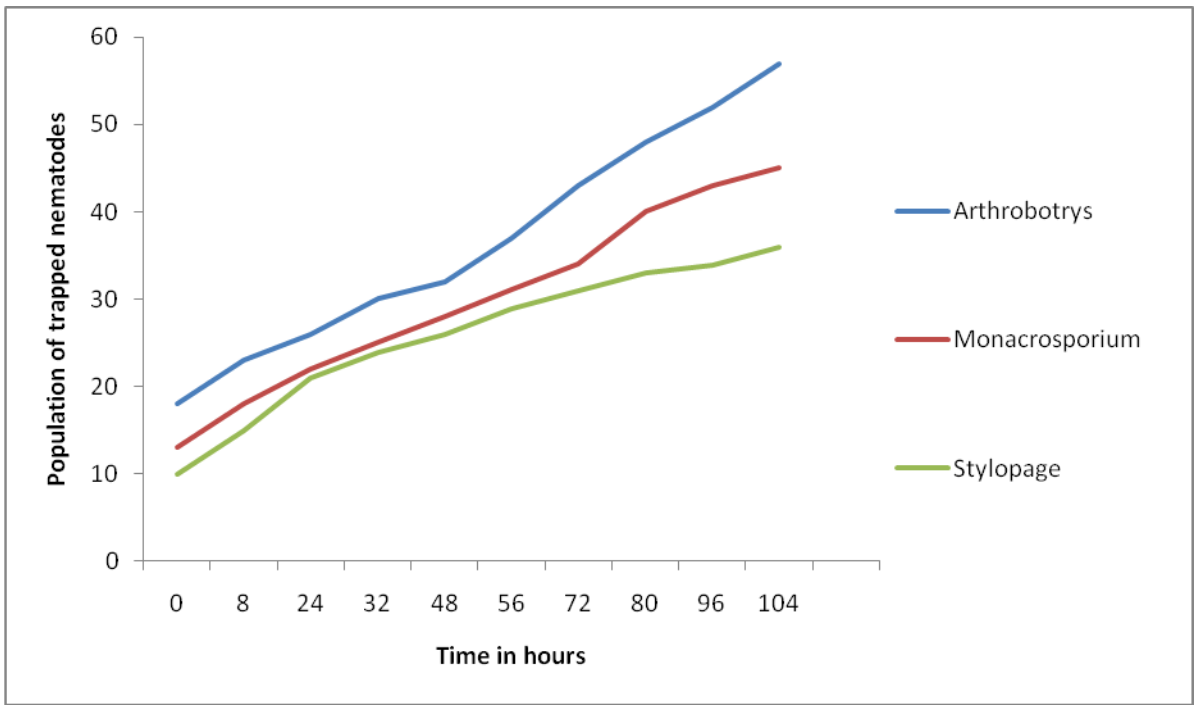

Figure 7: Population of trapped nematodes by *Stylopage*, *Monacrosporium* and *Arthrobotrys* after 104 hours of incubation

The three genera were significantly (P=0.003) different in their efficacy of nematode trapping with an overall mean of 6.2. *Arthrobotrys* recorded the highest mean of 7.3 followed by *Monacrosporium* and the least mean recorded by the *Stylopage* which means of 6.0 and 5.2.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

From this study, it was noted that farmers use diverse methods of soil fertility and pests management namely chemical fertilizers, cow manure and pesticides. This was in bid to increase farm yields either for own of commercial purposes. In Kinale, Athiriver and Kibwezi all the farmers applied chemical fertilizers. This could be attributed to the fact that the three zones supplies vegetables to Nairobi and Mombasa respectively. Kales are now one of the most consumed leafy vegetable in the city as they are easy to prepare and affordable to the consumer hence making them a major commercial crop. Athiriver, is one of the towns supplying vegetables to the city. A number of farmers in Athiriver use untreated effluent water to grow kales. Liquid slurry from sewers is also used, which apart from providing water to the crop, is also regarded to be rich in nutrients (Daily nation, 2012). According to FAO (2013) farmers in Kibwezi practice irrigated horticultural production with water from Kibwezi and Athi rivers and also use chemical fertilizers to increase yields from their farms. Horticultural production is mainly for commercial purposes and also for local consumption. The study demonstrated that nematode destroying fungi occurred in all farms in the five vegetable growing areas; with different diversity, occurrence and abundance. The study therefore confirmed that the nematode destroying fungi are saprophytic and cosmopolitan in nature. Nematode trapping fungi are quite common in natural soils, agricultural soils and all kinds of rotting organic debris (Jaffee *et al*., 1998). Because of their presence in agricultural soils, they have been playing a significant role in maintaining the natural balance of plant parasitic nematodes, which are responsible for mild to severe damage to crops.

These findings are also consistent with previous reports indicating that nematode destroying fungi were present in all habitats but at different densities and diversities (Birgit *et al.,* 2002). The fungi demonstrated various nematode destroying structures like, adhesive networks, constricting rings and non- constricting rings. Such structures had previously been reported in a study carried by Wachira *et al*., (2009) that fungi isolated had several mechanisms of capturing and destroying plant parasitic nematodes which included constricting rings, adhesive nets and non-constricting ring. A similar result was also reported by Yu'eHao *et al*., (2005) when they investigated ecology of aquatic nematode trapping fungi in Southwestern China. *Arthrobotrys oligospora* although the most abundant genera of nematode destroying fungi was not significantly affected by the land use. Other studies on nematode destroying fungi have also reported *A.oligospora* as the most frequently isolated nematode destroying fungi**.** These findings are consistent with previous report by Wachira (*et al*., 2008) indicating that *A. oligospora* was the most abundant species of nematode destroying fungi in the study area. Farrel *et al.,* (2006) also made similar observations, that *A. oligospora* was very abundant in Bodega marine reserve and attributed it to the organic matter of the soil which was estimated to be 6.5 % apart from presence of organic matter, the fungi also obtain its carbon and energy from two sources, organic matter (saprotrophs) and also from trapping nematodes (parasites), making it adaptable to wide range of habitats. Contrarily *A. dactyloides* was the only nematode destroying fungi seen to be affected by the agro ecological zones although it was recorded in all the agro ecological zones. The highest occurrence was recorded in Kabete while the least number was in Athiriver. Contrary to the findings of this study, a study carried by Wachira *et al*., (2008) reported that *A.dactyloides* was affected by land use. Another study by Wachira *et al*., (2009) working on

influence of land use and soil management practices on the occurrence of nematode destroying fungi in Taita Taveta, showed that *A. dactyloides* was affected by land use and organic input.

From the study Kabete had the highest fungal population while Kinale had the lowest. This difference in population might have been as a result of the different agricultural practices which included application of fertilizer, organic manure and application of pesticide could have resulted to the difference in the fungi frequency in the areas. In Kabete application of cow manure was identified as the only means of soil fertility management. Organic amendments have been demonstrated to increase biological activity and soil structure hence the high fungal population. Organic amendments stimulate the occurrence of nematode destroying fungi in the soil and reduce plant parasitic nematodes (Wachira *et al*., 2009). Application of organic amendments is not only beneficial to disease management but also improves the plant growth and productivity. They lead to the buildup of beneficial micro flora, that keep the plant healthy and vigor which will reduce the plant parasitic nematodes in the soil (Pakeerathan *et al*., 2009).In a related study Jaffee (2006) also showed that organic amendments enhanced build up of nematode destroying fungi. Good soil structure makes easier for the plant roots to reach moisture and to absorb the nutrients in the soil (Jedidi *et al.*, 2004). Although the diversity of vegetables was high in Kinale compared to other vegetable cultivation zones, low population of nematode destroying fungi was observed. This could be attributed to the application of chemical fertilizer and pesticides applied in the soil in order to increase vegetable yields. Application of chemicals in the soil has been reported to reduce soil biodiversity. Modern agricultural practices rely heavily on the use of chemical fertilizers to meet the rising demand for food which is estimated to be 40 – 45%. Chemical fertilizers cause farmland degradation and reduced soil fertility and biodiversity (Life

science, 2013). Current practices continue with the use of harsh chemicals and ignore the delicate balance of humus, microbes, trace minerals and nutrients in the soil. Such management has resulted in marked losses in soil organic carbon and greatly reduced diversity and abundance of microbes and larger organisms in the soil food web (Ingham, 2006). Agricultural activities such as rotation, drainage use of pesticides and fertilizer have a significant implication for the microorganisms present in the soil (Hengeveld, 1996). The chemical fertilizers are inhibitory to spore germination of nematode trapping fungi or lethal at the concentrations used in soil, may influence the natural predation of nematodes in soil and thereby bring imbalance in the natural equilibrium. Similar observations were reported by Kumar *et al*., (2005) that soils amended with higher concentrations of fertilizers such as urea, diammonium phosphate and muriate of potash adversely affected the spore germination of fungi. Amendment of soil with urea at the concentrations of 1.0%, 0.5% and 0.1% reduced spore germination of all the isolates of *Arthrobotrys dactyloides.* Inhibition of germination of spores by urea at the concentrations used may be attributed to direct toxicity of urea on the spores. From the observations he made it was very clear that application of fertilizers would reduce the population of nematode trapping fungi in the soil. According to Schinner and Sonnletner (1996), soil microorganisms are sensitive to changes in the surrounding soil and have been shown that the microbial population changes after fertilization (Hyman *et al.,* 1990, Kumar *et al*., 2005).). In summary, according to Sanchez (1997) agricultural practices have positive or negative impacts on microorganisms in the soil.

The highets number of nematodes was recoreded in Kinale while the least population was in Kibwezi. Application of chemical fertilizers as evidenced in Kinale would have resulted to the high population of the nematodes. This could also have been attributed to the high diversity of

vegetable grown hence diverse and increased root mass for the nematodes to feed on. Chemical fertilizers have been reported to increase the nematode feeding sites on the roots leading to their increases in number. A study carried by Griffin *et al.,* (1997) states that there is a direct relationship between root growth and nematode population densities. Studies have shown that areas with low temperatures like Kinale area have high soil moisture content. Soil moisture availability has been found to be one of the most significant ecological factors directly affecting nematode abundance and community composition (Steinberger *et al.,* 2001). Variations in temperature affect nematode development, reproduction and the length of life cycle (Freckman *et al*., 1990). Availability of soil water results to an increase in root growth leading to increased nematode population densities (Griffin *et al.,* 1997). Moisture is critical for nematode movement because they need a water film in the interstitial spaces of soil for effective propulsion. The moisture content, (grams water per100 gram dry soil), for different soil types gives little indication of the percentage of pores that contain water or air (moisture characteristic), for example sandy soils have large pore spaces but less total pore space than clay soils (Kung *et al*., 1990). When the soil becomes dry nematode movement is inhibited because there is no water film available. Oxygen becomes the limiting factor for nematodes in clay soils, water saturated soils or soil with high organic content. Temperature is also affected by moisture since solar heat penetrates deeper in wet soil but produces a smaller rise in temperature than in dry soil (Kung *et al*., 1990). Therefore availability of soil moisture which is affected by temperature, explains the difference in nematode population in the different temperate areas. This difference in nematode population could be attributed to the difference in temperature and soil moisture in Kinale and

Kibwezi. Therefore temperature affected nematode population in the different vegetable growing zones.

Soil nematode communities are sensitive to changes in food supply and environment (Freckman *et al*., 1993). This is evident in the five areas since occurrence, population and diversity of nematodes differed in all the five areas. Predator feeders, plant parasitic feeders and bacterial feeder's nematodes were present in all agro-ecological zones, with different populations. Plant parasitic and predator nematodes were the most abundant while the least were the bacterial feeders.

From the study it is evident that in areas with high fungal population, there was low plant parasitic nematode population. In Kabete and Machakos manure was applied to the vegetables to increase yields; the population of plant parasitic nematodes was low while fungi population was high. In Kinale and Athiriver where fertilizers were applied in the soil, the population of plant parasitic nematodes was high while fungi population was low. In Kabete and Machakos where manure was applied, fungi population was high and a low population of plant parasitic nematodes was recorded. These results concur with other reports that suggest that numbers of plant parasitic nematodes decrease after additions of organic amendments (Bohlen and Edwards, 1994). Adesiyan, (1990) reported that organic manure in the soil produces residues in form of decomposed products which may be detrimental directly to root-knot nematode on any susceptible crop. Akhtar and Alam (2003) reported that organic manure in form of livestock waste when incorporated into the soil stimulates the generation of plant parasitic nematode predators hence decreasing the population of plant-parasitic nematodes with consequent growth and yield increase. Beneficial micro-organisms are abundant in soils amended with different

organic matter. Some beneficial fungi and bacteria parasitize nematode eggs and also prey on the nematodes. The predatory nematodes that prey on other nematodes are high in organic amended soil. Thus organic amendment enhances biological suppression of parasitic nematodes in soil (Summer, 2011). Suppression of soil borne pathogen via incorporation or simple mulching of composted amendment are reputedly based on enhanced microbial activities and increased number of antagonists generated by decomposition of the amendments in soil (Wachira *et al*., 2009).Despite them increasing soil fertility, they also increase suppression of plant diseases by adding competitive, predaceous, or antagonistic microbes to soil (Clark *et al*., 1998). Therefore application of organic amendments leads to an increase in nematode destroying fungi. A high nematode destroying fungi population could have been the result of a reduction in plant parasitic nematode population in Kabete and Machakos.

From this study the genera *Arthrobotrys* was reported as the most effective genera in trapping nematodes, followed by *Monacrosporium* and the least in efficacy was the *Stylopage*. Nematode destroying fungi are well known parasites of nematodes, for example fungi in the genera *Arthrobotrys*, *Dactylella*, *Duddingtonia* and *Monacrosporium* (Timper and Davies, 2004).One potential of nematode destroying fungi is their utilization in biological control of plant-parasitic nematodes (Birgit *et al.,* 2011).

Studies on efficacy elsewhere agreed with the observation that the genera *Arthrobotrys* had a higher potential for application as a bio-control. A study by Tsay *et al*., (2006) working on a new method for isolating and selecting agents with high antagonistic ability against plant parasitic nematodes, concurs with this study that *Arthrobotrys* had the best nematode-trapping activity. A bioassay designed by Clark *et al*., (1996) to investigate the predatory response of several isolates

of nematode-trapping fungi against 3 mutants of *Caenorhabditis elegans* found that *Arthrobotrys* responded rapidly compared to *Monacrosporium*. As realized in this study the variation in the ability of different nematode destroying fungi isolates and species to trap parasitic nematodes has also been reported (Gonzalez *et al*.,1998). From this study it's evident that genera *Arthrobotrys* is effective in trapping nematodes and can be developed as an agent for the management of plant parasitic nematodes.

In conclusion, it is evident from this study that temperature and agricultural activities such as application of manure, pesticides and fertilizers affect fungal population. Application of fertilizer leads to low fungi population while organic manure leads to their enhancement in nature. Nematodes population densities were affected by temperature, since in areas with low temperatures, there is high soil moisture leading to high nematode populations and in areas with high temperatures there is low soil moisture leading to low nematode populations, this can explain the high nematode population in Kinale and low population in Kibwezi.

It is hypothesized that nematode destroying fungi might be affecting the population of plant parasitic nematodes in nature. This is because in areas with high population of nematode destroying fungi there was a low population of plant parasitic nematode. It is also evident that the genera *Arthrobotrys* had a higher potential for development in the management of plant parasitic nematodes.

5.1 RECOMMENDATION

From this study it is recommended that

i. Farmers adopt soil fertility strategies that promote soil biodiversity for example the application of organic amendments.

ii. A further study to develop *Arthrobotyrs oligospora* as an addition alterative for the management of plant parasitic nematodes.

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APPENDIX 1: KEY TO NEMATOPHAGOUS FUNGI

(Adapted and simplified from the key of Cooke and Godfreys (1964)

1. Endoparasitic fungi (mycelium in the life cycle predominantly inside nematode host)	2
1. Predatory fungi (mycelium in the life cycle predominantly outside nematode host)	13
2. Assimilative hyphae within host transformed into fertile hyphae, extended out of host slightly, producing adhesive cells or ingestive conidia	3
2. Vegetative hyphae within host transformed into sporangia producing zoospores, or producing conidia, zygospores or azygospores	9
Endoparasitic Fungi with Adhesive Cells or Ingestive Spores	
3. Hyphae aseptate	
3. Hyphae septate	4
4. Hyphae with clamp connection	5
4. Hyphae without clamp connection (*Nematoctonus*)	7
5. Hyphae bearing adhesive cells (knobs) a. *Nematoctonus robustus* Jones b. *N. concurrens* Drechs. c. *N. haptocladus* Drechs. d. *N. campylosporus* Drechs.	
5. Hyphae lacking adhesive cells, but producing adhesive knobs on conidium	6

6. Chlamydospores produced a. *Nematoctonus pachysporus* Drechs. b. *N. tylosporus* Drechs.	
6. Chlamydospores not produced a. *Nematoctonus leiosporus* Drechs. b. *N. leptosporus* Drechs.	
7. Conidia borne on strigmata, no phialides a. *Meria coniospora* Drechs.	
7. Conidia borne on phialide	8
8. Conidia adhesive a. *Hirsutella rhossiliensis*	
8. Conidia filiform a. *Harposporium helicoids* Drechs. b. *H. oxycoracum* Drechs. c. *H. subuliforme Drechs*.	
8. Conidia arcuate a. *H. anguillulae Lohde (Karling)* b. *H. liliputanum* Dixon c. *H. crassum* Shepard	
8. Conidia straight or slightly curved a. *H. baculiforme* Drechs. b. *H. sicyodes* Drechs.	

8. Conidia pea-pod, barbed at one or both ends a. *H. bysmatosporum* Drechs. b. *H. diceraeum* Drechs. (See Species of *Harposporium* spp.in Esser, 1992)	
9. Vegetative hyphae within the host developed into conidiophores that pass out of host, producing conidia. a. *Meristracum asterospermum* Drechs.	
Endoparasites that Produce Encysting Spores	
9. Vegetative hyphae within the host transformed into sporangia producing spores (See Fungi that utilize zoospores to parasitize nematodes by Esser and Schubert, 1983)	10
10 Sporangium (zoosporangium) producing motile zoospores	11
10. Sporagium producing inmotile spores	12
11. Zoospores uniflagellate, no zygospores, no resting spores. a. *Catenaria anguillulae Sorokin* (see Pathogenicity of selected nematodes by *Catenaria anguillulae*, Esser and Ridings, 1973) b. *Rhizophydium sp.*	
11. Zoospores biflagellate, may form zygospres, produce resting spores. a. *Lagenidium caudatum Barron* b. *Myzocythium vermicola* (Zopf) *Fischer* c. *M. glutinosporum* Barron d. *M. humicola* Barron & Percy e. *Nematophthora gynophila* Kerry & Crump	

12. Spores globular or polyhedral with a lobed appendages. a. *Haptoglossa heterospora Drechs.*	
12. Spores clavate. a. *Protascus subuliformis Dangeard*	
Nematode-Trapping Fungi	
13. Morphologically unmodified hyphae	14
13. Morphologically modified hyphae forming traps	17
14. Hyphae aseptate with yellow adhesive substances at contact	15
14. Hyphae septate	16
Adhesive Mycelia	
15. Produce conidia on simple conidiophore. a. *Stylopage hadra* Drechs. b. *S. leiohypha* Drechs. c. *S. grandis* Drechs.	
15. Without conidia, but chlamydospores formed. a. Chlamydospores formed laterally: *Cystopage lateralis* Drechs. b. Chlamydospores formed intercalary: *C. intercalaris* Drechs. c. Chlamydospores on crooked branches or intercalary: *C. cladospora* Drechs.	
16. Conidia bifurcate a. *Triposporina aphanopaga* Drechs.	
16. Conidia furcated, trident-like. a. *Tridentaria implicans* Drechs.	

17. Hyphae aseptate, lateral branches bearing poorly differentiated adhesive knobs. a. *Acaulopage pectospora* Drechs.	
17. Hyphae septate	18
18. Hyphae forming adhesive branches, sometimeS forming simple 2-dimensional network; conidiophore simple, single terminal conidium.	
Adhesive Branches a. *Monacrosporium cionopagum (Drechs) Subram.* b. *Dactylella gephyropaga* Drechs. c. *Dactylella lobata* Duddington	
18. Hyphae forming stalked or sessile adhesive knobs	19
18. Hyphae forming stalked non-constricting rings, sometimes accompanied by stalked adhesive knobs	21
18. Hyphae forming stalked constricting rings	22
18. Hyphae anastomosing to form 2 or 3 dimensional adhesive networks	23
Adhesive Knobs	
19. Conidiophore branched a. *Dactylaria haptospora* Drechs. b. *D. haptotyla* Drechs. c. *D. sclerohypha* Drechs.	
19. Conidiophore simple	20
20. Adhesive knobs always sessile a. *Monacrosporium phymatopagum* (Drechs.) Subram.	

20. Adhesive knobs sessile or short-stalked, often forming short chains of adhesive cells. a. *M. parvicollis* (Drechs.) Cooke & Dickinson	
20. Adhesive knobs always stalked, simple conidiophore. a. *M. ellipsosporum (Grove) Cooke & Dickinson* b. *M. mammilatum* (Dixon) Cooke & Dickinson	
20. Adhesive knobs always stalked, conidiophore branched. a. *Dactylella asthenopaga* Drechs.	
Non-constricting Rings	
21. Adhesive knobs not present. a. *Dactylella leptospora Drechs.*	
21. Adhesive knobs present, conidiophore simple. a. *Monacrosporium lysipagum (Drechs.) Subram.*	
21. Adhesive knobs present, conidiospore branched. a. *Dactylaria candida (Nees) Sacc. Drechs*.	
Constricting Rings	
22. Conidia borne in a terminal cluster on conidiophore. a. *Arthrobotrys anchonia* Drechs. b. *A. dactyloides Drechs*. c. *A. brochopaga* (Drechs.) Schenk, Kendrick, & Pramer d. *A. gracilis (Dudd.) Schenk, Kendrick, & Pramer*	
22. Conidium borne singly on a simple conidiophore. a. *Trichothecium polybrochum* Drechs.	

b. *Monacrosporium acrochaetum* (Drechs.) Cooke c. *M. doedycoides* (Drechs.) Cooke & Dickinson e. *M. stenobrochaum* (Drech.) Subram. f. *M. bembicodes* (Drech.) Subram g. *M. turkmenicum* (Sopronov) Cooke & Dickinson h. *M. coelobrochum* (Drechs) Subram. i. *M. acrochaetum* (Drechs.) Subram.	
3-dimensional Networks	
23. Conidia with one septum a. *Trichothecium cystoporium* Dudd. b. *T. flagrans* Dudd. c. *T. pravicovi* Soprunov d. *T. globosporum* var *globosporum* Soprunov e. *T. globosporum* var *microsporum* Soprunov f. *T. globosporum* var *roseum* Soprunov g. *Arthrobotrys arthrobotryoides* (Berl.) Lindau Drechs. h. *A. conoides* Drechs. i. *A. oligospora Fresenius* j. *A. superba* (Corda) Drechs. k. *A. longispora* Soprunov l. *A. oviformis* Soprunov m. *A. doliformis* Soprunov	

n. *A. kirghizica* Soprunov o. *A. cladodes* var *cladodes* Drechs. p. *A. cladodes* var *macroides* Drechs. q. *A. robusta Dudd.* r. *A. musiformis* Drechs.	
23. Conidia with more than one septum. a. *Dactylaria eudermata Drechs.* b. *D. psychrophila* Drechs. c. *D. megalospora Drechs.* d. *D. reticulata* Drechs. e. *D. thaumasia* Drechs. f. *D. polycephala* Drechs. g. *D. pyriformis* Juniper h. *D. scaphoides* Peach i. *D. gampsospora* Drechs.	

Source: Ching and Wang, (2013).