EFFECT OF LAND USE AND SOIL FERTILITY MANAGEMENT PRACTICES ON NEMATODE DESTROYING FUNGI IN TAITA TAVETA, KENYA

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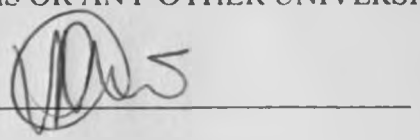

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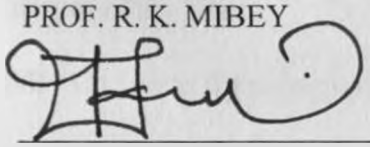

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DEDICATION

This work is dedicated to my family:

My dear wife, Lillian Njeri, my beloved children, Joy Murugi and Earnest Wachira, for their encouragement, patience and support on a course to follow the desires of my heart

May God bless them forever.

"Change has a considerable psychological impact on the human mind. To the fearful it is threatening because it means that things may get worse. To the hopeful it is encouraging because things may get better. To the confident it is inspiring because the challenge exists to make things better".

King Whitney Jr.

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ACRONYMS

ANOVA	Analysis of Varianace
$CACL_2$	Calcium Chloride
CAN	Calcim Ammonium Nitrate
CEC	Cation Exchangeable Capacity
CSM – BGBD	Conservation and Sustainable Management of Below Ground Biodiversity Project
DAAD	German Academic Exchange Service
GEF	Global Environmental Facility
ICIPE	International Center for Insect Physiology and Ecology
J2	Nematode second Juvenile Stage
KARI	Kenya Agricultural Research Institute
LUT	Land Use Type
MAVUNO	A blend of fertilizer containing 11 nutrients in balanced proportions and is suitable for most soil conditions in Kenya
MB	Methyl Bromide
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
RKN	Root Knot Nematodes
TSBF	Tropical Soil Biology and Fertility
TSP	Tripple Super Phosphate
UNEP	United Nations Environmental Programme

ABSTRACT

Plant-parasitic nematodes are recognized as one of the greatest threat to crops throughout the world. In Kenya, they cause 50 – 60 % of yield losses in heavily infected areas. Chemical nematicides have been banned from the shelves due to their negative impact on the soil biodiversity, high cost and the resistance by the plant parasitic nematodes, leading to the search for biological control methods. This study was therefore undertaken to investigate how land use and soil fertility management practices affect the occurrence of nematode destroying fungi aiming at harnessing the potential of these fungi to control the plant parasitic nematodes in the study area which is a very important vegetable catchment in the Coast Province of Kenya.

Soil samples were collected from the various land uses in the study area. Nematode destroying fungi were isolated from the soil using the soil sprinkle technique. Eighty five isolates, distributed in eight genera and fourteen taxa were identified as nematode destroying fungi. It was found that land use significantly (P= 0.05) affected the occurrence of nematode destroying fungi in the region. The highest diversity index was observed in the highly disturbed land uses, horticulture, maize bean, napier, shrub and forest in that descending order. The diversity profiles of nematode destroying fungi shows that maize/bean and the vegetable fields exhibited the highest diversity, followed by napier, shrub and forest respectively. The evenness profiles shows that the forest was the most even land use type followed by maize /bean, shrub, napier and vegetable in that order. The principal component analysis (PCA), showed two main factors with increased agricultural

intensification accounting for 84.12%, as the main factors affecting the occurrence of nematode destroying fungi in the area. Nematode destroying fungi are also affected by the soil chemical properties. Land uses with organic amendments (cow manure) had the highest number of nematode destroying fungi compared to the ones with chemical fertilizers. Land uses with organic amendments (cow manure) would be recommended for their role in increasing the population of nematode destroying fungi which reduced the population of plant parasitic nematodes. They also encouraged more predator and free living nematodes than the chemical fertilizers which were associated with increase of plant parasitic nematodes. The nematode trapping fungi were found to be associated with the plant parasitic nematodes while the endo-parasitic nematode destroying fungi were associated with the fungi feeding nematodes. *Arthrobotrys oligospora* was the most occurring nematode destroying fungi in the area. It occurred in all land uses and would therefore be recommended as the best candidate for development of a biocontrol. From this study, land uses that use organic amendments are recommended to the farmers since they are naturally able to control the population of plant parasitic nematodes. The amendments stimulated the population of native nematode destroying fungi and enhanced the population of predator nematodes which both attack the plant parasitic nematodes. This is because the success of sustainable agriculture will be due to conservation of natural resources and greater dependence on natural ecosystem processes.

Key words: *Diversity, evenness, plant parasitic nematodes, bio-control, organic amendments*

CHAPTER ONE

1.1 Introduction

Nematodes are invertebrate roundworms that inhabit marine, freshwater, and terrestrial environments. The name "nematode" comes from the Greek words: *nema*, which means "thread", and *toid*, which means "form" (Dufour *et al.*, 2003). Nematodes are usually vermiform, long and slender, but some species are swollen. They are multicellular worm like animals which comprise the phylum Nematoda and include parasites of plants, animals, humans, bacteria, fungi, algae, and on other nematodes. The majority of nematodes are microscopic, averaging less than a millimeter in length, but some of the animal parasites are quite large and visible to the naked eye (Platt, 1994; Dufour *et al.*, 2003).

Plant parasitic nematodes are belowground, invisible to the naked eye, yet causing huge economic losses. The most widespread and economically important nematode species is the root-knot nematode, *Meloidogyne* spp. which is a hidden enemy to growers of horticultural crops throughout the tropical world. They cause yield losses of up to 30% and burrow tiny holes in plant roots that let in soil fungi and bacteria causing diseases such as bacterial wilt (Dufour *et al.*, 2003; Coyne *et al.*, 2005). Root knot nematodes are severe pests of agricultural crops, especially in tropical countries causing heavy root destruction and reduced yields. Crop production problems induced by these nematodes therefore generally occur as a result of root dysfunction, reducing rooting volume and foraging and utilization efficiency of

water and nutrients. In Kenya, particularly, they have been reported to cause up to 50% and 60% yield loss in both maize and common beans respectively in heavily infested fields (Kimenju *et al.*, 1998). They have also been recorded as the responsible organisms for the huge crop loss in tomato for smallholder growers (Oruko and Ndungu, 2001). Plants with the root system damaged by nematodes often show retarded growth, chlorosis and reduced yield due to inability of the root to deliver water and nutrients and thus may be confused with similar symptoms resulting from poor soil conditions of nutrient deficiency.

Due to the losses caused by these nematodes on agricultural crops, their control is a major concern in crop production (Garcia *et al.*, 2004). For decades now, their control has mainly depended on chemical nematicides (Akhtar and Malik, 2000). Although chemical nematicides are efficient and fast-acting, they are currently being reappraised with respect to the environmental hazards associated with them. Some of these nematicides have been detected in the underground water (Harrison, 1995). The populations of the nematodes have also developed resistance to these nematicides with time. In addition they are relatively unaffordable to many small-scale farmers. This has lead to banning of most efficient nematicides. There is therefore a persistent pressure on farmers to adopt strategies that are affordable and do not pollute the environment. This pressure has increased the urgency in the search for alternative sustainable methods to control plant parasitic nematodes. As a result, the stature of biological control of plant parasitic nematodes as a viable practice in modern agriculture and horticulture has increased dramatically (Pinkerton *et al.*, 2000; Mashela *et al.*, 2008).

On biological control of plant parasitic nematodes, nematode antagonists have been used. Examples are bacteria and fungi. One group of fungi, nematophagous fungi, has attracted a lot of interest for their interactions with nematodes (Elshafie *et al.*, 2006). Nematode destroying fungi are cosmopolitan natural enemies of plant parasitic nematodes (Nordbring-Hertz *et al.* 2002). So far more than 160 fungal species that live on nematodes partially or entirely have been reported (Elshafie *et.al.* 2006). Some of these fungi use adhesive conidia, branches, knobs and mycelia to parasitize nematodes. These devices are used to capture and destroy nematodes by means of an adhesive layer covering part or all of the device surfaces (Yang *et al.*, 2007). Other fungi immobilize or kill nematodes by releasing toxins (Luo *et al.*, 2004). Consequently, this group of fungi has drawn much attention because of their potential as biological control agents of nematodes that are parasitic on plants and animals (Araújo *et al.*, 1999; Jansson & Persson 2000; Sanyal 2000; Masoomeh *et al.*, 2004; Yan *et al.*, 2005). This group targets the plant parasitic nematodes before they attack the plant roots that is, the second juvenile stage (J2). This is the most vulnerable period of nematodes when they are actively searching for host roots and when surviving unfavourable growing seasons and also because of their small size (Jasson *et al.*, 2000).

Taita Taveta, South West of Kenya, is a major vegetable growing area for the coast of Kenya (Pellikka *et al.*, 2004). Plant parasitic nematodes contribute greatly to loss of vegetable crops in Taita Taveta and the cost of production has increased due to the cost of chemical nematicides (Republic of Kenya Development Strategy 2002-2006). The aim of this study was therefore to investigate the occurrence and diversity of

nematode destroying fungi as affected by land use and soil fertility management in Taita Taveta with the aim of harnessing them for biological control of plant parasitic nematodes.

1.2 Justification

Available reports show a huge annual crop loss due to nematode damage worldwide (Sasser and Freckman, 1987; Stirling, 1992; Maqbool *et al.*, 1988; Saxena, *et al.*, 1988; Eissa, 1988). In Kenya, plant parasitic nematodes are responsible for up to 50% yield loss in maize. They also cause up to 60% yield loss on common bean (Kimenju et al, 1998, 1999). Oruko and Ndungu, (2001), have reported that, though tomatoes are economically important for smallholder growers in Kenya, crop loss from root knot nematodes is a problem.

Only few studies in agricultural fields have been conducted to show the effect of organic amendments on nematode destroying fungi with no reference to plant parasitic nematodes. Therefore, the data available on nematode destroying fungi is minimal and not replicated in other areas (Dackman *et al.*, 1997; Jaffee *et al.*, 1998) and completely not available from Kenya. Unlike the other studies mentioned, this study will focus on the nematode destroying fungi and plant parasitic nematodes. The study will create an understanding of the effect of different fertility management, to the interaction of the nematodes and the fungi. It will also form the basis of biological control of plant parasitic nematodes on vegetable farms in Kenya.

It will provide information that will be very useful for the initiation of biological control of nematodes in Kenyan agroecosystems.

Related studies in the area have reported increased nematode populations in the vegetable gardens and high cost of chemical control of nematodes. Most of the non-chemical controls used by the farmers are crushing leaves of some plants and pouring solution around the root area, application of ash at the planting season and application of animal manure (Mutsotso *et al*, 2005). The community is also aware of biological control method on vegetables for example the use of the destructive diamond – black moth (*Plutella xylostella*) with a parasitic wasp (*Diadegma semiclausum*) (ICIPE, 2005).

The studies will create an understanding of the occurrence and diversity of nematode destroying fungi and the farming practices that will boost their population in the soil for the biological control of plant parasitic nematodes.

1.3 Hypothesis

1. Land use has no effect on abundance and diversity of nematode destroying fungi.
2. Soil fertility management practices have no effect on populations of nematode destroying fungi
3. Population dynamics of nematode destroying fungi in the soil has no effect on the population of the phytoparasitic nematodes

1.3.1 Main objective

To enhance the establishment of biological control of plant parasitic nematodes in agricultural ecosystems.

1.3.2 Specific objectives

1. To identify and characterize the commonly occurring species of nematode destroying fungi under different land use systems in the area
2. To determine the occurrence of nematode destroying fungi under existing different soil fertility management practices.
3. To evaluate the effect of recommended soil management practices on the population dynamics of nematode destroying fungi.
4. Formulate potential nematode biocontrol strategies by enhancing the populations of the nematode destroying fungi.

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CHAPTER TWO

2.0 Literature review

2.1 Soil biodiversity

Biodiversity is usually defined as the variety and variability of living organisms and the ecosystems in which they occur. The soil represents a favourable habitat for microorganisms and is inhabited by a wide range of them (Davet and Francis, 2000). Although not generally visible to the naked eye, soil is one of the most diverse habitats on earth and contains one of the most diverse assemblages of living organisms (Giller *et al.*, 1997). A typical healthy soil contains several species of vertebrate animals, several species of earthworms, 20-30 species of mites, 50-100 species of insects, tens of species of nematodes, hundreds of species of fungi and perhaps thousands of species of bacteria and actinomycetes. In one gram of productive soil there is a complex web that can exceed over 100 million microorganisms that may represent over 1000 species (Nannipieri *et al.*, 1990). No-where in nature are species so densely packed as in soil communities (Hagvar, 1998). Hawksworth and Mound (1991) reported some of the available estimates on the number of species presently described of selected soil biota that have been better studied. However, it is a fact that these estimates are still preliminary and much lower than the estimated total number of species within each group. For example, the described number of soil dwelling fungal species ranges from 18-35,000, while the projected number may be greater than 100,000 (Hawksworth 1991). Other organisms that are thought to be much more species-rich are the nematodes and mites, with only 3 and 5%, respectively, of the total species presently described (Hawksworth and

Mound, 1991). The estimates for bacteria and archea species are particularly problematic because of the differences in opinion as to what criteria should be used to define a species, and the present unculturability of many of these organisms (Hawksworth and Kalin-Arroyo, 1995). Soil biodiversity therefore reflects the mixture of living organisms in the soil. All these living things interact with one another and also with plants forming a web of biological activity.

These soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from each of them. In particular, they play an active role in soil fertility as a result of their involvement in the cycle of nutrients like carbon and nitrogen, which are required for plant growth. For example, soil microorganisms are responsible for the decomposition of the organic matter entering the soil and therefore in the recycling of nutrients in soil (Okoth, 2004). Other beneficial effects of the soil microorganisms include organic matter decomposition and soil aggregation, breakdown of toxic compounds both metabolic by-products of organisms and agrochemicals, inorganic transformations that make available nitrates, sulphates, and phosphates as well as essential elements such as iron and manganese and nitrogen fixation into forms usable by higher plants (Anderson, 1994). In summary, soil microorganisms improve the entry and storage of water, resistance to erosion, plant nutrition and break down of organic matter. Other microorganisms will provide checks and balances to the food web through population control, mobility and survival from season to season. In this regard a healthy soil has been defined as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and

promote plant and animal health (Doran *et al.*, 1996). Soil health has also been defined as "the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal, and human health" (Pankhurst *et al.*, 1997).This particular definition shows the importance of the soil biota to soil functioning.

Despite their usefulness, many groups of soil inhabiting organisms are also detrimental to plant growth. For example some moles and rodents may seriously damage crops, snails and slugs are serious pests as well as some ants, aphids and nematodes. Micro-flora, bacteria and actinomycetes cause some plant diseases while fungi account for most soil -borne crop diseases such as wilts, root rot, clubrot and blight. Soil organisms may compete for nitrogen with higher plants and under conditions of poor drainage, soil organisms compete for limited oxygen. Agricultural practices as well have significant positive and negative impacts. For example, high external-input agriculture can overcome specific soil constraints through the use of inorganic fertilizers, pesticides, and other amendments, in order to meet plant requirements (Sanchez, 1994 and 1997). Although these practices have led to considerable increases in overall food production worldwide, they also tend to decrease or disregard the potential benefits of soil biological activities in maintaining soil fertility and enhancing crop production. Furthermore, the misuse or overuse of these practices has led to soil and environmental degradation (depletion or loss of soil fertility and its physical and biological components, contamination of surface and ground water) and declines in productivity in certain areas of the world. In

specific cases, the use of pesticides and herbicides kill other beneficial predator organisms which serve to check and balance various pest species. Fungicides and fumigants have greater impact on soil organisms (Sanchez, 1997).

2.2 Economic importance of nematodes

Nematodes are examples of soil microorganisms which are common in all soils the world over and play an important role in essential soil processes (Dropkin, 1980). They represent a central position in the soil food web and correlate with ecological processes such as nitrogen cycling and plant growth. Their direct contribution to nitrogen mineralization and distribution of plants has been demonstrated in controlled experiments. Under field conditions, bacterivorous and predatory nematodes are estimated to contribute about 8% directly and 19% indirectly to nitrogen mineralization in conventional and integrated farming systems, respectively. They contribute to nitrogen mineralization indirectly by grazing on decomposer microbes, excreting ammonium and immobilizing nitrogen in live biomass (Dufour *et al.*, 2003). They are good bioindicators because of their permeable cuticle, which allows them to respond to pollutants and correspond with the restorative capacity of soil ecosystems. Other nematodes have resistant stages that allow them to survive unfavorable environmental conditions. They also have heat chock proteins that are highly conserved. Due to their position in the trophic level, nematodes have become excellent bioindicators (Bongers, 1990). Light, sandy soils generally harbor larger population of plant parasitic nematodes than clay soils. This is attributed to the more efficient aeration of sandy soils, the presence of fewer organisms that compete with

and prey on nematodes and the ease with which nematodes can move through the root zone (Dropkin 1980; Dufour *et al.*, 2003). Nematodes can be grouped according to what they eat. The different groups are fungal feeders, bacterial feeders, predators, animal parasites, algal feeders, omnivores, and plant parasites. Of our interest, in this work, are the plant parasitic nematodes

Parasitic nematodes are some of the examples of the harmful soil microorganisms. Plant-parasitic nematodes, the majority of which are root feeders, completing their lifecycle in the root zone, are found in association with most plants (Sasser, 1990; Kimenju *et. al.*, 2004). They enter the roots as juveniles, select feeding site of the three to eight cells, and swell up in their chosen spot as they progress towards adulthood. They introduce hormone like substances into the plant cells, causing the plant to produce galls or root knots. It is at this stage that the male regain their slender profile and leave after adulthood but the female remain to produce the eggs to the soil and after the juveniles are hutched, the cycle repeats (Sasser 1990). Some are endoparasites, living and feeding within the tissue of roots, tubers, buds and seeds. Low numbers may appear inconsequential, but in high numbers, parasitic nematodes can damage crops. Others are ecto-parasites feeding externally through plant walls. A single endo-parasitic nematode can kill a plant or reduce its productivity, while several hundred ecto-parasitic species might feed on a plant without seriously affecting production. A few species are highly host-specific, such as *Heterodera glycines* on soybeans and *Globodera rostochiensis* on potatoes. But in general, nematodes have a wide host range. Plant-parasitic nematodes, like root-knot nematodes and cyst nematodes are global pests in agriculture and horticulture,

causing severe yield losses. They must be addressed in crop production and integrated pest management (IPM) systems if agriculture is to meet the world demands for increasing food and fiber production since they are known to cause serious yield loss.

2.3 Control of Nematodes

Nematode control is generally preventive because once a plant is parasitised it is impossible to kill the nematode without destroying the host plant. Several methods have been tried on the control of plant parasitic nematodes. They are chemical control, cultural practices, which include crop rotation, addition of organic amendments, use of trap crops and antagonistic crops, use host-resistant varieties and biological control (Mweke *et al.*, 2008; Kimenju *et al.*, 2008)

2.4 Evolution of nematode control

The control of nematodes dates back to 1869 when the use of carbon bisulphide for soil fumigation was first suggested (Fleming and Baker, 1935). The most popular and successful application method was injection of carbon bisulphide into holes which were 50 centimeters apart. From these application points, fumes diffused through the soil. Application by spraying the undiluted liquid into the soil while plowing was later tried but found to be often ineffective (Newhall, 1955). Antagonists of nematodes, especially nematode-trapping fungi, were considered interesting wonders of nature with little relevance to agriculture. During this period, nematicides were inexpensive and effective, and little research effort was devoted to studying the effect of antagonists on nematode populations in the soil. The second

phase began in 1977, which was characterized by the restriction of several important nematicides.

The research in the second phase, which continues into the present, is typified by attempts to replace nematicides with antagonists. Thus far, few of these efforts have resulted in effective biological control and the research has done little to increase our understanding of how biological control may or may not be achieved. Our greatest need is for sound, in-depth biological information on how organisms, populations, and communities operate in the soil. The nematologists today are faced with challenges trying to control the effect of nematodes on crop plants. These challenges include the development of nematode resistant varieties of crop plants but these varieties are unavailable to farmers and are only resistant to a particular group of nematodes making them ineffective. Chemicals are expensive and uneconomical to small-scale farmers. There is also continuous discovery of new species that seeks for new approaches (Baker *et al*, 1974). To add on this, the chemical materials used to control these pathogens are either banned or are subject to rigid control in some countries for example many nematicides, like methyl bromide (an ozone depleter) has been banned because of health and environmental concerns (Stirling 1992). Methyl bromide (MB), a pesticide that is used for soil fumigation, quarantine of high value export crops, and for grains and other commodities in storage, releases emissions that contribute to the depletion of the ozone layer (Crow *et al.*, 1995). In many cases, it is used for crops that are not indigenous to a particular climate system. In Kenya, methyl bromide is used for soil fumigation and treatment for the production of strawberries and cut flower for export. What is alarming, however, is

that Kenya uses 5% of its foreign exchange earnings to import these harmful substances (Stats. Abstr, 2004).

2.5 Biological control of nematodes

Biological control offers a good alternative for nematodes management. This includes the manipulation of certain native microorganisms present in the soil that are antagonistic to soil pathogens and can prevent the infection of crop plants (Siddiqui, 1996). Collectively, these soil microorganisms have been termed bio-pesticides and represent an emerging and important bio-control. Examples of these organisms are the fungi. Since most plant parasitic nematodes remain mobile throughout their life cycle, microbial control agent must produce traps or adhesive spores to infect them which is a major characteristic of the fungi (Dropkin, 1980). Those with sedentary stages like cyst and root – knot nematode may be parasitized from vegetative hyphae with the production of appresoria. Though nematologists have identified natural enemies with a range of modes of action, it has been streesed that several organisms that have effective natural enemies of nematodes in the field may have limited potential as biological control agents for application by growers. An example is *Nematophthora gynophila*, which is a causal agent of cereal cyst nematode decline in many soils. It is known to have limited host range; a complex requirement for in vitrol culture and requires specific soil moisture level.

2.6 Fungi as biological control agents

Fungi have the highest biomass in most ecosystems. It plays an important role in decomposition and nutrient cycling (Rayner and Boddy, 1988). Fungi possess a

number of characteristics that make them potentially ideal biocontrol agents. First, many saprophytic species antagonize, representatives of all the pest organisms, including plant pathogenic fungi, weeds and insects; secondly fungi can be readily grown in culture so that large quantities can be economically produced for release, mainly as spores or mycelial fragments, into the environment. These inoculants then germinate or grow to produce active mycelium which can parasitise or otherwise inhibit the pest without damaging the non-target organisms. Fungi also survive for relatively long periods as resting bodies, and can then germinate to grow and control the target population thereby making continual reinoculation with the biocontrol agent unnecessary. On biological control of nematodes, Wakelin *et al.*, 2008, have reported five mechanisms used by fungi to suppress nematode population. Nematophagous fungi that feed on nematodes directly, fungi that kill nematodes by mycotoxin, fungi that destroy the feeding sites of sedentary nematodes in roots, fungi that are nonpathogenic to plants, but compete with nematodes in roots, mycorrhizal fungi that improve the growth of nematode infected plants. Many of these fungi are used as potential nematode biocontrol agents.

2.7 Nematode destroying fungi

Nematode destroying fungi are microfungi that are natural enemies of nematodes (Birgit *et al.*, 2002). They feed on nematodes by capturing, killing and digesting them (Rodrigues *et al.*, 2001). Special mycelial structures or spores are used to trap vermiform nematodes or hyphal tips to attack nematode eggs and cysts before penetration of the nematode cuticle, invasion and digestion. They all share the ability to attack living nematodes and use them as nutrients. Three main groups of fungi are

involved, the nematode trapping and the endoparasitic fungi that attack vermiform living nematodes by using specialized structures. and the egg-and cyst-parasitic fungi that attack these stages with their hyphal tips (Birgit *et al.*, 2002; Masoomeh *et al.*, 2004).

Nematophagous fungi have been found in all regions of the world, from the tropics to Antarctica (Gray 1985; Birgit *et al.*, 2002). They have been reported from agricultural, garden and forest soils, and are especially abundant in soils rich in organic material. In agricultural soils in temperate regions the nematode-trapping fungi follow a seasonal variation, with highest densities and number of species in late summer and autumn, possibly due to the higher soil temperature and increased input of organic debris. These fungi are most frequent in the upper 20 cm of the soil and appear to be almost absent below 40 cm (Barron 1977; Gray 1985). The saprophytic (forms adhesive nets) are found in soil with low organic matter and low moisture. They compete with other soil organisms by feeding on the nematode population. Those that form the rings are common in soil with high organic matter and moisture. Endoparasite fungi that produce conidia are strongly influenced by organic matter. Those that form ingestive spore are dependent on nematode density. Parasitic nematophagous fungi show low saprophytic ability, but forms traps spontaneously. This group form constricting rings, adhesive branches and are more effective nematode trappers than the saprophytic types (Jasson, 1982).

2.8 Mode of action to control nematodes

The question of how nematode destroying fungi recognize their prey is complex. No simple host specification has been found in any of the nematode trapping species. The most popular hypothesis is that, nematodes are attracted to the mycelia of the fungi in which they may induce trap formation and they are attracted even more to fully developed traps and spores. After the attraction, adhesion of the nematode to the fungi occurs leading to nematode capture. After the capture, an infective bulb is produced from the internal wall of the trap and the nematode cuticle is broken and penetrated. Assimilative hyphae are produced along the nematode body and finally the content is absorbed by the fungus (Gomes *et al.*, 2001; Birgit *et al.*, 2002).

The reason for the continuing interest in these fungi is, in part, their potential as biocontrol agents against plant and animal parasitic nematodes. Another reason for the continued fascination in nematophagous fungi is the remarkable morphological adaptations and the dramatic capturing of nematodes. In addition, both fungi and nematodes can be grown in the laboratory easily, providing an excellent model system for interaction studies. They have also attracted attention in controlling nematodes in livestock after prolonged drug residuals in animal tissues and pasture due to intensive use of anthelminthin treatment in livestock (Duddington, 1994, Masoomeh *et al.*, 2004, Rodrigues *et al.*, 2001).

2.9 Research Site

2.9.1 Location of the study

The study was conducted in Taita Taveta district for three years, from 2006 to 2008. The district covers an area of 16,965 km^2 and is divided into six (6) administrative divisions namely Wundanyi, Mwatate, Mwambirwa, Voi, Tausa and Taveta. The population of the district 30 years ago was approximately 45,000 persons but this has shot up to well over 250,000 persons with population densities ranging from 3 persons per km^2 to more than 800 persons per km^2. This is due to the varied rainfall and terrain with the lower zones receiving an average 440 mm of rain per annum and the highland areas receiving up to 1900 mm of rain. The total population of the district is currently estimated to be 266,107 people. The district is mainly dominated by mixed farming livelihoods, a little bit of irrigated cropping in Taveta division and Wildlife conservation which covers 62 % of the total district. Of the remaining 38% of the district, two individuals own 20 % of the land and are engaged mainly in sisal plantation and intensive sheep farming. This implies that the total population is concentrated in 18 % of the total district area, majority of which is marginal agriculture to semi-arid. The problem of poor land tenure system and squatters especially in sisal plantations is a big issue and has a negative implication on food production and household food security in the district. Wundanyi division although described as a medium potential highland, 40% of the division bordering with Tausa realized total crop failure in 2005. Farm holdings in Wundanyi are small and are mainly used for smallholder dairy and some horticulture. The major food crops grown in Taita Taveta districts include; maize, beans, green grams and cowpeas.

Maize accounts for 60 % of household food consumption while pulses account for 30 %. The remaining 10 % constitutes root crops such as cassava and sweet potatoes. Although pigeon peas and cassava are important, farmers are reluctant to grow them due to destruction by elephants. The main cash crops include: cotton (Voi, Taveta, Tausa and Mwatate Divisions), bananas (Taveta and Wundanyi), sunflower (Taveta) and horticultural products from irrigated areas which include: onions, kales and tomatoes. Crops grown under irrigation schemes are maize, beans, tomatoes, onions, vegetables, babycorn, Asian vegetables, bananas and passion fruits. The district was split into two districts (2007) of Taita and Taveta and have a combined area of 16,959 square km. The population of Taita and Taveta Districts is 210,370 and 57,623, respectively.

The main area of the study was along the valley bottoms of Werugha and the Ngangao forest, Wundanyi divison. Wundanyi is a town lying in the Taita Hills of southern Kenya west of Voi and near Ngerenyi. It is also the headquarters of Taita district. The town has a population of 4000 (1999 census). This is the centre of an agricultural area, with the surrounding slopes being terraced. Being the highest point in Coast province (at an altitude of about 2,000m above sea level), Wundanyi, one of the six divisions of Taita district has a low temperature, ideal for the cultivation of horticultural crops. Farmers in this town concentrate on growing *Brassica* cabbages and, kales (sukuma wiki). These two vegetables find ready markets in Coast provinece of Kenya. Werugha (1652 m) is located between Yale (2104 m) and Ngangao (1952 m) mountains of the Taita Hills. These valleys are rich in vegetable cultivations (Pellikka *et al.*, 2004) (Fig 1).

Adopted from Pellikka *et al.*, 2004

The arrow shows increased land intensification; from the forest to the valley bottoms.

Fig 1. A map of the study area

2.9.2 Biodiversity importance of the area

The Taita Hills forests are located in Southeastern part of Kenya. 25kms west of Voi town in the Taita-Taveta District, at approximately 03 degrees -20'S, 38 degrees -15'S. In total they are 48 forests, of which 28 are gazetted under government

protection management. They are the only part of the Eastern ARC Mountain Forests-which run from south eastern Kenya to the Usambara region of Tanzania. The hills rise abruptly from the plains to a series of ridges, culminating in Vuria peak at 2228m above sea level (Beentje, 1988). These hills were created around 290 – 180 million years ago due to faulting. Due to their age, isolated location and comparatively stable conditions, they contain unique plant and animal species with very high levels of endemism (Mwangombe and Mwanyumba, 1999). In previous studies, 74 endemic vertebrates, 265 endemic invertebrates and 66 endemic trees have been reported. 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 forests have been acknowledged as one of the 25 biodiversity (eighth globally) 'hotspots' in the world (Rogo and Oguge, 2000) while International Conservation has identified the area as top-ten biodiversity hotspot in the world (Pellika and Clark, 2004).

2.9.3 Soils and rainfall of the study area

The soils are composed of a high-humic A-horizon overlaying a pinkish acid sandy loam. These sandy loams are generally deep, with high infiltration rates, a low pH (3-4), a low water holding capacity, and they are low in nutrients due to excessive leaching. The soils are also characterized by the presence of high aluminum levels, low calcium levels, and unavailable potassium, causing a low cation exchange capacity (Muya *et al.*, 2006, Spoerry 2006). Two rain seasons are experienced; (i) Long rain season- March to May, (ii) Short rains - November to December. In the Taita hills each month of the year receives some form of precipitation allowing

continuous cropping. Highlands receive more rain than lowland areas. Lowlands are mainly arid and semi arid lands. The district's mean annual rainfall is about 55mm. However, in the highland areas, it can be as high as 1500mm and 250mm for the lowlands. The district-cropping calendar is short rains dependent especially in the lowlands of Voi, Mwatate, Tausa and parts of Taveta. This general rainfall trend has been declining in the last four years and worsened in the last two years.

2.9.4 Farming activities in the study area

Most of the land under agriculture is on the hill slopes and the valley bottoms, which falls in the eco-climatic zones 2 and 3. The crops planted are maize, beans, sweet potatoes, cassava, arrowroots, bananas, fruit trees and horticulture crops like tomato, kale, cabbage, lettuce and are limited to valley bottoms. This forms the vegetable catchment area for southern part of Kenya and supplies vegetables to the Kenyan coast. These crops have been planted since 1967 to the present and farm inputs have remained the same (Ortiz1 *et al.*, 2007). The intercropping is haphazard even though farmers believe it is a response to land scarcity and not related to belowground biodiversity functions. Agriculture contributes 95% of household income in Taita Taveta (Mutsotso *et al.*, 2005). In the District Development Strategies 2002-2006 for Taita, crop production was identified as an area of focus while high input costs, soil degradation, inadequate technical know how and low soil fertility were identified as contributing to low production. Majority of the farmers are small-scale farmers with very little or no inputs in the agricultural activities. A 2005 social survey indicated that farmers used chemicals and non-chemical methods to control nematodes at the valley bottoms which had resulted in reduction of profit from the sale of vegetables

(Mutsotso *et al.*, 2005). There are high populations of parasitic nematodes in the vegetable gardens compared to where maize/beans and cassava are grown (Kimenju *et al.*, 2005).

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CHAPTER THREE

3.0 Materials and Methods

3.1 Description of the study Area

The study was conducted in Taita district, Werugha location of Wundanyi division along a land use intensity gradient spanning from the valley bottoms of Werugha and the Ngangao forest, of the Taita hills. A preliminary survey of nematode destroying fungi was first conducted in the area to establish the factors affecting the occurrence of nematode destroying fungi. During the study, the area was stratified into five strata representing the five main land use types in the area. The five land use types selected were natural forest, shrub, vegetable, napier grass and maize/bean intercrop. The natural forest consisted of a broad diversity of indigenous trees. Natural shrub consisted of mainly *Croton megalocarpus (Euphorbiaceae), Lantana camara (Verbenaceae), Sporoblus pyramidalis (Gramineae)* and *Ficus thoningii (Moraceae).* The vegetable gardens were mainly dominated by cabbage, tomato, kale and cucumber, grown separately in randomly selected rotation systems. Maize intercropped with beans was selected because it was the main food production system in the study area. Napier grass fields (*Pennisetum purpureum*) are also widely distributed in the area and serve to supply fodder to the dairy animals under restricted grazing systems.

3.2 Soil Sampling Method.

To investigate the effect of land use on the occurrence and diversity of nematode destroying fungi, eight soil samples were taken from each of the five land uses. In total, 40 main sampling points were randomly identified from which five sub samples were taken. One sub - sample was taken from the center and four sub-sampies at a distance of 3 meters from the centre (Fig. 2). An auger was used to take soil cores from the 0 -10 and 10-20 cm soil depth.

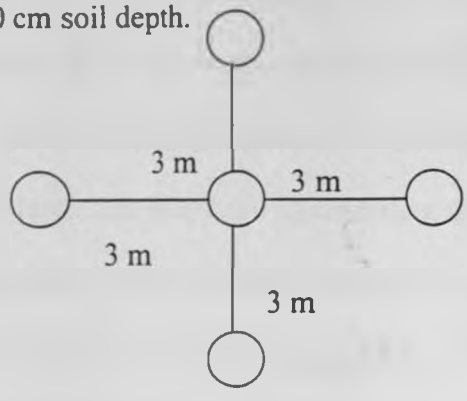

Fig. 2. Schematic representation of the five soil sampling points which comprised one main sampling point.

The five sub-samples were mixed homogeneously to constitute a composite sample from which 500g soil was taken, placed in a plastic bag and then placed in a cool box. The auger was sterilized by dipping it in ethanol between sampling points to avoid cross contamination.

3.3 Effect of farmer soil fertility practices on the occurrence of nematode destroying fungi and nematode community

To investigate the effect of existing farmers soil fertility management practices on occurrence and diversity of nematode destroying fungi, 40 farms under different soil fertility management and under horticulture were randomly selected in the area. The farms were sampled for nematode destroying fungi and the nematode community by picking nine sub samples in each farm on 0 -10 and 10-20 cm soil depth and making a composite sample. From the composite sample, 500 grams of soil was sampled and taken to the laboratory for isolation of both nematode destroying fungi and nematodes. Soil sampling was done for three during the short rain (between October and December), long rain (between March and May) and again during the next short rain (October and December) consecutively. The nematode community was grouped in terms of their mode of feeding, the plant parasitic, the fungal feeders, the bacterial feeders and the predators (Mai and Mullin. 1996) while the nematode destroying fungi were grouped as either trapping or endoparasitic nematode destroying fungi(cooke and Godfrey, 1964).

3.4 Experimental effect of soil fertility management practices on the nematode destroying fungi

Ten experimental farms were randomly selected in the area to investigate the effect of soil fertility management practices on occurrence and diversity of nematode destroying fungi. From these farms, the soils were characterized for nitrogen, phosphorous, carbon and potassium before the treatments were laid. Total organic carbon was estimated through calorimetric method where all organic carbon in the

soil sample was oxidized by acidifying dichromate at 150^0c for 30 minutes to ensure complete oxidation. Barium chloride was added to the cool digests. After mixing thoroughly, digests were allowed to stand overnight. The carbon concentration was read on the spectrophotometer at 600nm (Anderson and Ingram 1993). To get the total nitrogen, Kjeldahl method was used (Kamnierer *et al.*, 1967). Soil samples were digested with concentrated sulphuric acid containing potassium sulphate, selenium and copper sulphate hydrated at approximately 350^0c. Total nitrogen was then determined calorimetrically on a flow analyzer (Hinga *etal.*, 1980; Keeny and Nelson, 1982). Soil pH – Water was determined in 1:1 (w/v) soil – water suspension with pH meter. To get the amount of extractable phosporus in the soil, Olsen method was used, and in the extract determined spectrophotometrically (Watanabe and Olsen, 1965; Hinga *et al.*, 1980).

The ten farms were then divided into 4 plots measuring 3 x 3 meters with 1 meter path around each plot. In one of the plots, 9 kg of cow manure was broadcasted all over the plot (10 tons per ha), on the next, 0.8 kg of triple super phosphate (TSP) and 0.5 kg of calcium ammonium nitrate (CAN) were broadcasted together (KARI Recommenced farmer practices). The third plot was the control while the fourth was broadcasted with 0.9 Kg Mavuno (blend of fertilizers containing 11 nutrients in balanced proportions and is suitable for most crops and soil conditions in Kenya).

After broadcasting all the inputs, they were mixed with the soil with a rake. The plots were then planted with maize at a spacing of 90 x 30 cm, two seeds per hole and beans at spacing of 30 cm in alternate rows. Soils were collected at the harvest period from each plot for estimation of nematode destroying fungi for three seasons.

(Jaffee *et al.*, 1964. Watanabe and Olsen. 1965; Hinga *et al.*, 1980). The treatments were then applied and characterization was then done at the end of the experiment.

3.6 Isolation and characterization of nematode destroying fungi and nematode community

After collecting soil samples from each activity, they were transported to the laboratory where they were kept in a cold room at about 10^0C before isolation of the nematode destroying fungi.

Isolation of the fungi was done using the soil sprinkle technique as described by Jaffee *et al.*, (1996). Tap water agar was prepared by dissolving 20 grams of agar in one liter of tap water. The medium was autoclaved and cooled to 45^0 C before amending it with 0.1 g/L of streptomycin sulfate to suppress bacterial growth. Approximately one gram of soil from each sampling point was sprinkled onto the surface of water agar in Petri dishes. A pure culture of plant parasitic nematodes (*Meloidogyne spp*) was added into the Petri dish as baits. The plates were incubated at room temperature and observed daily under a microscope at low (40 x) magnification, from the third week up to the 6th week. The examination was focused on trapped nematodes, trapping organs and conidia of the nematode destroying fungi that grew from the soil.

After the sixth week, all the fungal colonies that had emerged were sub-cultured on potato dextrose agar to obtain pure cultures. To verify the status of the fungal isolates as predators of nematodes, observations were made on a daily basis, after the third day, for trapped nematodes, trapping organs and conidia. Photographs of trapped nematodes, trapping organs and conidia were taken for use in identification of the

3.5 Greenhouse and field experiment on stimulation of nematode destroying fungi for the control of plant parasitic nematodes

Greenhouse and field experiments were carried out in the period between August 2007 and February 2009 on stimulation of nematode destroying fungi at the University of Nairobi, Kenya. In the green house, the amendments namely chicken manure, cow manure and their combinations were dried at 70^0C until a constant weight was achieved. The amendments were then applied at the rate 5% w/w (Kimenju *et al.*, 2004) into soil that was naturally infested with nematodes and nematode-trapping fungi. The pots were irrigated and two-week old tomato seedlings (cv Moneymaker) were transplanted into them. Un-amended soil was used as a control. Treatments were arranged in a completely randomized design with five replications. Soil samples were taken at the maturity of the tomatodes for estimation of both nematode edstryoing fungi and nematodes. Growth of tomato plants was monitored at the 4^{th} and 7^{th} weeks by assessing the plant height, leaf width- apical leaf of 3^{rd} branch, internodal length (between 3^{rd} and 4^{th} branch) and the type of flower/flowering pattern. Shoot and root dry weights were taken at the end of the experiment after drying the samples at 70 ^{0}C to constant mass.

This experiment was repeated in the field conditions in plots of 3 x 3 meters with addition of inorganic fertilizer as a treatment. The five treatments (chicken manure, cow manure, their combination, inorganic fertilizer and the control) were replicated five times in a completely randomized design. The soil was characterized in terms of nematode destroying fungi, nematodes and the chemical characteristics of the soil

nematode destroying fungi. Identifation was doen according to the key by Cook and Godfrey, 1964. Nematodes were extracted from 200 cm3 soil using the modified Baermann technique as described by Hooper *et al.*, (2005). The nematodes were identified to genus level using the descriptions described by Mai and Mullin (1996), and then counted.

3.7 Data analysis

All the data obtained from the study were analyzed by calculating the frequency of occurrence, evenness, Renyi profiles and the Shannon diversity index (Kindt and Coe, 2005). The R Statistical Computing package, Version 2.1.1 (2005-06-20), ISBN 3-900051-07-0 and XLSTAT version 2008.3.01were used.

Where the overall F test was significant, means were compared using the Tukey Honest Significance test (HSD) at $P \leq 0.05$. Multivariate analysis using ADE4 software was done on the temporal association of nematode-trapping fungi and nematodes and also the nematode destroying fungi and land use and soil characteristics (Thioulouse *et al.*, 1997).

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CHAPTER FOUR

4.0 EFFECT OF LAND USE ON OCCURRENCE AND DIVERSITY OF NEMATODE DESTROYING FUNGI IN TAITA TAVETA, KENYA

4.1 Abstract

Due to the increased concerns about the effect of agro-chemicals on soil health and soil biodiversity, use of biological methods has become most acceptable alternative methods for farmers to control soil pathogens during crop production. In Taita Taveta, the study area, horticulture is the main economic activity. It is characterized by heavy use of agrochemicals and nematicides with parasitic nematodes cited as causing the biggest draw back to the economic returns. This study was therefore undertaken with the objective of determining the occurrence of nematode destroying fungi in soil under different land use systems, with the ultimate goal of harnessing their potential in the control of plant parasitic nematodes. Given that the intensity of land cultivation is continually increasing in the study area, it is prudent to document the status of the nematode destroying fungi before the remaining forest habitats are ultimately lost. Soil samples were collected from an indigenous forest, maize/bean, napier grass, shrub and vegetable fields, which represented the main land use types in Taita Taveta district of Kenya. The nematode destroying fungi were identified to species level and were grouped into seven genera. The species identified were, *Acrostalagmus obovatus, Arthrobotrys. dactyloides*, *A. oligospora*, *A. superba*,

Dactyllela lobata, *Haptoglosa heterospora*, *Harposporium anguillulae*, *Harposporium* sp, *Monacrosporium cionopagum* and *Nematoctonus georgenious*. Occurrence of nematode destroying fungi was significantly (P: 3.81 x 10^{-7}) different among the land use systems in the study area. Out of the isolates that were positively identified, 33.7 %, 27.9 %, 20.9 %, 11.6 % and 5.8 % were from fields under vegetable, maize/bean, napier grass, shrub and forest, respectively. The diversity of nematode destroying fungi was highest in the maize/bean fields and lowest in forest soil. Fungal isolates from vegetable gardens were most diverse but the least even while the forest land use was most even but least diverse. The total richness of nematode destroying fungi was 9, in vegetable and maize/bean fields while 7, 6, and 3 were recorded in napier, shrub and forest habitats, respectively. Land use with organic inputs had more nematode destroying fungi than those with inorganic. This study has established that nematode destroying fungi are widely distributed and that land use and soil fertility management has a significant effect on their diversity.

Key words: *Arthrobotrys oligospora*, evenness, vegetable field, natural forest, organic input

4.2 Introduction

The Taita Hills are located in Southeastern Kenya, 25km west of Voi town in the Taita-Taveta District, at approximately 03 degrees -20°S, 38 degrees -15°S. Due to their age (290 – 180 million yrs), isolated location and relatively stable conditions, they contain unique plants and animals with very high levels of endemism. The forests have been recognized as one of the 25 biodiversity 'hotspots' in the world

(Rogo and Oguge 2000). Subsequent work ranked the area among the top-ten biodiversity hotspots in the world (Mittermeier *et al.*, 2005). According to Pellikka *et al.* (2004), the area had 74 endemic vertebrates, 265 endemic invertebrates and 66 endemic trees. Some species, however, are critically endangered, like the Taita Thrush, *Turdus helleri* (Bytebier 2001). Taita Hill forests hold a unique biodiversity with 13 taxa of plants and nine endemic animal species.

Although such inventories of aboveground biodiversity have been documented in this area, none of the studies has focused on soil biodiversity despite its importance (Davet and Francis 2000; Moreira *et al.*, 2006). Currently the forest area is under serious threat from fragmentation through agricultural activities leading to loss of biodiversity (Githiru and Lens 2007). Hence there is urgent need for documenting the belowground biodiversity.

Nematodes are microscopic multicellular roundworms that inhabit marine, freshwater and terrestrial environments. Some are beneficial soil microorganisms that play an important role in essential soil processes while others cause plant diseases (Dufour *et al.*, 2003). Plant parasitic nematodes infect the root tissues of the plant causing root galls that lead to reduced water and mineral uptake in the plant root system. They have been reported to cause up to 50% and 60% yield loss in both maize and common beans respectively in heavily infested fields in Kenya (Kimenju *et al.*, 1998). They are also associated with huge crop loss in tomato for smallholder growers in Kenya (Kimenju *et al.*, 1998; Oruko and Ndungu, (2001). In Taita Taveta, horticulture is the main source of livelihood accounting for 95% of household income, Spoerry (2006). However, Kimenju *et al.*, (2005), reported high

populations of plant parasitic nematodes in horticulture farms in this area, while Spoerry, (2006) reported that nematodes are the major soil pests identified by the farmers that cause low vegetable production. These soil pest problems together with the poor degraded soils found in the area has led to heavy use of agro chemicals in the farms. The negative effects of this land intensification on soil health has however been recorded over the years in the study area (Pellikka *et al.*, 2004) and include decrease in useful organisms in the soil and soil erosion, (Sirvio *et al.*, 2004). In agreement with these reports, the District Development Strategies 2002-2006 for Taita, crop production was identified as an area of focus while high chemical costs, soil degradation, inadequate technical know how and low soil fertility were identified as factors contributing to low farm production.

There is considerable concern about the use of chemical nematicides globally, to address the nematode loses (Pinkerton *et al.* 2000) while some nematodes may have developed resistance to these chemicals (Kerry 2000; Larsen 2000). Thus, alternative nematode control strategies are being sought. About 70% of fungal genera and 160 species are associated with nematodes but only a few of them are suitable for trial in biological control of nematodes (Elshafie *et al.*, 2006). They continuously destroy nematodes in virtually all soils because of their constant interaction in the soil rhizosphere.

Nematode destroying fungi are a group of cosmopolitan microfungi that are natural enemies of plant parasitic nematodes (Birgit *et al.*, 2002; Yang *et al.*, 2007). They comprise fungi which parasitise nematode eggs and other life stages (Jansson and Persson. 2000). Although taxonomically diverse, this group of microorganisms is

capable of destroying, by predation or parasitism, microscopic animals such as nematodes, rotifers and protozoans. Collectively, they have the unique ability to capture and infect nematodes in the soil and appear to be widespread in distribution (Birgit *et al.*, 2002). The actual mechanisms by which the fungi are attracted to the nematodes have not been fully understood. However, it is generally accepted that the cuticle is penetrated and the nematode is immobilized through infection bulbs, being finally digested by the trophic hyphae produced by the fungus (Bordallo *et al.*, 2002). Some fungi use adhesive conidia, branches, knobs and mycelia to capture nematodes (Jaffe and Muldoo, 1995). In some cases, nematode destroying fungi produce toxins that immobilize or kill nematodes (Araújo *et al.*, 1999). The group also includes endoparasitic species in such genera as *Harposporium*, *Nematoctonus* and *Meria* (Timm *et al.*, 2001) which spend their entire vegetative lives within infected nematodes.

Nematode destroying fungi have drawn much attention due to their potential as biological control agents of nematodes that parasitize plants or animals (Jansson and Persson, 2000; Sanyal, 2000; Masoomeh, *et al.*, 2004). Unfortunately, there exist multidimensional drawbacks to the realization of the full potential of the nematode destroying fungi. Unavailability of reliable methods to visualize the fungi and demonstrate their activity in their natural habitats 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 (Jaffee *et al.*, 1998). Although fluorescence microscopy can be used to monitor the nematode destroying fungi in the soil, the sampling procedure available is inappropriate due to

its destructive nature and heterogeneity of the soil (Jensen *et al.*, 1998). Apart from disagreements on methods that can be used in monitoring organisms in the soil, the process is cumbersome (Persson *et al.*, 2000). On quantification, some authors have recommended the soil dilution method and the most probable number as well as Polymerase Chain reaction (PCR) for the estimation of the nematode destroying fungi population in the soil (Mauchline *et al.*, 2002). Bioassay for conidia and parasitism assay or predatory index has also been recommended (Jaffee, 1999; Sanyal, 2000). Above all, the gaps in knowledge of the ecological factors that influence the occurrence and abundance of nematode destroying fungi are largely unclear.

The objective of this study was therefore to determine how land use and soil fertility practices influences the diversity and occurrence of nematode destroying fungi in the soil.

4.3 Materials and methods

Description of study area

The study was conducted in Taita district, Werugha location of Wundanyi division located at 03^0 23'S 380 18'E, along a land use intensity gradient spanning from the valley bottoms of Werugha and the Ngangao forest, of the Taita hills (Beentje, 1988). The valleys are rich in vegetable cultivation which is the main economic activity of the Taita community (Pellikka *et al.*, 2004). The study area was stratified into five strata based on the land use types (Plate 1 a, b, c, d, e); maize/bean intercrop, vegetable, shrub, natural forest and napier grass. The natural forest consisted of a broad diversity of indigenous trees, which included; *Strombosia*

scheffleri (Olacaceae), Dicralonepis usambarica (Thymelaceae), Craibia zimmermanii (Papilionaceae), Oxyanthus speciosa (Rubiaceae), Dracaena deremensis (Dracaenaceae), Rauvolvia mannii (Apocynaceae), Rytiygynia schumanii (Rubiaceae) and *Chassalia discolor (Rubiaceae).* Natural shrub consisted of mainly *Croton megalocarpus (Euphorbiaceae), Lantana camara (Verbenaceae), Sporoblus pyramidalis (Gramineae)* and *Ficus thoningii (Moraceae).* The vegetable gardens were mainly dominated by cabbage (*Brassica oleraceae*), spinach (*Chenopodium spinacia*), tomato (*Solanum lycopersicum*), kale (*Brassica oleraceae var. acephala*) and cucumber (*Cucumis sativus*), grown separately in randomly selected rotation systems. Maize intercropped with beans was selected because it was the main food production system in the study area. Napier grass fields (*Pennisetum purpureum*) are also widely distributed in the area and serve to supply fodder to the dairy animals under restricted grazing systems.

Information on soil fertility management at the sampling points was obtained using questionnaire, observation and interviews. The following attribute data was collected: land use, organic inputs used, inorganic input (fertilizer) and crop rotation.

a. Maize/bean

b. Vegetable

c. Shrub

d, Natural forest

e, Napier

Plate 1: Main land use types (LUT) in Taita Taveta

Eight soil samples were taken from each of the five land uses. In total, 40 main sampling points were randomly identified from which five sub samples were taken. One sub- sample was taken from the center and four sub-samples at a distance of 3 meters from the centre (Fig. 3). An auger was used to take soil cores from the 0-20 cm soil depth.

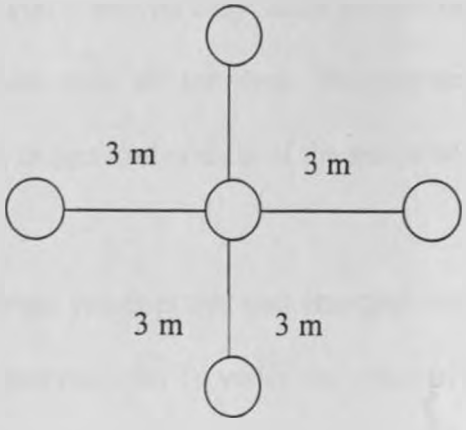

Fig.3 Soil sampling procedure.

The five sub-samples were mixed homogeneously to constitute a composite sample from which 500g soil was taken, placed in a plastic bag and then placed in a cool box. The auger was sterilized by dipping it in ethanol between sampling points to avoid cross contamination. The soil samples were transported to the laboratory where they were kept in a cold room at about 10^0c before isolation of the nematode destroying fungi.

Isolation of the fungi was done using the soil sprinkle technique as described by Jaffee *et al.*, (1996). Tap water agar was prepared by dissolving 20 grams of agar in

one liter of tap water. The medium was autoclaved and cooled to 45^0c before amending it with 0.1 g/L of streptomycin sulfate to suppress bacterial growth. Approximately one gram of soil from each sampling point was sprinkled onto the surface of water agar in Petri dishes. A suspension of 500 - 1000 juveniles of *Meloidogyne incognita* was added into the Petri dish as baits. The plates were incubated at room temperature and observed daily under a microscope at low (40 x) magnification, from the third week up to the 6th week. The examination was focused on trapped nematodes, trapping organs and conidia of the nematode destroying fungi that grew from the soil.

After the sixth week, all the fungal colonies that had emerged were sub-cultured on potato dextrose agar to obtain pure cultures. To verify the status of the fungal isolates as predators of nematodes, observations were made on a daily basis, after the third day, for trapped nematodes, trapping organs and conidia. Photographs of trapped nematodes, trapping organs and conidia were taken for use in identification of the nematode destroying fungi. Identification of the fungi was done using a key by Cook and Godfrey, 1964. Pure cultures of each fungal species were later inoculated in tap water agar without the streptomycin but with nematode suspension to determine their predatory activity on the nematodes.

4.4 Data analysis

Generalized linear models were fitted to test the effect of land use on the occurrence of nematode destroying fungi since the data were found to be over dispersed. Data were also analyzed by calculating the frequency of occurrence, evenness, Renyi

profiles and the Shannon diversity index (Kindt and Coe, 2005). Principal component analysis using ADE4 software was done on the temporal association of nematode-trapping fungi, land use and soil chractristics (Thioulouse *et al.*, 1997).

4.5 Results

All the sampled land uses were significantly different in terms of occurrence of nematode destroying fungi (p-value: 3.81×10^{-07}). Organic inputs (cow manure and chicken manure) significantly affected the occurrence of nematode destroying fungi in the study area ($P \leq 0.05$). Inorganic inputs (chemical fertilizers and pesticides) and crop rotation did not show any effect on the occurrence of nematode destroying fungi ($P \geq 0.05$) (Table 1).

Nematode destroying fungi were present in all the land use types but at varying frequencies and abundance. The frequency of isolating nematode destroying fungi was 33.7% and 5.8% in vegetable and forest ecosystems, respectively. The vegetable ecosystem harbored all the species recorded during this study, apart from *Acrostalagumus obovatus* which was absent. With the exception of *Monacrosporium cionopagum*, all the other nine species were recovered from the maize/bean fields. The forest land use had the least counts of nematode destroying fungi, which were in the genera, *Arthrobotrys, Monacrosporium* and *Harposporium*. The proportions of nematode destroying fungi isolated from maize/bean, shrub land and napier grass plots were 27.9, 11.6 and 20.9 %, respectively (Table 2).

Differences in evenness were significant (P-value: 7.139×10^{-4}) among the five land use systems studied. Mean richness of 3.6, 2.3, 1.3 and 0.6 was recorded in

vegetable, maize/bean, napier, shrub and forest respectively. The diversity of nematode destroying fungi was also significant ($P = 1.062 \times 10^{-6}$) across the land use types. The total species richness ranged from three to nine being highest in the intensively cultivated ecosystems under maize intercropped with beans and in the vegetable fields. The total richness of the nematode destroying fungi was equal in maize/bean and vegetable fields but the evenness was slightly higher in the former than the latter (Table 2).

The diversity profiles of nematode destroying fungi in the five land uses shows that maize/bean and the vegetable fields exhibited the highest diversity, followed by napier grass fields. The diversity was lowest in the forest ecosystem (Fig. 4a). The evenness profiles showed two distinct categories in the study area (Fig. 4b). The evenness profile in the forest was distinct and above those of the other land uses. Evenness in the maize/bean field was almost equal to that in the shrub land.

Table 1: Effect of land use, inorganic and organic inputs and crop rotation on occurrence of nematode destroying fungi.

Source of variation	% Deviance explained	P- value
Land use	63.73	0.0003152
Inorganic inputs	13.47	0.1298
Organic inputs	59.32	2.123×10^{-05}
Crop rotation	9.5	0.1005

Table 2: Effect of land use on Frequency of isolation, richness and diversity of nematode destroying fungi in Taita Taveta district, Kenya

Land use	Frequency of isolation %	Mean evenness	Mean richness	Mean shannon
Forest	5.8	0.375	0.625	0.17.
Maize/bean	27.9	1.000	3.000	1.07
Napier	20.9	1.000	2.250	0.76
Shrub	11.6	0.625	1.250	0.36
Vegetables	33.7	1.000	3.625	1.26
P-value	3.81×10^{-07}	0.0007139	3.81×10^{-07}	1.062×10^{-06}

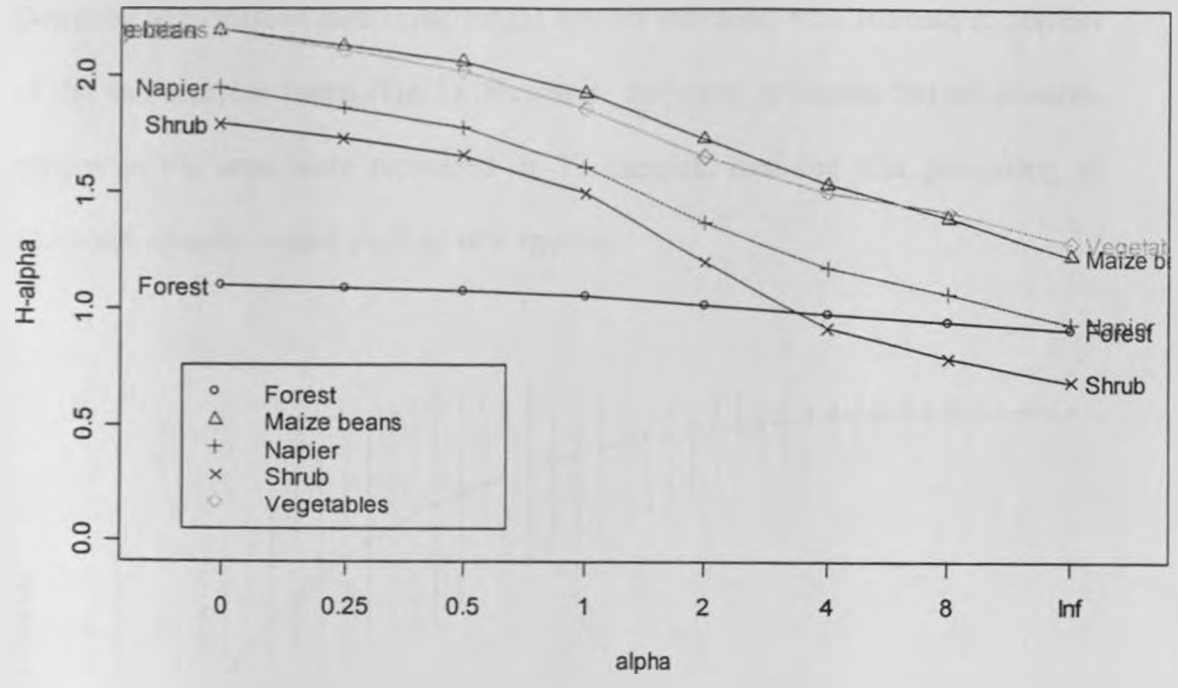

Fig. 4a. Diversity of nematode destroying fungal species under varying land use systems in Taita Taveta district, Kenya.

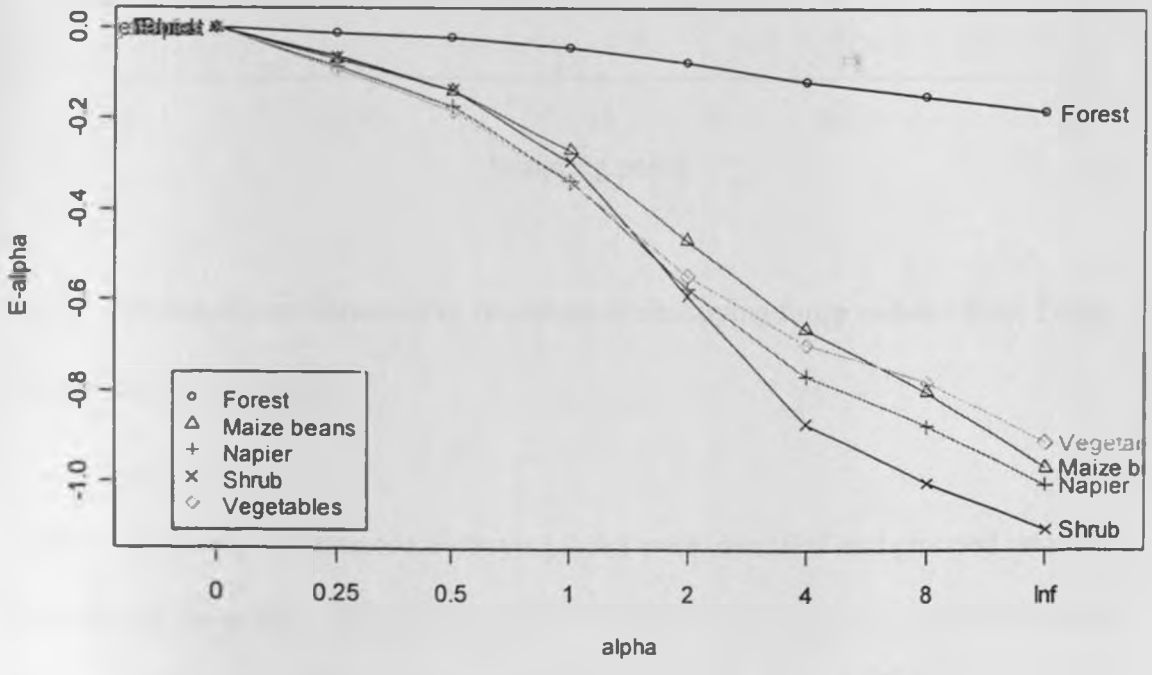

Fig.4 b. Evenness of nematode destroying fungal species isolated from soil under different land use systems in Taita Taveta district, Kenya.

Detection of nematode destroying fungal species increased with increase in number of the soil samples taken (Fig 5). However, the curve indicates that all possible species in the area were recovered in 37 samples, meaning that processing of additional samples would yield no new species.

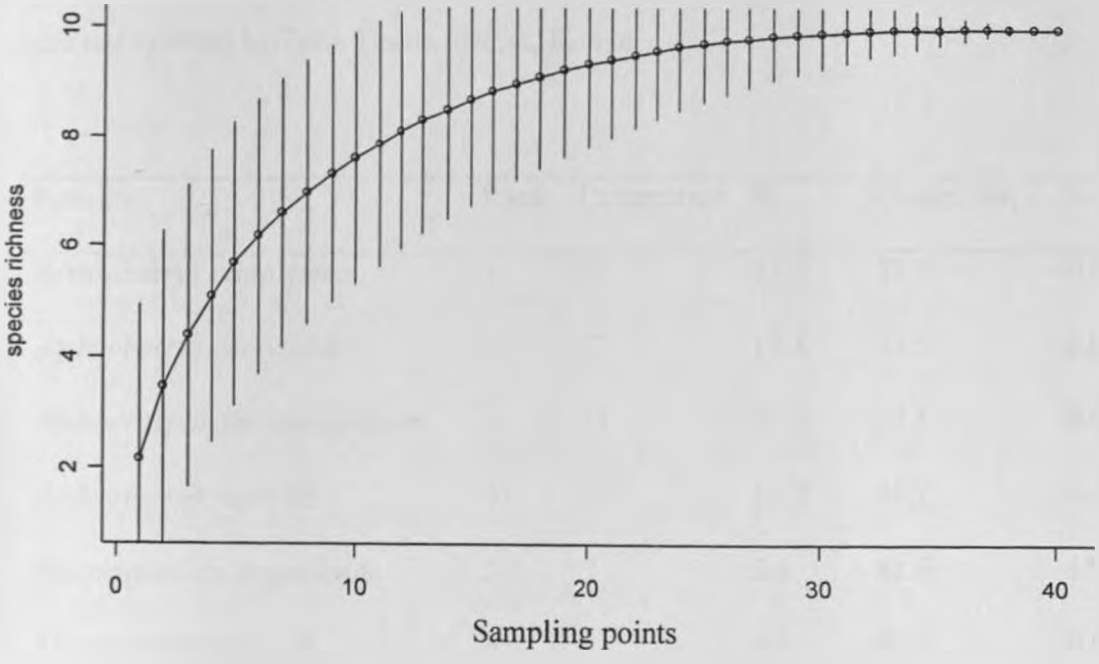

Fig.5. Species accumulation curve of nematode destroying fungi isolated from Taita Taveta district in Kenya.

Eighty six isolates of nematode destroying fungi were identified and grouped into ten taxa and seven genera. Fungi in the genus *Arthrobotrys* were the most frequently isolated, with a cumulative frequency of 64% (Table 3). Species in the genus were *A.oligospora*, *A.dactyloides* and *A. superba.* The genus was represented in all the land use systems. It was followed by the genus *Harposporium* which was

represented by *H. aunguillilae* and *Harposporium* sp. Members of the genus *Nematoctonus* were least frequent (2.3%), being isolated only in two samples in this study.

Table 3: Frequency of occurrence of nematodes destroying fungi in different land use systems in Taita Taveta district, Kenya.

Species	Rank	Occurrence	%	Cumm.freq.	P- value
Arthrobotrys oligospora	1	29	33.7	33.7	0.006872
Arthrobotrys dactyloides	2	17	19.8	53.5	0.001228
Monacrosporium cionopagum	3	11	12.8	63.3	0.01092
Arthrobotrys superba	4	9	10.5	76.7	0.03096
Harposporium anguillulae	5	5	5.8	82.6	1*
Harposporium.sps	6	4	4.7	87.2	0.005619
Dactyllela lobata	7	3	3.5	90.7	0.3382*
Acrostalagmus obovatus	8	3	3.5	94.2	0.3382*
Haptoglosa heterospora	9	3	3.5	97.7	0.018
Nematoctonus georgenious	10	2	2.3	100.0	0.1028*

* the species occurrence is not significantly affected by the land use types.

Arthrobotrys oligospora had the highest frequency of occurrence, followed by *A.dactyloides, Monacrosporium cionopagum, A.superba,* and *Harposporium*

aungullilae on the species rank curve (Table 3). Occurrence of *A.oligospora* had a P value of 0.006872 while *A.dactyloides, M.cionopagum* and *A.superba* had p-values of 0.001228, 0.01092 and 0.03096, respectively. Some rare isolates also reflected the effect of land use on their occurrence, *Harposporium* sp and *Haptoglosa heterospora* with a p-value of 0.005619 and 0.018 respectively. *Harposporium anguillulae, Dactyllela lobata, Acrostalagmus obovatus* and *Nematoctonus georgenious* were not affected by the land use.

The probability of isolating an *Arthrobotrys oligospora* from the vegetable, maize/bean and napier grass fields was above 0.8 while it was 0.2 in the forest soil (Fig. 6). The fungus was present in all the target land uses. *Arthrobotrys dactyloides* was most frequent in vegetable gardens, followed by napier grass fields, but very low in the shrub land and completely absent in the forest. Chances of isolating *Monacrosporium cionopagum* were 0.8 in vegetable gardens but below 0.3 in all the other land uses and absent in the maize/bean fields.

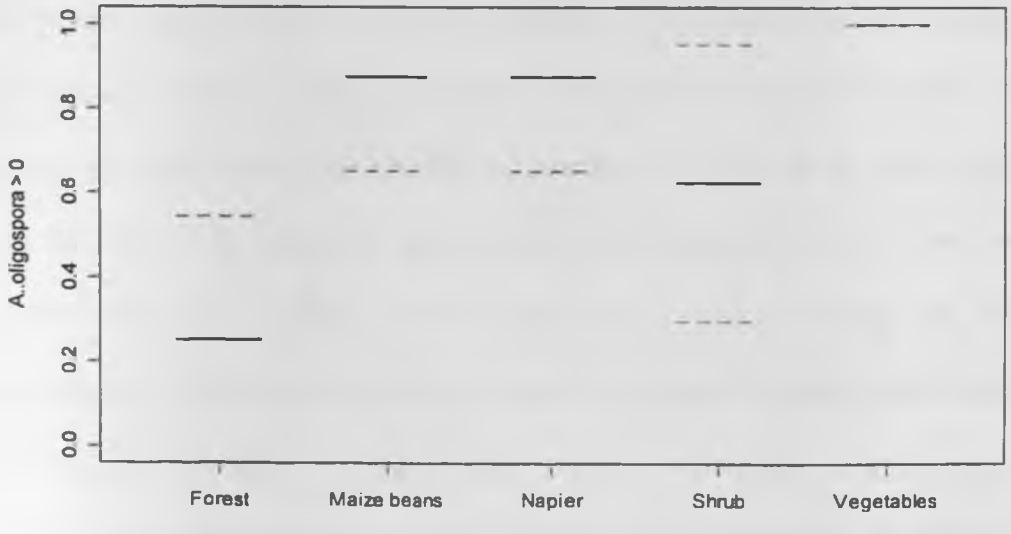

Fig.6. Probability of isolating *Arthrobotrys oligospora* in soil under different land use systems in TaitaTaveta district in Kenya.

Characteristics of isolates

Arthrobotrys oligospora formed adhesive nets, non constricting rings and three dimensional structures which caught nematodes. The Conidia were indented at the septa producing two distinct cells. The species was also differentiated by the upright conidiophores in which conidia were in groups of more than ten. It ensnared nematodes with three ring networs and adhesive mycelia (Plate 2a, b, c). *A.dactyloides* developed an-upright and un-branched conidiophores. The conidia were ellipsoid and slightly curved and almost equal two cells. It produced three cell constricting rings (Plate 3). The *Monacrosporium* species mycelium developed adhesive columnar branches, which looked like ladder or a mesh where all the nematodes were held. Conidia are two to many celled, conidiophores colourless, erect, bearing single terminal conidia (Plate 4 a,b). The genus *Nematoctonus* produced mycelial net work originating from the destroyed nematode. Its conidia germinated after being released from the destroyed nematode (Plate 5a). The nematode was attacked by germinated adhesive hour-glass shaped knob conidia. The mycelium showed a clamp connection. The adhesive conidia of endo parasitic nematode destroying fungi identified, *Harposporium and Meria* attach themselves on the body of the nematode and germinate penetrating the cuticle of the nematode (Plate 5b,c). The hyphae of the fungus live in the nematode and only the conidiophores that come out through natural openings of the nematodes and release the conidia for another cycle to start. On the other hand, trapping nematodes, *Arthrobotrys* and *Monacrosporium*, immobilized the nematodes by trapping them

with their adhesive mycelia of rings. Other nematode destroying fungi could not be identified (Plate 6 a - b)

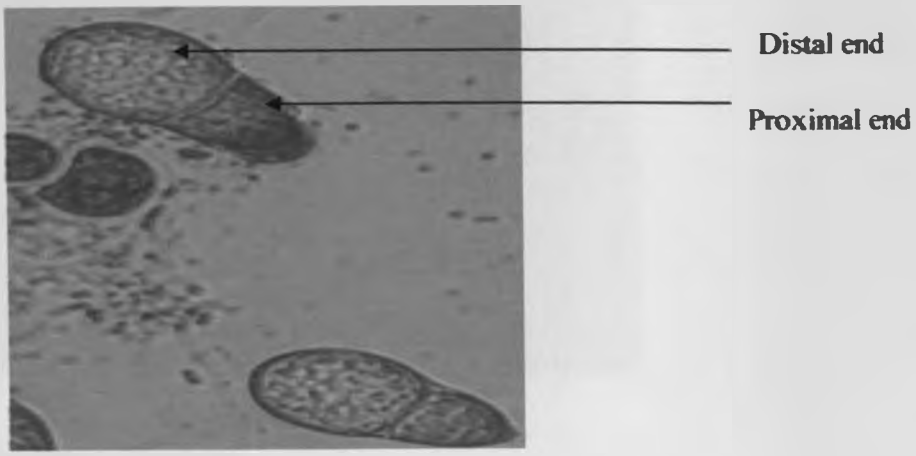

Plate 2a. *Arthrobotrys oligospora:* Conidia with two distinct cells, the distal cell; is almost twice as big as the proximal cell.

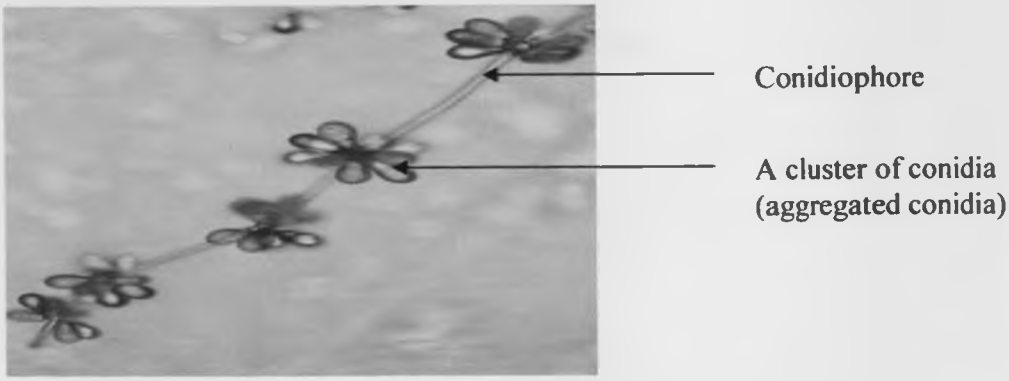

Plate 2 b. A Conidiophore of *Arthrobotrys oligospora* with groups of conidia.

The conidia occur in series of more than ten in each conidiophore

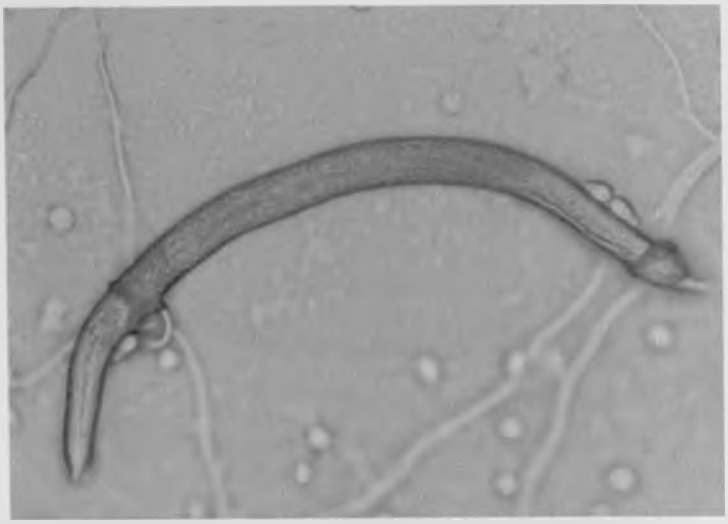

Plate 2 c. *Arthrobotrys oligospora*

Anematode ensnared at two points by the nematode trapping fungi *Arthrobotrys oligospora*

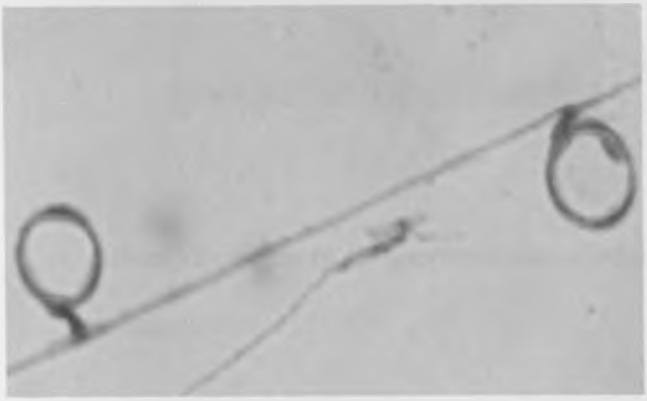

Plate 3. Constricting rings of *Arthrobotrys dactyloides*

The ring is made up of three cells which expand towards the centre and squeeze the nematode when touched from the inner side.

Plate 4 a. Conidia of *Monacrosporium cionopagum*

The conidia have two to several cells. The conidia are strongly spindle shaped

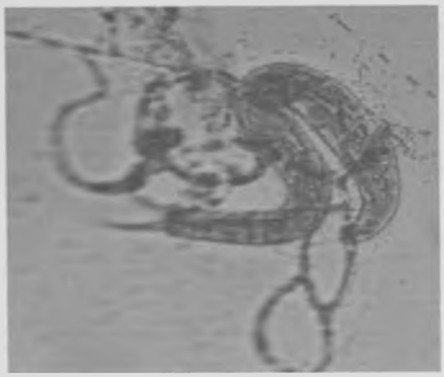

Plate 4b: Adhesive column of *Monacrosporium cionopagum* with a trapped nematode

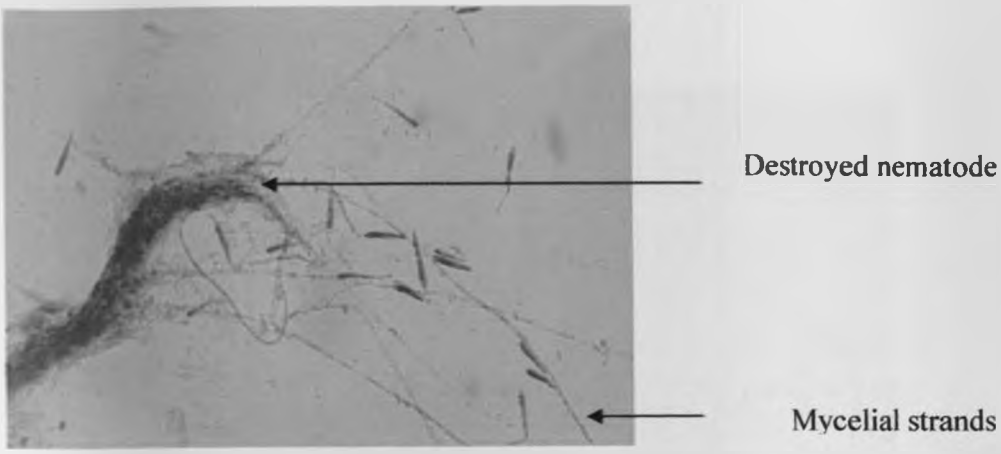

Plate 5a: A nematode destroyed by *Nematoctonus leiosporus*.

The conidia of the fungus germinate on the soil after leaving the nematode.

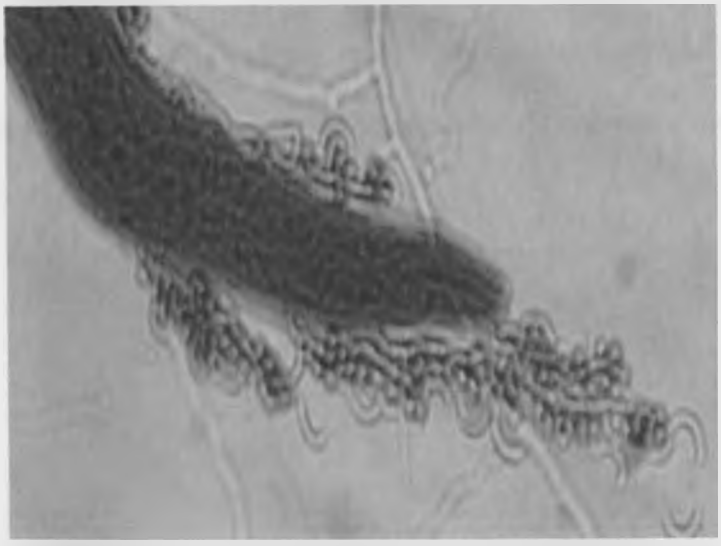

Plate 5 b. *Harposporium aungullilae*.

An endo-parasitic nematode destroying fungi, The conidia are half-moon shaped and are produced outside the infected nematode. All the other vegetative phase of the fungi remains inside the nematode.

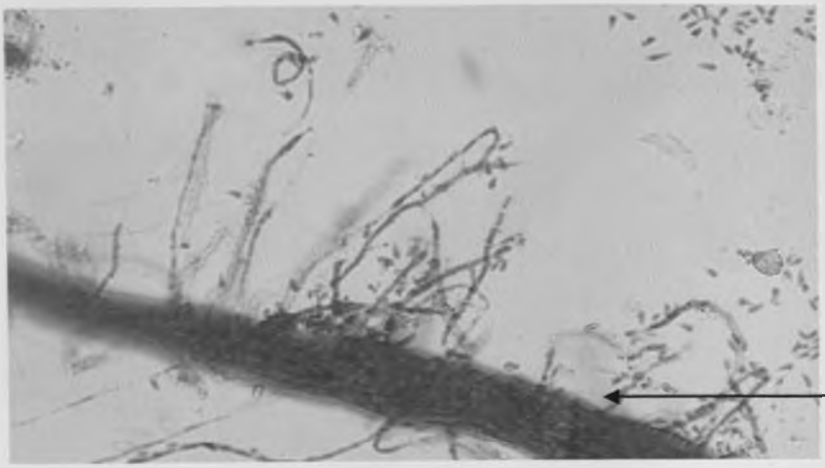

Plate 5c. *Meria coniospora.*

An endo-parasitic nematode destroying fungi. The conidiophore and the conidia appear through the pierced skin of the infected nematode and other openings. The myclia lives inside the nematode.

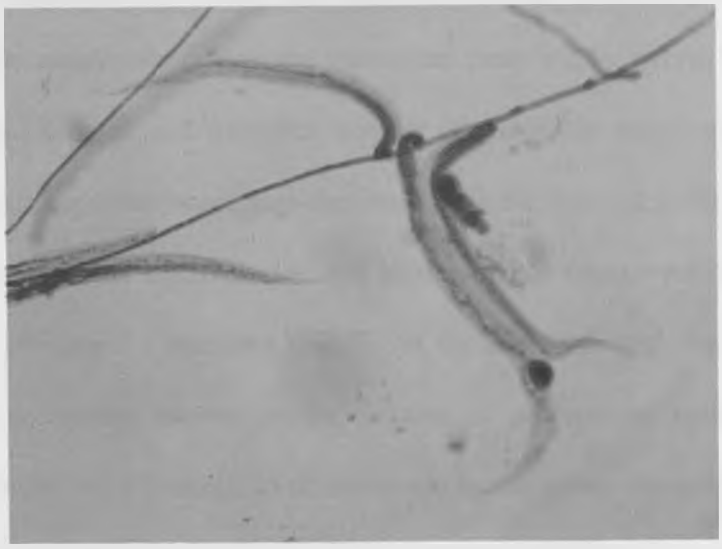

Plate 6a. Un-identified nematode destroying fungi (i).

This fungi repeatedly tied nematodes along the hyphae

Plate 6 b. Un-identified nematode destroying fungi (ii).

This fungus did not produce any conidia. It was an endo parasitic.

***In vitro* studies of selected isolates**

A.oligospora formed adhesive nets, non constricting rings and three dimensional structures which caught nematodes and consumed them within twelve hours. The number of traps increased with increased number of nematodes reaching the highest pick on the eighth day (400 traps), that also resulted in the increase in the number of trapped nematodes. After the ninth day, the number of the traps went down as the conidiophores and conidia increased (Fig 7). In the thirteenth day, the number of traps, trapped nematodes as well as the number of un-captured nematodes had declined. Time for the consumption of nematode by the genus *Nematoctonus* was between twenty-four and thirty six hours after incubation. Since the fungus is endo parasitic, the actual time when the adhesion of the conidia to the nematode and infection occurred could not be determined. In *Monacrosporium* spp the adhesion occurred on the ladder like mycelium. The nematodes were fully consumed between

twelve and fifteen hours. In all of them, the newly hatched juveniles were easily trapped and killed faster than the adult nematodes.

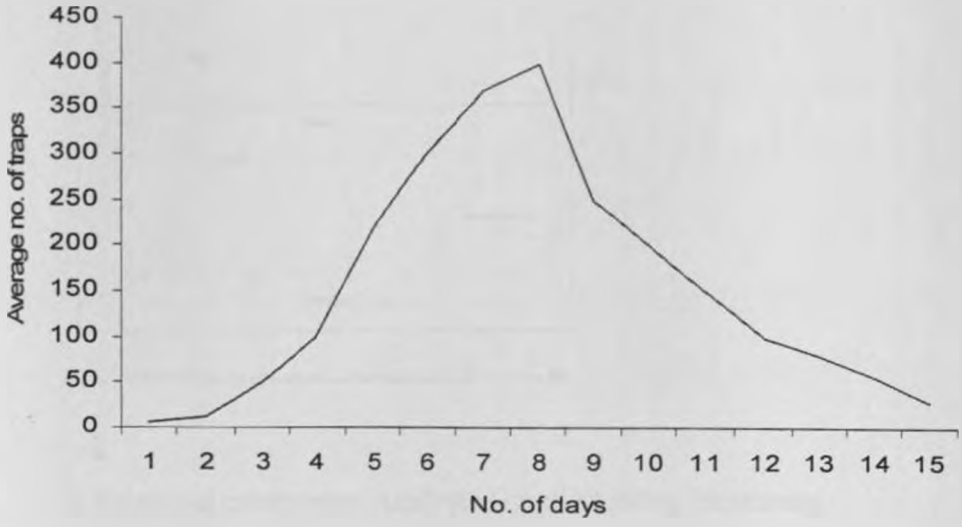

Fig.7. Traps formation in *A. oligospora*

Correlation of nematode destroying fungi, land use and soil chemicals.

The principal component analysis (PCA), shows two main factors explaining 84.12% of the total occurrence of nematode destroying fungi as affected by the land use (Fig. 8a). The first factor, explained 60.77 % while the second factor explained 23.35%.The first factor separated maize/beans and vegetables on one hand and shrub, napier and forest on the other. The nematode destroying fungi species loaded more towards the horticulture and the maize/bean land uses. *Monacrosporium cionopagum* and *Acrostalgmus obovatus* loaded strongly to vegetable and maize/bean land uses respectively. Sixty percent of all the species were associated with vegetable land use while 40 % loaded with maize/bean (8b).

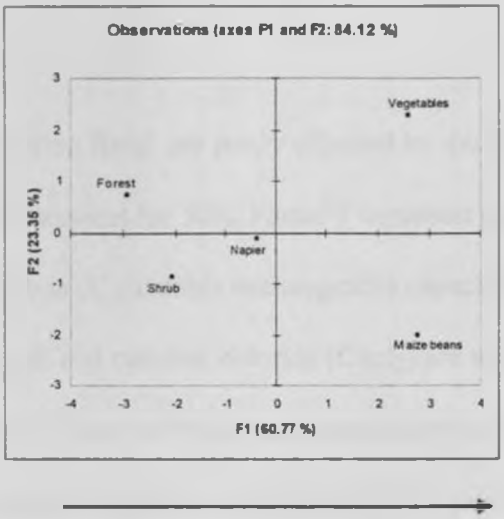

Fig. 8a. A Principal component Analysis Graph showing increasing agricultural intensification, increase soil disturbance, use of organic and inorganic fertilizer

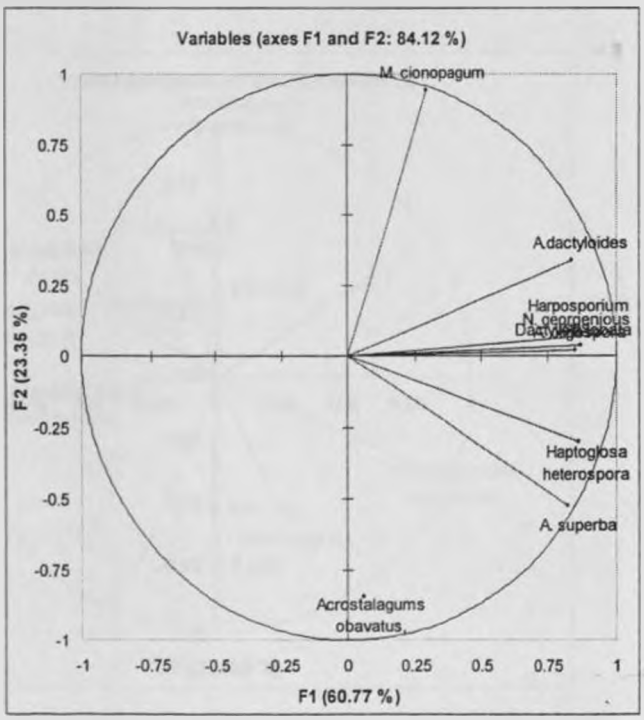

Fig. 8b. Effect of land use in distribution of nematode destroying fungi

Nematode destroying fungi are partly affected by the soil chemical properties as the two main factors account for 50%. Factor 1 separates phosphorus (P) on one side and nitrogen (N), carbon (C), cations exchangeable capacity (CEC) and potassium (K) on the other hand. pH and calcium chloride ($Cacl_2$) are not significant in the survival of fungi. The second factor which is the management of organic matter separates C, N and $Cacl_2$ on one side and P, K and pH and CEC on the other side. Some species (*A. oligospora, A. dactyloides, A. supreba, Dactyllela lobata, Haptoglosa heterospora, Acrostalagumus obovatus*) depend on soil pH, K and CEC for survival. *M. cionopagum, Harposporium aunguillulae* and *Harposporium georgenous* prefers soil with high N, C, and $Cacl_2$ while P soils seem to deter the growth of nematode destroying fungi (Fig. 8c)

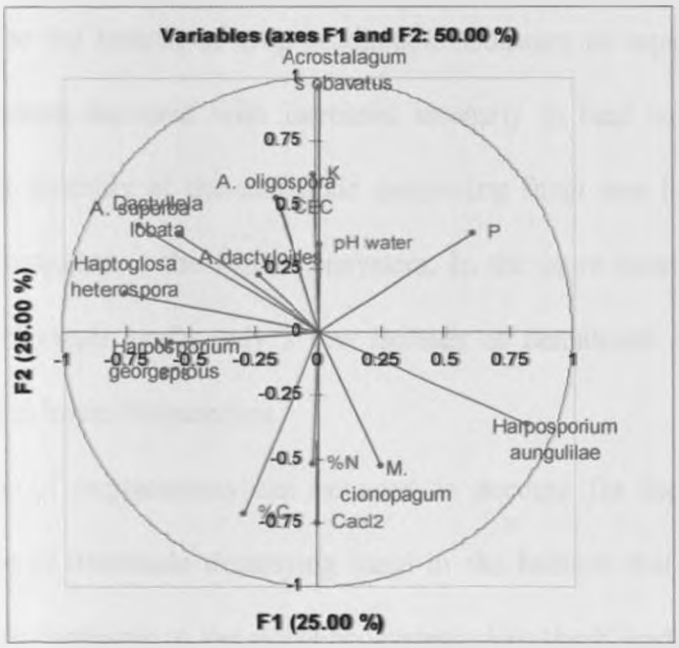

Fig. 8c. Canonical correlation analysis between the nematode destroying fungal species and soil chemical parameters

4.6 Discussion and conclusion

This study has demonstrated that nematode destroying fungi are wide spread in occurrence in the target habitats which were indigenous forest, shrub land, napier grass, maize/bean and vegetable fields. The fungi that were isolated exhibited several mechanisms of capturing and destroying plant parasitic nematodes which included constricting rings, adhesive nets, and non-constricting rings. The study has also revealed that increased land use intensity resulted in increased occurrence and diversity of nematode destroying fungi. These findings are consistent with previous reports indicating that nematode destroying fungi were present in all habitats but at different densities and diversities (Birgit *et al.*, 2002). Widespread occurrence and abundance of the fungi is thought to be an indicator of great potential that can be exploited to the benefit of crop production. Contrary to expectation that beneficial microorganism decrease with increased intensity in land use (Vandermeer *et al.*, 1998), the diversity of the nematode destroying fungi was higher in the vegetable gardens compared to the forest ecosystem. In the more intact and stable land uses (forest and shrub land), only a few isolates of nematodes destroying fungi were recovered at lower frequencies.

A number of explanations can be used to account for the higher frequency of occurrence of nematode destroying fungi in the habitats that are subject to regular disturbance compared to the stable ecosystems like shrub land and indigenous forest. Addition of farm inputs in the form of organic and inorganic compounds has an effect on indigenous microorganisms in the soil. According to Wang *et al.*, 2003,

some of the agricultural inputs stimulate build-up of nematode trapping fungi. It is also possible that fungal tissues are fragmented and scattered in the course of farm operations, thus increasing their frequency of isolation. Intensive cultivation is characterized by increased movement of soil which may result in increased spread of the microorganisms in the field. Soil disturbance, coupled with frequent changes in crop cover, subjects the soil biota to strees 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 nematode destroying fungi are able to establish and suppress other species that are poorly suited to compete effectively.

Evenness of the nematode destroying fungi was lower in the highly disturbed habitats like in vegetable gardens. According to Sanchez, 1997, agricultural practices can have positive or negative impacts on microorganisms in the soil. Intensive cultivation is usually accompanied by application of inorganic fertilizers and pesticides. Apart from the negative effects from synthetic inputs, human activities may also impose selective pressure on the naturally present microorganisms. Crop management practices (like addition of organic amendments) are known to have varying effects on indigenous microorganisms in the soil (Akhtar and Malik, 2000). This may account for the higher evenness of nematode destroying fungi in the forest, which is a more stable ecosystem, when compared to the vegetable gardens which are subject to the management practices adopted by farmers in a given area.

Arthrobotrys oligospora was the most abundant species of nematode destroying fungus in the study area. It was isolated from all the land uses with an overall

occurrence frequency of 33.7%. This finding was consistent with results from similar studies conducted in South Africa (Durand *et al.*, 2005; Farrell *et al.*, 2006). The genus *Arthrobotrys* was the most frequently represented in all the habitats that were the subject of this study. It's possible that members of the genus were the best adapted to the biotic and abiotic conditions prevailing in the study area. This finding is of practical value to the search and utilization of biological agents for the control of plant parasitic nematodes. Apart from introduction of particular species from the genus, agricultural practices that stimulate build-up of the fungi could be identified and recommended for adoption by farmers.

Factors affecting the occurrence of nematode destroying fungi in the soil would be separated into two as indicated by the principle component analysis. One would be the increased agricultural intensification caused by soil disturbance and addition of fertilizes. Since the vegetable and maize /bean are frequently disturbed by digging, weeding and even planting of many host plants, chances of detecting the nematode destroying fungi becomes higher than the other land uses. The many crop plants are hosts of many nematodes which are in constant interaction with the nematode destroying fungi. Forests, shrub and napier do not receive a lot of disturbance as compared to the maize/bean and vegetables.

The other factor is the amount of moisture content in the soil (Grey, 1985). The soil in natural forest contains high moisture content due to the forest canopy which is more closed than in shrub and napier land uses. This reduces the amount of evaporation from the soil. The soils in the vegetable farms also have high moisture content due to the constant irrigation and soil cover due to mulching and cover crops

unlike in the maize/bean land use that depend on rain.Soil moisture has been reported to be a major factor determining the occurrence of fungi in the soil (Gray, 1985)

There is no evidence so far to support the suggestion that the high numbers of nematode destroying fungi observed in the field result to suppression of plant parasitic nematodes as our work did not look at the population changes of both the nematodes and the nematode destroying fungi. Strong indications of nematode trapping fungi suppressing nematodes have been demonstrated in the laboratory using Petri dishes (Elshafie *et al.*, 2006). Jaffee and Strong, 2005 and Jaffee *et al.*, 2007 found no direct involvement of nematode destroying fungi in nematode suppression in Bodega Marine Reserve. More studies on biological interactions are recommended in this area. Studies should focus on improving the fungal quantification method (Jaffee *et al.*, 1996).

Additional evidence has been provided from this study that nematode destroying fungi are naturally occurring and widespread in agricultural and forest habitats. The fungi were more frequently isolated from the intensively cultivated land under annual and vegetable crop production. It indicates also that soil disturbance and moisture are the main factors affecting the occurrence of nematode destroying fungi. The study indicates that the fungi are able to survive in highly disturbed ecosystems. This unique observation sets the justification for continued work to establish the potential of nematode destroying fungi in regulation of plant parasitic nematodes.

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CHAPTER FIVE

5.0 EFFECT OF SOIL FERTILITY MANAGEMENT PRACTICES ON NEMATODE DESTROYING FUNGI AND NEMATODE COMMUNITIES IN TAITA TAVETA

5.1 Abstract

The effect of soil fertility management practices on the dynamics of nematode destroying fungi and the nematode community was investigated for three seasons in Taita, Kenya. The study was aimed at identifying soil fertility practices that promote the nematode destroying fungi and reduce the population of plant parasitic nematodes. Soil samples were randomly collected from fourty farms that were under vegetable cultivation in the study area. Isolation of nematode destroying fungi was done using the soil sprinkle technique and the identification done using the key of Cooke and Godfrey (1964). Nematodes were extracted from soil using the modified Baermann technique, identified to genus levels using the descriptions described by Bongers (1988) and Mai and Mullin (1996), and then counted. Two major soil fertility management practices are used in the area, application of commercial fertilizers and application of animal (cow and chicken) manures. Occurrence of nematode destroying fungi and nematode community was significantly (P= 0.05) different in the three seasons. The mean richness of the 1.4, 1.8 and 1.4 was recorded for the nematode destroying fungi in season 1, 2 and 3 respectively. Mean Shannon indices recorded for nematode destroying fungi was 1.6, 1.8 and 1.8 for

season 1, 2 and 3 in that order. Thirty seven genera of nematodes were recorded in this study. Of the three seasons, season 3 was the most diverse in terms of nematode genera with a diversity index of 2.9, while seasons 1 and 2 had 1.7 and 2.1, respectively. Application of chemical sprays affected both the nematode community and the nematode destroying fungi. The plant parasitic nematodes were associated with the nematode trapping fungi and commercial fertilizers and were negatively correlated with the cow manure. Chicken manure promoted the fungal feeding nematodes and the endo-parasitic nematode destroying fungi. The study has demonstrated that application of organic amediments encouraged the free living nematodes and nematode destroying fungi while commercial fertilizer encourages the presence of plant parasitic nematodes.

Key words: *Biological control, Plant parasitic nematodes, fertilizers, cow manure, chicken manure*

5.2 Introduction

Taita Taveta district is located in the Southeastern part of Kenya, 25kms west of Voi town. It is approximately 03 degrees -20'S, 38 degrees -15'S. It covers an area of 16,965 km^2 and is divided into five divisions. The main area of the study was along the valley bottoms of Werugha and the Ngangao forest in Werugha division. Werugha valley bottom (1652 m) is located between Yale (2104 m) and Ngangao (1952 m) hills. The soils in the surrounding hill forest are composed of a high-humic A-horizon overlaying a pinkish acid sandy loam. These sandy loams are generally deep, with high infiltration rates, a low pH (3-4), a low water holding capacity, and they are low in nutrients due to excessive leaching. The soils are also characterized

by the presence of high aluminum levels, low calcium levels, and unavailable potassium, causing a low cation exchange capacity (Muya *et al.*, 2005; Spoerry 2006). Two rain seasons are experienced in this area; long rain season which fall from March to May and short rains which falls from November to December. In the Taita hills, each month of the year receives some form of precipitation allowing continuous cropping. Highlands receive more rain than lowland areas. Lowlands are mainly arid and semi arid lands. The district's mean annual rainfall is about 55mm. However, in the highland areas, it can be as high as 1500mm and 250mm for the lowlands.

The area contains unique plant and animals with very high levels of endemism (Mwanyumba and Mwang'ombe, 1999). In previous reports on the area, 74 endemic vertebrates, 265 endemic invertebrates and 66 endemic trees have been recorded. A 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 the study site. The forests of the area have been acknowledged as one of the 25 biodiversity (eighth globally) 'biodiversity hotspots' in the world (Rogo and Oguge, 2000) while International Conservation has identified the area as top-ten biodiversity hotspot in the world (Clark and Pellika, 2005). Although such inventories of aboveground biodiversity have been documented in this area, none of the studies focused on soil biodiversity despite its importance (Davet and Francis 2000; Moreira *et al.*, 2006). There is evidence that soil biotic communities are associated with the vegetation, such that there is a mutual dependence between above-ground and below-ground communities. Therefore compromised soil

communities may curtail particular plant assemblages from forming. This justifies the importance of studying the belowground biodiversity of the study area.

In the continued effort to produce more and more crops in the area, major forest loss under land degradation has been experinenced since 1960s, thereby threatening the unique biodiversity in this area (Beentje, 1988; Githiru and Lens, 2007). The crops initially planted after clearing the forests were maize, beans, sweet potatoes, cassava, arrow roots, bananas, fruit trees and horticulture crops like tomato, kale, cabbage and lettuce among others. These crops have been planted since 1967 to the present and farm inputs have remained the same (Ortiz1 *et al.*, 2007). The intercropping of these crops is haphazard even though farmers believe it is a rational response to land scarcity but not related to belowground biodiversity functions (Mutsotso *et al.*, 2005). Poor soil management practices have resulted to continued soil erosion from the hills to the valley bottoms. As a result, soil particles accumulate at the valley bottoms along the main rivers. These deposits are the most fertile soils in this part of Taita Taveta which are exploited for horticulture (Pellikka *et al.*, 2004; Spoerry 2006). The valley bottoms are therefore the vegetable supply centre for coastal province of Kenya. Because of the economic value of vegetables production in this area, most attention has focused on changes in the abundance and diversity of the plant-parasitic nematode community. Plant parasitic nematodes contribute greatly to loss of vegetable crops in Taita hills (Mutsotso *et al.*, 2005) while the cost of production has increased due to the cost of chemical nematicides (Taita District Development Strategies 2002-2006). A social survey report from the area has indicated that farmers use chemical fertilizers, chemicals and non-chemical methods

to control nematodes at the valley bottoms and also to increase the soil fertility which have resulted to reduction of profit from the sale of vegetables (Mutsotso *et al.*, 2005). In recent years. there has been a great environmental concern on the use of chemical nematicides globally (Pinkerton *et al.*, 2000). In addition, some nematodes have developed resistance to these chemicals decreasing their effectiveness (Kerry, 2000; Larsen, 2000) and prompting the search for alternative methods for their control.

Globally, alternative nematode control strategies are being sort which are cost effective to the farmer and are environmental friendly. Of greater importance is the biological control where the natural enemies are stimulated in their natural habitat to control plant parasitic nematodes. A bout 70% of fungi genera and 160 species are associated with nematodes but only a few of them can be used as biological control agents of nematodes (Elshafie *et al.*, 2006). They continuously destroy nematodes in virtually all soils because of their constant association in the soil rhizosphere. Nematode destroying fungi are natural enemies of plant parasitic nematodes (Birgit *et al.*, 2002). They have drawn much attention because of their potential as biological control agents of nematodes that are parasitic on plants and animals (Jansson and Persson, 2000; Sanyal, 2000; Masoomeh, *et al.*, 2003; Yan *et al.*, 2005). Collectively, they have the unique ability to capture and infect nematodes in the soil and appear to be widespread in distribution (Birgit *et al.*, 2002).

The aim of the study was therefore to identify the soil fertility management practices which favour the buildup of nematode destroying fungi and at the same time reducing the population of the plant parasitic nematode.

5.3 Materials and Methods

A total of 40 farms under horticulture were randomly selected in the area. The farms were sampled for nematode destroying fungi and the nematode community for three consecutive seasons. Nine sub samples of soil were collected from each sampling point from every farm. The sub samples were mixed homogeneously to make one composite sample. From this composite sample, a 500 gram soil sample was taken, put in a polythene bag, sealed and labeled. Soil augers were sterilized with 75% ethanol before moving to another farm to avoid cross contamination. All the samples were transported to the laboratory and stored under room temperature, approximately 28^0c± 1. These soil samples were characterized for nematode destroying fungi and nematode community.

Isolation of the fungi was done using the soil sprinkle technique as described by Jaffee (1996). Approximately 1 g of soil from each sampling point was sprinkled onto the surface. Tap water agar (20 g of agar (Biotec, Biotec laboratories, United Kingdom) in L^{-1} tap water amended with 0.1 gL^{-1} streptomycin sulfate after autoclaving to suppress bacterial growth). A suspension of *Meloidogyne incognita*, about 1000 larvae, was added into the Petri dish as bait. The plates were incubated at room temperature, approximately 28^0c ± 1 and observed daily from the third week for 5 – 6 weeks under a Carl Zeis x 40 dissecting microscope for trapping organs, conidia and trapped nematodes. After the sixth week, all the fungal colonies that had emerged were sub-cultured on potato dextrose agar (Fluka, India) for pure cultures and multiplication. To confirm the status of the fungi, observations were made daily. Records were made after the third day for trapped nematodes, trapping organs and

conidia. Photographic records were made. Identification of the fungi was done using the key of Cooke & Godfrey (1964).

Nematodes were extracted from 200 cm^3 soil using the modified Baermann technique as described by Hooper *et al.* (2005). The nematodes were identified to genus levels using the descriptions described and counted (Bongers, 1988; Mai and Mullin 1996). The nematode community was then grouped in terms of their mode of feeding, the plant parasitic, the fungal feeders, the bacterial feeders and the predators, while nematode destroying fungi were grouped as trapping and endo parasitic nematode destroying fungi.

Experimental effect of soil fertility management practices on the nematode destroying fungi was also conducted. Ten experimental farms were randomly selected and divided into 4 plots measuring 3 x 3 meters with 1 meter path around each plot. In one of the plots, 9 kg of boma manure was broadcasted all over the plot (10 tons per ha), on the next, 0.8 kg of triple super phosphate (TSP) and 0.5 kg of calcium ammonium nitrate (CAN) were broadcasted together (KARI Recommended farmer practices). The third plot was the control and had no treatment, while the fourth was broadcasted with 0.9 Kg Mavuno fertilizer (blend of fertilizers containing 11 nutrients in balanced proportions and is suitable for most crops and soil conditions in Kenya).

After broadcasting all the inputs, they were mixed with the soil with a rake. The plots were then planted with maize at a spacing of 90 x 30 cm, two seeds per hole and beans at spacing of 30 cm in alternate rows. Soils were collected, as described earlier in 3.3, from each plot at the harvest period from each plot for estimation of nematode destroying fungi during the short rain, long rain and short rain consecutively. Nematode destroying fungi we isolated and characterized as earlier described.

5.4 Data analysis

Frequency of occurrence, evenness, Renyi profiles and the Shannon diversity index were determined (Kindt & Coe 2005). Principal component analysis and Multivariate analysis using ADE4 software was done on the temporal association of nematode-trapping fungi and nematodes and also the nematode destroying fungi (Thioulouse *et al.*, 1997).

5.5 Results

There were three main soil fertility management practices adopted by farmers in the area. Of the fourty farmers, thirty one farmers used cow manure on their farms, thirty eight farmers used commercial fertilizers either in combination with cow manure and chicken manure or alone. Only 9 farmers used chicken manure in combination with

cow manure and commercial fertilizers. Twenty-three farmers also used chemicals int their farms (Mocarp, Furadin. Bestocks, Diazinon and Achock)

in the cultivation of vegetables for control of pests. All except one farmer practice crop rotation in all seasons (Fig. 9). The major rotations observed were of vegetables with maize, vegetables with fallow and vegetables of different families where the farmers made the largest cluster after characterization of nematode destroying fungi (Fig. 10). Other rotations followed no specific order.

The three sampled seasons were significantly different in terms of occurrence of nematode destroying fungi ($P \leq 0.05$). The mean species richness recorded for nematode destroying fungi was 1.4, 1.8 and 1.3 in season 1, 2 and 3 respectively with season two (2) being the richest. The mean shannon of the three season was 1.6, 1.8 and 1.8 in season 1, 2 and 3 respectively, with season 3 being most diverse.

The nematode community was also significantly different in the three seasons ($P \leq 0.05$). In total. 37 nematode genera were recorded with a total abundance of 51,132. These genera were; *Meloidogyne, Scutellonema, Tyelchulus, Longidorus, Tetylenchus, Ditylenchus, Heterodera, Pratylenchus, Paratylenchus, Trichodorus, Dolichodorus, Helicotylenchus, Rotylenchus, Trophotylenchus, Hemicyclophora, Radopholus, Hoplolaimus, Paratrichodorus, Criconema, Criconemella, Iotonchus, Cryptonchus, Aphelenchus, Dorylaiminae, Teratocephalus, Tobrilus, Alaimus, Eucephlobus, Trypila, Plectus, Acrobeles, Achromadora, Mononchus, Nygolaimus, Odontopharynx, Rhabditis* and *Discolaiminae*

Season 3 was the most diverse and richest (Fig 11). All the different nematode genera in the area were recorded in this study site as indicated by the nematode genera accumulative curve (Fig. 12).

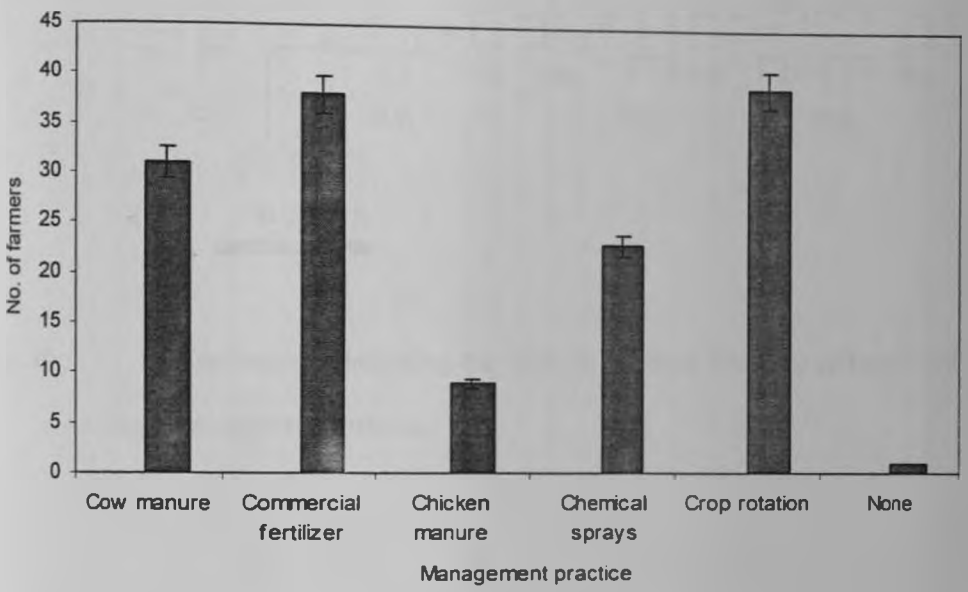

Fig. 9. Soil management practices in the 40 farms in Taita Taveta.

Fig.10. A dendrogram indicating the clusters of farms from the different soil fertility management practices.

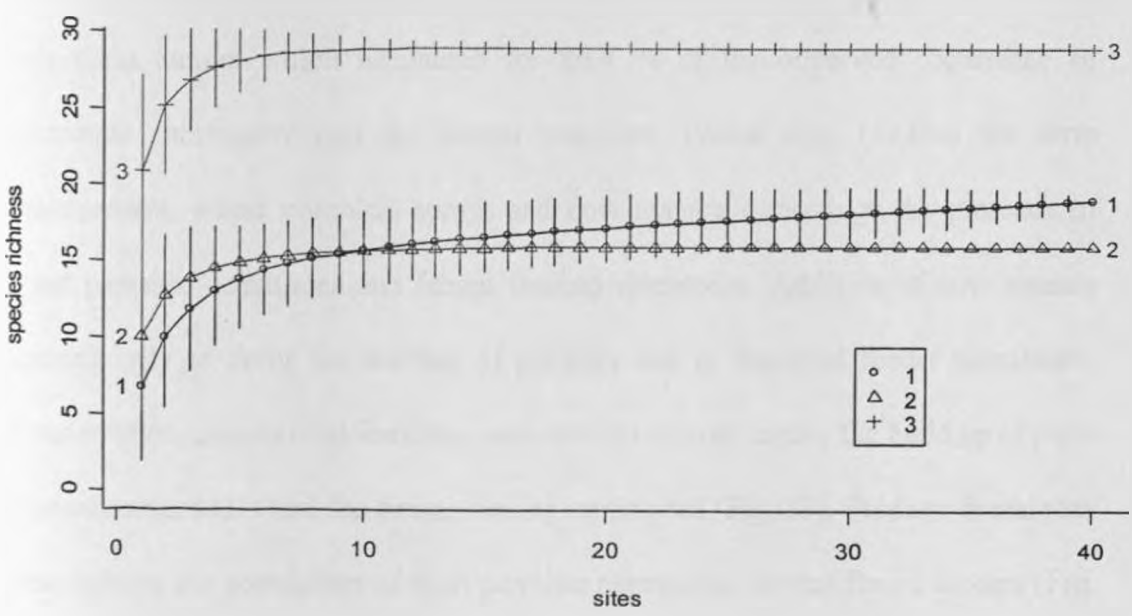

Fig.11. Nematode genera cumulative curves for the three seasons in Taita Taveta.

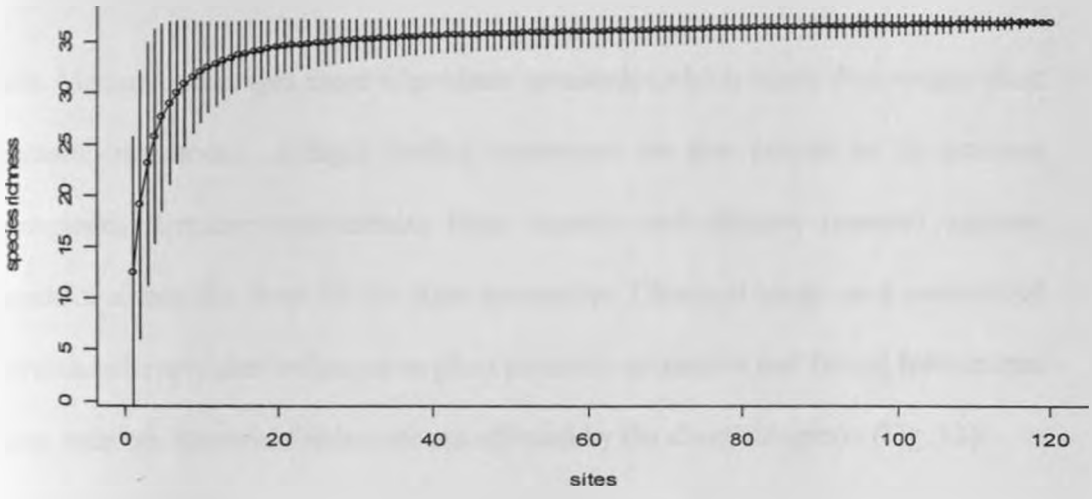

Fig.12. Combined season's genera cumulative curve for the nematode community in Taita Taveta.

The farmer soil fertility management practices affected the occurrence and diversity of both nematode destroying fungi and the nematodes. Canonical correlation showed two main factors which accounted for 86.4 % of the observed occurrence of nematode community and the farmer practices. Factor one, (52.6%) the farm management, where chemical sprays and cow manure discourage the presence of plant parasitic nematodes and fungal feeding nematodes. Addition of cow manure seemed only to favor the buildup of predator and of bacterial feeder nematodes. Crop rotation, commercial fertilizers and chicken manure causes the build up of plant parasitic nematodes and the fungal feeding nematodes (Fig. 13). Predator nematodes also reduces the population of plant parasitic nematodes and the fungal feeders (Fig. 13). Factor two (33.8%) shows the input of organic amendments in the management of plant parasitic nematodes. Chicken and cow manures encourage the predator nematodes that feed on plant parasitic nematodes reducing their population in the

soil. Manure encourages more of predator nematodes which intern discourages plant parasitic nematodes. Fungal feeding nematodes are also preyed on by predator nematodes. Organic amendments (cow manure and chicken manure) separate predator nematodes from all the other nematodes. Chemical sprays and commercial fertilizers have higher influence on plant parasitic nematodes and fungal feeders than crop rotation. Bacterial feeders are not affected by the chemical sprays (Fig. 13).

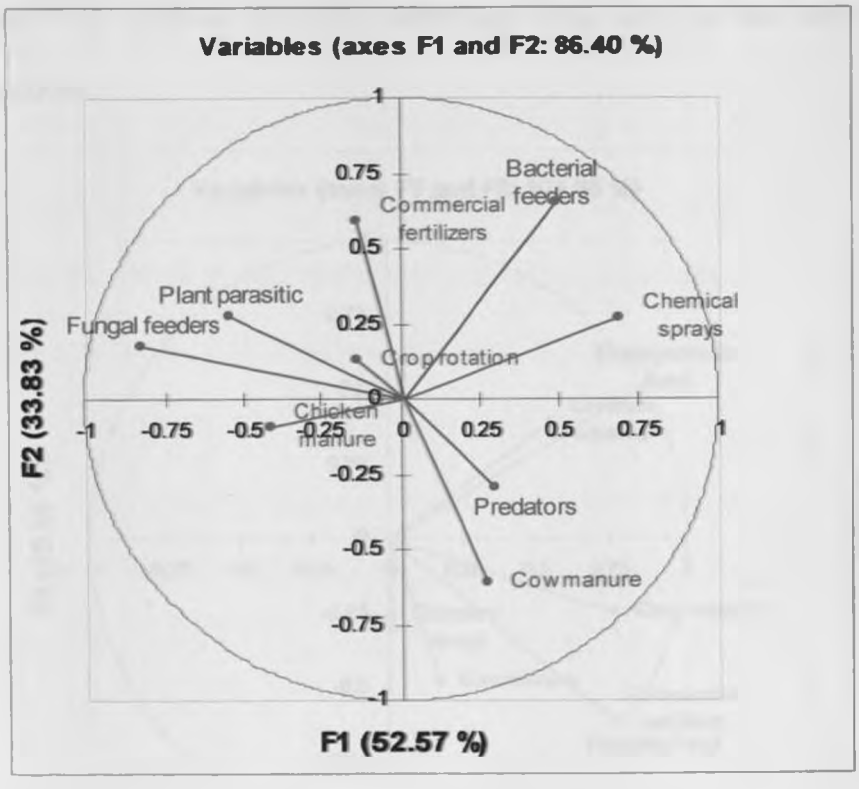

Fig.13. Canonical correlations for the nematode and soil management practices.

For the nematode destroying fungi, two main factors accounting for 100.00% with farm management practices were observed (Fig. 14). Factor one (80.7%) indicated the role of soil fertility management practices done by the farmer. The trapping nematodes loaded more with manure, commercial fertilizers and the crop rotation. Endo parasitic nematode destroying fungi are affected more by the chicken manure. Chemical sprays do not play a significant role in the distribution of the nematode destroying fungi. Factor two separates chicken manure and endo-parasitic nematode destroying fungi from trapping nematode destroying fungi and all the other management practices.

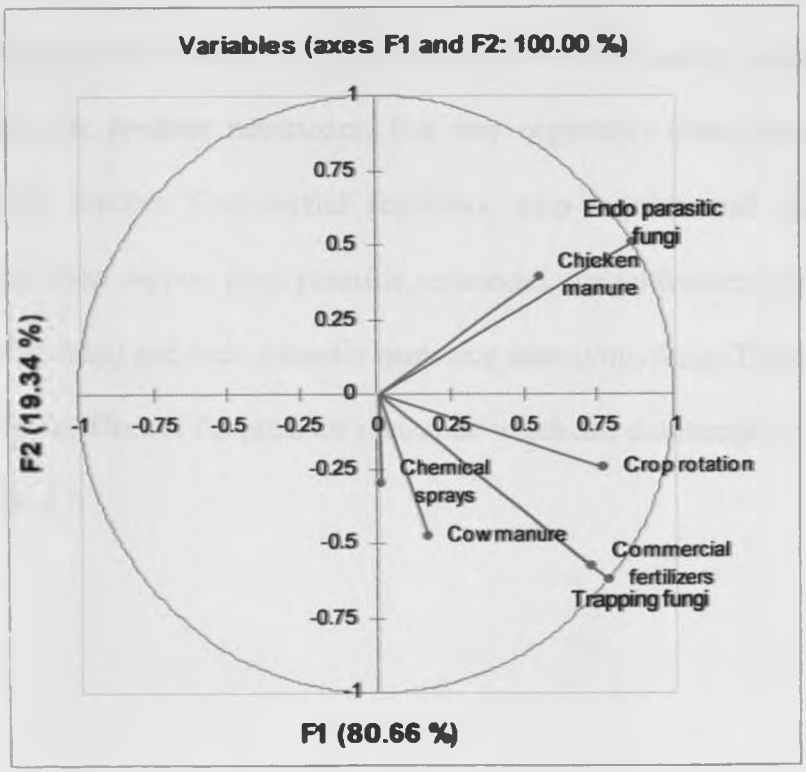

Fig.14. Canonical correlation for nematode destroying fungi and soil management practices.

When plotted together, factor 1 separates cow manure and chemical spray which encourages predator nematodes and bacterial nematodes, while commercial fertilizers, chicken manure and crop rotation encourage the plant parasitic nematodes and fungal feeding nematode which are associated with nematode trapping and endo parasitic fungi. Factor 2 separates cow manure harbors high population of predator nematodes, chemical sprays only encourage the bacterial feeders. Commercial fertilizers and crop rotation to a lesser extent increases the plant parasitic nematodes. The existence of plant parasitic nematodes encourages the nematode trapping fungi while fungal feeders stimulated buildup of the endo-parasitic fungi. The trapping fungi seemed to load with plant parasitic nematodes while the endo-parasitic fungi loaded with the fungal feeders. Chemical sprays do not affect the bacterial nematode feeders and neither the predator nematodes. But they negatively affect the plant parasitic and fungal feeders. Commercial fertilizers, crop rotation and chicken manure on the other hand support plant parasitic nematodes, fungal feeders, trapping nematode destroying fungi and endo parasitic nematode destroying fungi. This could be due to the negative effect of the predator nematode which are discouraged by the same practices (Fig .15)

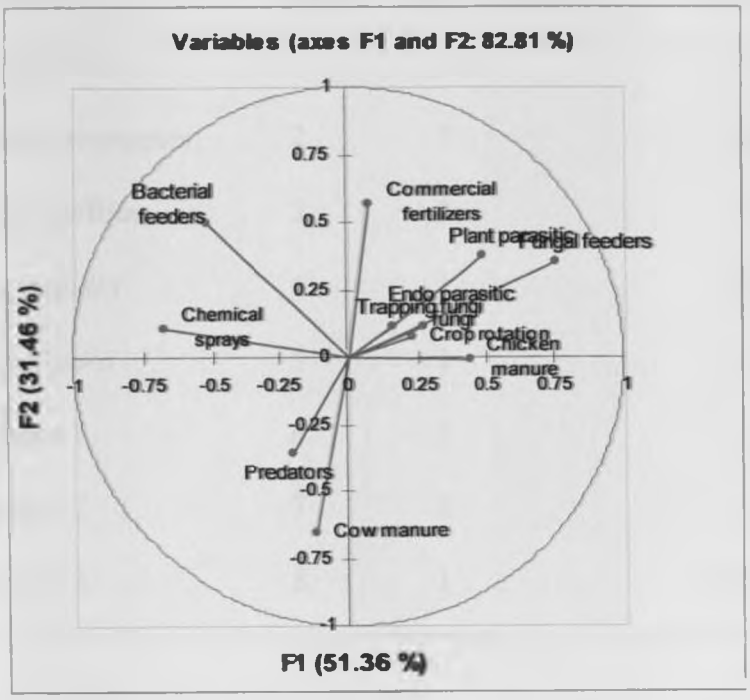

Fig.15. Canonical correlation for nematode destroying fungi, nematode community and soil management practices

After characterization of the experimental farms, the ten farms were significantly different in terms of occurrence of nematode destroying fungi (P = 0.01815). The two levels sampled did not show any significant differences in the occurrence of nematode destroying fungi. A total of twenty eight isolates were identified which belonged to five species and three unidentified species. *Arthrobotrys oligospora* had 53% of the total occurrence while the rest ranged between 10.7 and 3.6 % (Table 4.)

Table 4: Species of nematode destroying fungi identified in Taita Taveta.

Species Identified	Rank	Total abundance	Total percentage
Arthrobotrys oligospora	1	15	53.6
Monacrosporium cionopagum	2	3	10.7
Harposporium aungullilae	3	2	7.1
Arthrobotrys dactyloides	4	1	3.6
Arthrobotrys longispora	5	1	3.6
Unidentified species 1	6	2	7.1
Unidentified species 2	7	2	7.1
Unidentified species 3	8	1	3.6

After the treatment, cow manure had the highest means of occurrence of nematode destroying fungi, 1.4 while control had the least, 0.65. Though the means were not statistically different in all the treatments, mavuno fertilizer had the least mean of 1 while TSP+CAN had 1.10 (Fig. 16). The total number of isolates had increased to 64. *Arthrobotrys oligospora* was still the highly isolated isolate with an occurrence frequency of 40.6 %. There was no significant difference between the two levels of soil sampling, 0 – 10 and 10 – 20 though the upper level, 0 – 10 had the highest mean of occurrence, 1.13 compared to the second level 10 – 20 which had 1.0. All the farms were statistically different in the occurrence of nematode destroying fungi after the treatment with farm 2, 1 and 10 having the highest frequency while farms 3, 5, 7, 8 and 9 had the least Farms 4 and 6 were evarage. All the farms and treatments were positive of nematode destroying fungi.

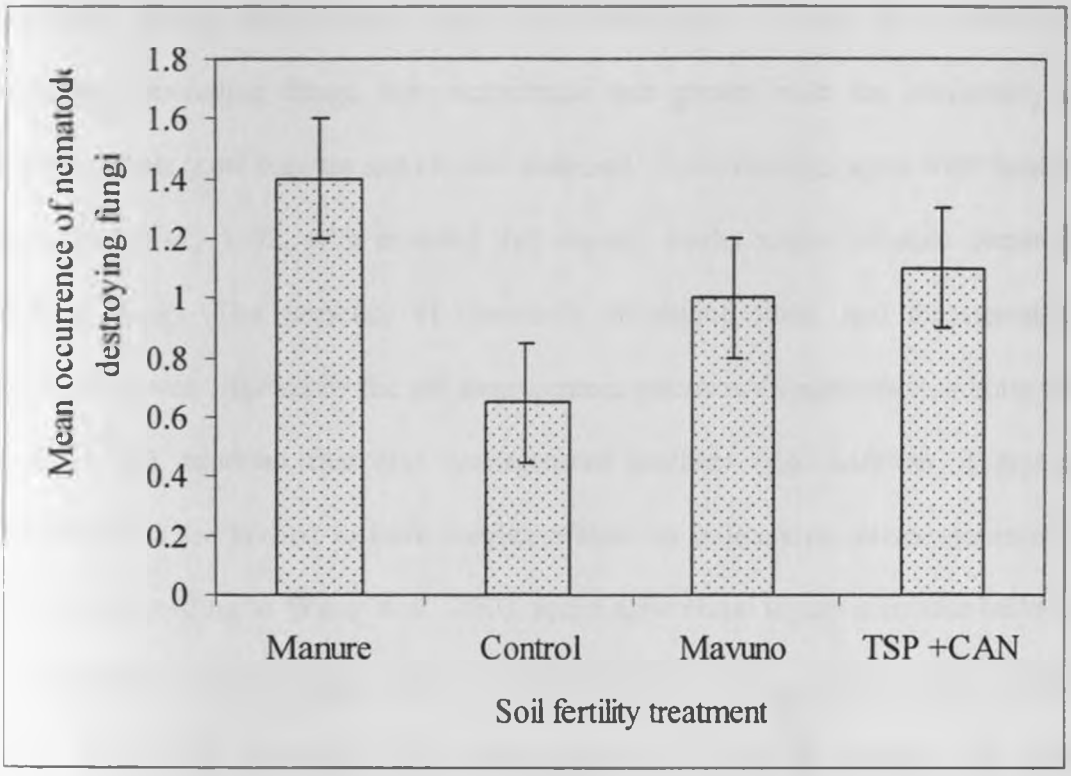

Fig.16. Effect of soil fertility treatment in Taita Taveta.

5.6 Discussion and conclusion

The nematodes destroying fungi occurred in all land uses in the study area, though their diversity varied. This shows the cosmopolitan nature of the fungi indicating their survival and adaptability in all ecosystems. These fungi are able to survive as saprophytes when the nematodes are not present or survive as resting spores until the nematode host is identified. Land use and organic inputs were found to be significant factors affecting the occurrence of the nematode destroying fungi. Land uses that received cow and chicken manures (organic inputs) favored the presence of

nematode destroying fungi. These were the horticulture, napier and the maize beans land uses. Though the inorganic inputs also significantly affected the occurrence of nematode destroying fungi, their occurrence was greater with the availability of organic inputs (cow manure and chicken manure). These findings agree with those of Dackman *et al.*, 1992, who reported that organic matter might enhance nematode trapping fungi. The presence of nematode destroying fungi and the nematode community was affected by the soil management practices. In agreement, Akhtar and Malik, 2000, reported that crop management practices (e.g. addition of organic amendments) are known to have varying effects on indigenous microorganisms in the soil. According to Wang *et al.*, 2003, some agricultural inputs stimulate build-up of nematode trapping fungi hence the observed diversity, evenness and richness. Also agricultural practices can exert positive or negative impacts on other microorganisms in the soil (Sanchez 1997; Akhtar & Malik 2000). The horticulture, maize/ bean and napier land uses receives more attention in terms of inputs since they are the main source of income (horticulture and napier) and food (maize/bean) (Mutsotso *et al.*, 2005; Sylvie, 2006).

From this study, the trapping nematode destroying fungi were strongly associated with plant parasitic nematodes while the endo-parasitic nematode destroying fungi were associated with the fungal feeding nematodes. This observation is of ecological importance because the trapping fungi which are the majority are able to select the plant parasitic nematodes from the other free living nematodes. This could be explained by the fact that the plant parasitic nematodes live outside the host after hutching from the eggs (J2). This is the most destructive stage of the nematode since

it leads to the attack of the plant roots by the nematodes. Unfortunately, the nematodes are at their weakest stage as they hunt for a host to feed. They are small in size and weak from hunger and are therefore easily caught by the fungi traps and adhesive mycelia. This observation was in agreement with the study conducted by Jasson *et al.*, 2000, who demonstrated that the nematode size determines the possibility of its capture with the big nematodes escaping the ring traps formed by the *A.dactyloides*. Since the fungal feeding nematodes feed on fungi fragments in the soil, the trophic phase of thc fungi is found on the body of the nematode. Only the conidia and conidiophores exist outside the nematode. Ecologically, these nematodes facilitate the distribution of these fungi in the environment where the fungi will live saprophytically in the absence of the nematodes. Upon their destruction by the fungi, the nematodes contribute indirectly to nitrogen mineralization (Dufour *et al.*, 2003). Since nematodes occupy a central position in the soil food web by occurring at multiple trophic levels, they have a unique potential to provide insights into the condition of the soil food webs (Dufour *et al.*, 2003). The nematode destroying fungi can also be used as indicators. In the association between nematode trapping fungi and the nematodes, the presence of trapping fungi could be associated with the presence of plant parasitic nematodes while endoparasitic nematode destroying fungi would indicate the presence of fungal feeding nematodes. Organic amendments (cow manure and chicken manure) that stimulated the nematode destroying fungi also stimulated the presence of free living nematodes and more so to the predator nematodes and the bacterial feeders. The predator nematodes predated on plant parasitic nematodes and the fungal feeders. All organic amendments tend to increase availability of nutrients, such as nitrogen, microbial biomass and abundance of

bacterivore nematodes (Bulluck *et al.*, 2002). On synthetic fertilizers, pesticides, and herbicides they are important inputs in conventional agricultural systems. They have been shown to impact on diversity and abundance of nematode trophic groups (Yeates and Bongers, 1999) therefore affecting the occurrence of the related nematode destroying fungi. Commercial fertilizers encouraged the plant parasitic nematodes therefore increasing the trapping nematode destroying fungi and reduced the free living especially the predator nematodes by the presence of salts. The salt in the fertilizers have a powerful reaction in the soil and actually can have more pulling power than a root of a plant. Therefore, a salty soil solution can hold water away from a root, and even dehydrate the root itself. Once the salty soil dehydrates the root, the bark will slide off, allowing the nematode to enter the plant. Another aid to nematodes is too much nitrogen which makes roots and tubers to grow faster than normal, so they crack open, and then comes the nematodes to kill the crop. The nematode will therefore dwell in areas where the soils are salty due to fertilizers (Garcia *et al.*, 2004). In agreement with the results from this study, Cheng *Zhi*-Ping (2008) reported that one major negative impact of chemical fertilizer on soil health was the increase in the relative abundance of plant-parasitic nematodes compared to the compost treatment. Wang *et al.*, 2006, noted that relative abundance of plant-parasitic nematodes was greater in the chemical fertilizer treatment. Chemical fertilizers promote vigorous plants growth because more root biomass is produced providing more feeding sites for plant-parasitic nematodes (Cheng *Zhi*-Ping, 2008)

From the experimental farm, addition of cow manure in the soil stimulated the population of nematode destroying fungi more compared to the other practices. Soils with high humus and low salt will not allow nematodes to live. Humic acid produced

on high humus soil will destroy harmful nematodes. In high humus soil, beneficial fungi, such as the fungi imperfecti families thrive. These fungi are "nematode eaters" - they can remove all active nematodes. Bacterial-feeders predominated in the nematode community, while the least opportunistic groups had a very low occurrence. The differences among the fertilizer treatments included in the study were not statistically significant. In the context of agrarian practices in organic agriculture, the use of organic amendments is considered a way to restore biodiversity in the edaphic environment (Garcia *et al.*, 2004).

In conclusion, it is evident that soil fertility management practices have an impact on nematode destroying fungi and the nematode community. Farm activities that include the use of animal manures could be recommended to the farmers as they are useful in restoration and maintenance of natural plant parasitic nematode regulatory processes.

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CHAPTER SIX

6.0 EFFECT OF ORGANIC AMENDMENTS ON NEMATODE-DESTROYING FUNGI AND PLANT PARASITIC NEMATODES

6.1 Abstract

A screenhouse and a field experiment were conducted to evaluate the effect of cow manure, chicken manure and their combinations (and a commercial fertilizer in the field) on nematode destroying fungi, nematode community and growth of tomato (*Solanum lycopersicum* L.). The amendments were applied at the rate of 5% w/w in all the treatments. Isolation of nematode destroying fungi was done using the soil sprinkle technique. Nematodes were extracted from soil using the modified Baermann technique. Tomato growth was estimated through plant height and dry weight. Application of the organic amendments resulted in significant differences ($P \leq 0.05$) in occurrence of nematode destroying fungi amongst the treatments. From the green house experiment, the nematode destroying fungi occurred at frequencies of 50, 29.4, 17.6 and 2.9% in soil amended with chicken manure, cow/chicken combination, cow manures and the control, respectively. Eight species of nematode destroying fungi were identified in this study. The fungus *Arthrobotrys oligospora* (Fresenius) was most dominant fungus in all the treatments including control pots with an isolation frequency of 38.2%. Addition of organic amendments into the soil also resulted in an increase of bacterial and fungal feeding nematodes and reduction of plant parasitic nematodes. Specifically there was a 225, 96 and 62% increase in

bacterial feeding nematodes and 391, 96 and 74% increase in fungal feeding nematodes in soil amended with chicken manure alone, combination of chicken and cow manure alone in that order. Numbers of plant-parasitic nematodes were 92% lower in soil treated with chicken manure compared to the control. Plant height and leaf widths were highest in plants treated with combination of cow and chicken manures. The plants mean dry weight were 6.6, 5.6, 2.0 and 1.5 in combination of chicken and cow manure, chicken manure alone, cow manure alone and control, respectively. Similar trends were observed from the field experiment with mean occurrence of nematode destroying fungi being 2.9, 2.5, 2.2, 1.8 and 1.1 in chicken manure, combination, mavuno fertilizer, cow manure and control in that order. The plant prarsits nematodes had mean of 183, 112, 95.2, 90, and 79 in control, combination, mavuno plots, cow manure and chicken manure in that decreasing order. The plots with mavuno fertilizer produced the highest marketable fruits with a mean weight of 7 kilograms, followed by chicken manure, combination, cow manure and the control with mean weights of 4.9, 4.8, 3.8 and 1.6 kilograms respectively. This study has, therefore, revealed that organic amendments stimulate the occurrence of nematode destroying fungi in the soil and reduce plant parasitic nematodes. In addition, the combination of cow and chicken manure stimulates plant growth.

Key words: *Biological control, Arthrobotrys oligospora, chicken manure, nematode community, mavuno fertilizer*

6.2 Introduction

Although nematodes are generally regarded as silent enemies, losses of up to 80% have been associated with them in vegetable fields that are heavily infested (Siddiqi 2000; Kaskavalci 2007). For decades, the control of plant-parasitic nematodes has mainly depended on chemical nematicides such as carbon disulphide, methylobromide, achock and others (Akhtar and Malik, 2000). Although nematicides are efficient and fast-acting, they are currently being reappraised with respect to the environmental hazards associated with them. In addition they are relatively unaffordable to many small-scale farmers. The persistent pressure on farmers to adopt strategies that do not pollute the environment has increased urgency in the search for alternative sustainable methods to regulate plant parasitic nematodes (Pinkerton *et al.*, 2000; Mashela *et al.*, 2008).

One of the alternative strategies for management of plant parasitic nematodes is the application of organic amendments in the soil (Agyarko and Asante, 2005). Oka *et al.*, (2000) pointed that organic amendments have consistently been shown to have beneficial effects on soil nutrients, soil physical conditions, soil biological activity and thereby improving the health of plants and reducing populations of plant parasitic nematodes. On the other hand, populations of free-living nematodes have also been shown to increase rapidly following the addition of organic substrates (Akhtar and Malik, 2000). Kimenju *et al.*, (2004) reported that application of organic amendments stimulated the activity of natural antagonists of plant parasitic nematodes. However the available reports do not mention the contribution of nematode destroying fungi in the reduction of plant parasitic nematodes yet they are

known to destroy nematodes in the soil. In vitro experiments have shown that nematode destroying fungi increase in numbers or activity when organic substrates are incorporated into the soil (Gomes *et al.*, 2001; Timm *et al.*, 2001). In a related study, Jaffee (2006) reported that alfalfa (*Medicago sativa* L.) leaves enhanced the populations of *Dactylellina candidum* (Nees) but the study did not mention other nematode destroying fungi.

Nematode destroying fungi are natural enemies of plant parasitic nematodes (Birgit *et al.* 2002). Some of these fungi use adhesive conidia, branches, knobs and mycelia to parasitize nematodes. These devices are used to capture and destroy nematodes by means of an adhesive layer covering part or all of the device surfaces (Yang *et al.*, 2007). Other fungi immobilize or kill nematodes by releasing toxins. This group of fungi has recently drawn much attention because of their potential as biological control agents of nematodes that are parasitic on plants and animals (Jansson and Persson, 2000; Sanyal, 2000; Masoomeh, *et al.*, 2004). This study was undertaken with the aim of determining the effects of organic amendiments pus a commercial fertlizer on occurrence of nematode-destroying fungi, nematode community in general and plant growth.

6.3 Materials and methods

Screenhouse experiments were carried out in the period between August 2007 and April 2008 at the University of Nairobi, Kenya. The amendments namely chicken manure, cow manure and the combination of chicken and cow manures were dried at 70 ^{0}C until a constant weight was achieved. The amendments were then applied at

the rate of 5% w/w (Kimenju *et al.*, 2004) into soil that was naturally infested with nematodes and nematode-destroying fungi from a vegetable farm. The pots were irrigated and two-week old tomato seedlings (cv Moneymaker) were transplanted into them. Un amended soil was used as a control. Treatments were arranged in a completely randomized design with five replications. This experiment was repeated in the field conditions with addition of mavuno fertilizer as a treatment. The five treatments (chicken manure, cow manure, their combination, inorganic fertilizer and the control) were replicated five times in a completely randomized design in a 3m x 3m plots. The fertilizer was applied at the manufactures recoomebdade rate. Soil was characterized in terms of nematode destroying fungi, nematodes and the chemical characteristics of the soil. Characterization was again done at the end of the experiment.

Isolation of nematode destroying fungi was done using the soil sprinkle technique as described by Jaffee *et al.*, (1996). Tap water agar was prepared by dissolving 20 grams of agar in one liter of tap water. The medium was autoclaved and cooled to 45^0C before amending it with 0.1 g/l of streptomycin sulfate to suppress bacterial growth. Approximately one gram of soil from each sampling point was sprinkled onto the surface of water agar in petri dishes. A suspension of *Meloidogyne incognita,* about 1000 larvae, was added into the Petri dish as bait. The plates were incubated at room temperature, approximately 28^0c± 1, and observed daily from the 3rd week to the 6th week under a microscope at low (40 x) magnification. The examination was focused on trapped nematodes, trapping organs and conidia of the nematode destroying fungi that grew from the soil. Identification of the fungi was

done using the key described by Cooke and Godfrey (1964). Nematodes were extracted from 200 cm^3 soil using the modified Baermann funnel technique as described by Hooper *et al.*, (2005). The nematodes were identified to genera levels using the descriptions described by Bongers and Bongers (1988) and Mai and Mullin (1996), counted and the grouped according to feeding habits. Growth of tomato plants was monitored at the 4^{th} and 7^{th} weeks by assessing the plant height, leaf width- apical leaf of 3^{rd} branch, internodal length (between 3^{rd} and 4^{th} branch) and the type of flower/flowering pattern. Shoot and root dry weights were taken at the end of the experiment after drying the samples at 70 ^0C to constant mass.

6.4 Data Analysis

All the data were tested for homogeneity and subjected to analysis of variance (Kindt and Coe, 2005). Where the overall F test was significant, means were compared using the Tukey Honest Significance test (HSD) at $P \leq 0.05$.

6.5 Results

From the screen house experiment, differences in occurrence of nematode destroying fungi was significant ($P = 0.05$) among the treatments with means of 3.4 in chicken manure alone, 2.0 in combinations of cow and chicken manure, 1.2 in cow manure and 0.2 in control (Table 5).

Table 5. Effect of organic amendments on occurrence of nematode destroying fungi.

Amendment	Mean occurrence of nematode destroying fungi
Control	0.2
Cow Manure	1.2
Cow/Chicken manure	2.0
Chicken manure	3.4
P - value	3.604×10^{-05}

The nematode destroying fungi occurred in frequencies of 50, 29.4, 17.6 and 2.9% in chicken manure, cow/chicken combination, cow manures and control respectively (Fig 17). Out of the nematode destroying fungi isolated, 71% were in the category of nematode trapping fungi while 29% of them were endo-parasitic. Fifty percent of all the nematode destroying fungi were recorded in the soil treated with chicken manure. The mean richness of nematode destroying fungi was 3.4 in chicken manure and 0.2 in the control. Combinations of cow and chicken manure had a mean richness of 2.0 while cow manure had 1. Soil amended with chicken manure was the most diverse in terms of nematode destroying fungi with mean shannon of 1.2. Combinations of cow and chicken was 0.64 diverse followed by cow manure alone with, 0.28 while the control had 0.

Of all the fungal isolates, *Arthrobotrys oligospora* (Fresenius) was most dominant and its occurrence was significantly different ($P \leq 0.05$) across the treatments with an isolation frequency of 38.2%. The other nematode destroying fungi had isolation frequencies of 26.5, 17.6, 8.8, 5.9 and 2.9% in *Harposporium aunguillulae* (Lohde), *Arthrobotrys dactyloides* (Drechsler), *Monacrosporium cionopagum* (Drechsler), *Adhesive* hyphae and *A.superba* (Corda), respectively. *Arthrobotrys superba, H. aunguillulae, M. cionopagum,* and the Adhesive hyphae did not seem to respond to the treatments. The occurrence of *Athrobotrys dactyloides* was significantly different ($P = 6.837 \times 10^{-05}$) in all the treatments. Soils amended with chicken manure alone were characterized by presence of *A. oligospora, H. angullilae, A. superba, A. dactyloides, M. cionophagum* and nematode trapping structures such as adhesive hyphae. Soils amended with the combination of chicken and cow manure harbored populations of *A. oligospora,*

A. dactyloides, *H. angullilae* and Adhesive hyphae. Nematode destroying fungi, *A. oligospora, H. angullilae and A. dactyloides* were isolated from soils amended with cow manure alone.

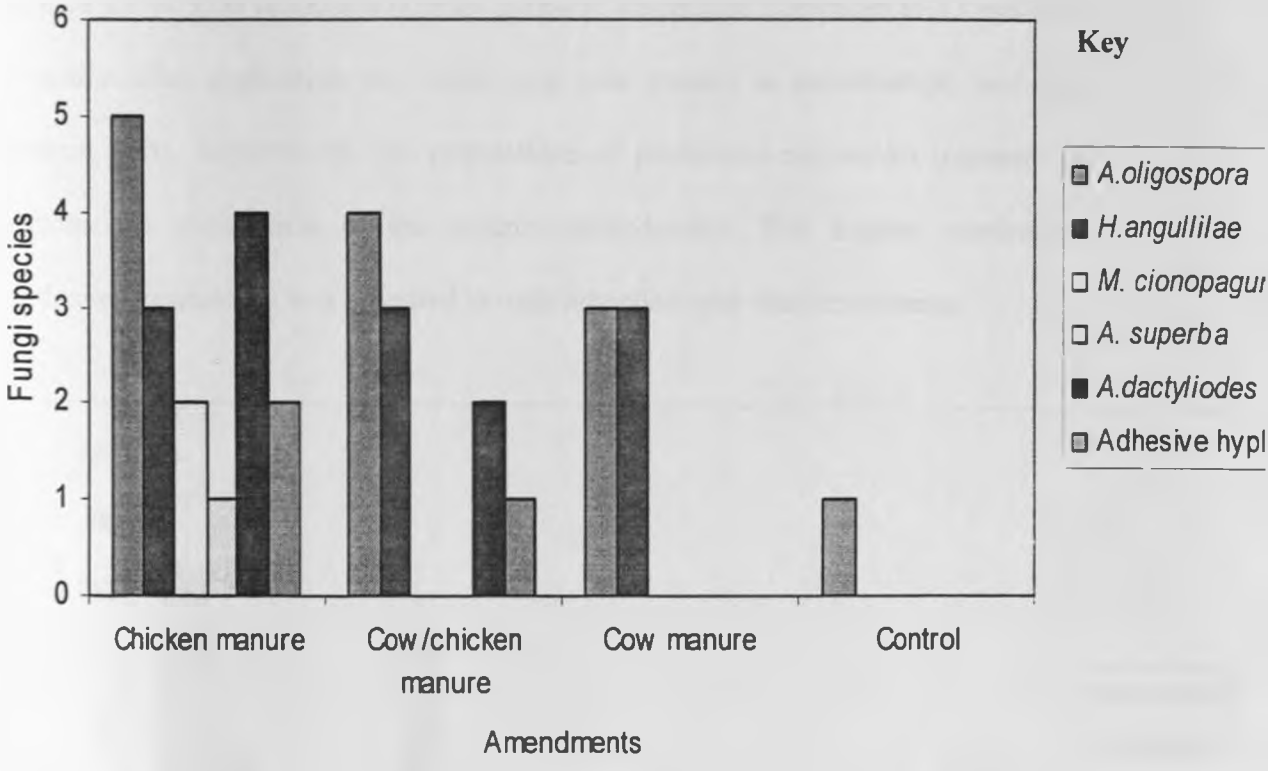


Fig.17. Effect of organic amendments on occurrence of nematode destroying fungi

On nematode community, organic amendments resulted in a significant change in composition of the nematode community (Fig. 18). Application of the organic amendments caused an increase in numbers of bacterial and fungal feeding nematodes. There was an increase of 225, 96 and 62% in bacterial feeding nematodes in soils amended with chicken manure, combination of chicken and cow manure and cow manure, respectively. Similarly, application of chicken manure

alone, combination of chicken and cow manure and cow manure alone led to a 391, 96 and 74% respective increase in fungal feeding nematodes. In addition, application of the amendments suppressed the numbers of plant parasitic nematodes. Chicken manure led to 92% reduction in plant parasitic nematodes compared to 73 and 55% reduction after application of chicken and cow manure in combination, and cow manure alone, respectively. The populations of predacious nematodes increased in response to application of the organic amendments. The highest number of predacious nematodes was recorded in soils amended with chicken manure.

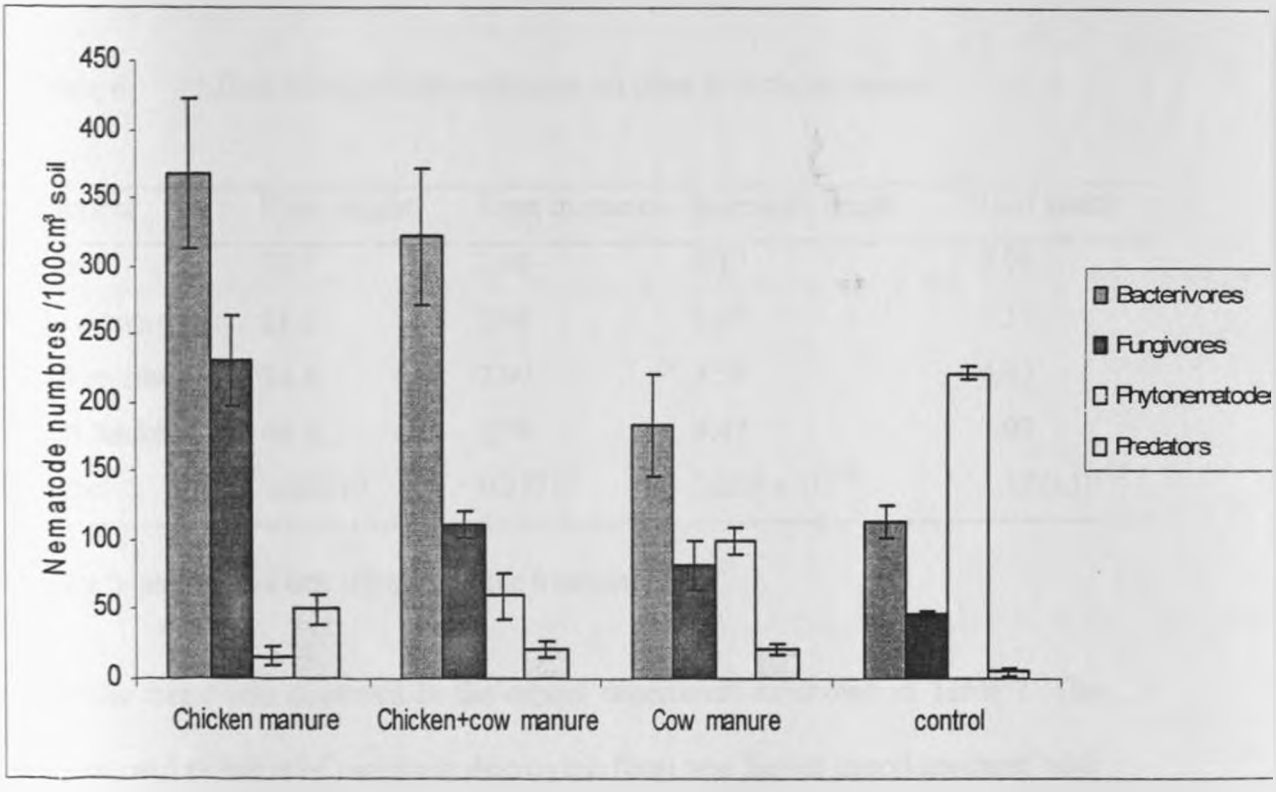


Fig.18. Effect of organic amendments on nematode community structure.

Further results showed that organic amendments caused significant differences ($P \leq 0.05$) in the plant height. The mean heights were 46.8, 34.8, 31.5 and 32.1cm in

combinations of cow and chicken manure, chicken manure alone, cow manure alone and control, respectively (Table 6). The number of branches had significantly increased in all the treatments in the 7th week than 4th week ($P = 2.2 \times 10^{-16}$). In the 7th week, the plants were significantly taller and with thicker shoot than in 4th week. Flower induction was earliest and more pronounced in tomato plants grown in soil amended with chicken manure, chicken/cow manure, cow manure and least in control pots. The combination of chicken and cow manure had the highest mean dry weight of 6.6 kilograms. Chicken manure alone recorded 5.6, cow manure alone 2.0 and 1.5 kilograms in control in descending order.

Table 6. Effect of organic amendments on plant growth parameters

Treatment	Plant height	Stem diameter	Internode length	Leaf width
Control	32.1	2.46	6.17	3.99
Cow manure	31.5	2.68	3.67	3.52
Chicken manure	34.8	2.90	3.59	4.43
Cow/Chicken	46.8	2.76	4.47	5.03
P value	0.03319	0.2778*	3.830×10^{-08}	1.822×10^{-08}

* Growth parameters not affected by the treatments.

A similar trend was observed in the repeat experiment as shown in Table 7. The diversity and richness of nematode destroying fungi was higher in soil amended with chicken manure alone, combination of chicken and cow manure and cow manure alone as compared to the control. Plant parasitic nematode numbers were significantly lower ($P = 3.039 \times 10^{-09}$) in soils amended with chicken manure alone and all the other amendments compared to the control.

Plant growth parameters were significantly higher in soils with organic amendments compared to the control. The plant height and mean dry weight were higher in chicken manure treatments, followed by a combination of cow and chicken manure, cow manure and least in the control. Amendments with chicken manure also recorded more flowers than cow manure alone and control.

Table 7. Effect of organic amendments on plant growth, diversity and richness of nematode destroying fungi and plant parasitic nematodes.

Treatment	Plant height	Flower production	Dry weight	Mean Shannon index of NDF	Mean richness of NDF	Mean PPN
Control	30.3	0.1	1.36	0.4564	1.6	222.0
Cow manure	42.6	0.5	2.25	0.7049	2.0	141.5
Chicken and cow	53.3	1.0	3.37	0.9247	2.7	84.0
Chicken manure	57.3	1.0	4.23	1.0450	3.0	58.5
P-Value	6.189×10^{-07}	7.906×10^{-08}	1.33×10^{-05}	0.02466	0.03596	3.039×10^{-05}

* PPN Plant parasitic nematodes.
* NDF Nematode destroying fungi

In the field experiment, a total of 105 nematode destroying fungi were isolated. They belonged to which included five genera, *Arthrobotrys, Meria, Harposporium, Monacrosporium* and *Nematoctonus*. Two isolates could not be identified. *Arthrobotrys oligospora* was the most occurring nematode destroying fungus. It had a proportion of 44.8% of the total nematode destroying fungi isolated. *Nematoctonus leiosporus*, and the two unidentified nematode destroying fungi were the least in occurrence with a proportion of 1% each (Table 8). The occurrence of nematode

destroying fungi was significantly different across the treatments ($P = 3.724 \times 10^{-4}$). The mean occurrence of these fungi was ; 2.9, 2.5, 2.2, 1.8 and 1.1 in chicken manure, combination, mavuno fertilizer, cow manure and control in that order . The occurrence of *A.dactyloides, A. longispora, Harposporium aunguillulae, Meria coniospora*, was affected by the treatments (significant codes, 0.001, 0.05) while *A. oligospora, A. superba, Monacrosporium cionopagum, Nematoctonus leiosporus* were not affected by the treatments.

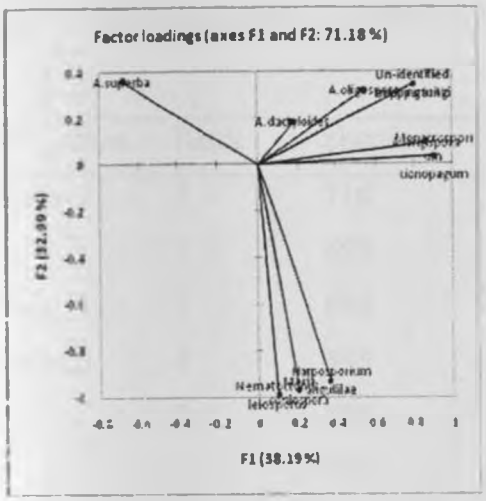
Table 8: Rank and abundance table of the nematode destroying fungi isolated in the field.

Isolate	Rank	abundance	Proportion%
Arthrobotrys oligospora	1	47	44.8
Arthrobotrys longispora	2	29	27.6
Meria coniospora	3	9	8.6
Harposporium aunguillulae	4	6	5.7
Monacrosporium cionopagum	5	5	4.8
Arthrobotrys dactyloides	6	3	2.9
Arthrobotrys superba	7	3	2.9
Un-identified trapping fungi	8	1	1.0
Nematoctonus leiosporus	9	1	1.0
Un-identified endoparasitic fungi	10	1	1.0

Factor analysis indicated that two main factors accounted for 71.19 % of the occurrence of nematode destroying fungi. Factor one, accounted for 38.19 % of the observed variation. It separated chicken manure, mavuno and combination from the control and the cow manure. The second factor separated the chicken manure from mavuno, combination, cow manure and the control. The endo-parasitic fungus

(*Harposporium aunguillulae, Meria coniospora* and *Nematoctonus leiosporus*) loads more with chicken manure. The trapping fungi (*A. oligospora, A. dactyloides and Monacrosporium cionopagum*) loaded more with the combination of chicken and cow manure and very slightly on Mavuno fertilizer. *Arthrobotrys superba* loaded with the cow manure. Chicken manure and combination attracted all the nematode destroying except for the *A. superba* which was attracted more by the cow manure and the control. The combination attracted trapping nematode destroying fungi (*A.oligospora, Monacrosporium cionpagum and A. dactyloides*) (Fig. 19 a,b).

The soil parameters estimated did not show any significant difference with the treatments. The soil pH was least in mavuno, cow manure, control, comination and highest in chicken manure. While phosphorus was highest in mavuno fertilizer, it was least in cow manure. The soil characterization parameters showed that Mavuno fertilizer has high amount of phosphorus and magnesium. Increasing the use of mavuno fertilizer in farm would therefore lead to increased pH with soil becoming alkaline. It would also reduce the amount of calcium chloride and calcium in the soil. The control plots had high potassium and cat ion exchangeable capacity which reduced the availability of nitrogen and carbon. Chicken manure, cow manure and combination were associated with increased nitrogen, carbon, pH, calcium and calcium chloride. The addition of organic amendments, (chicken manure, cow manure and their combination), reduces the amount of magnesium and phosphorus in the soil (Fig. 20 a,b)



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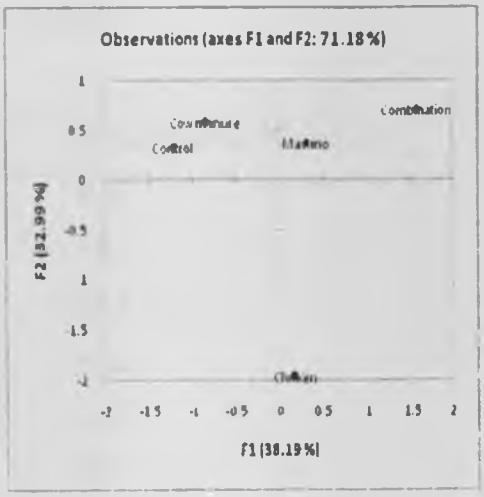

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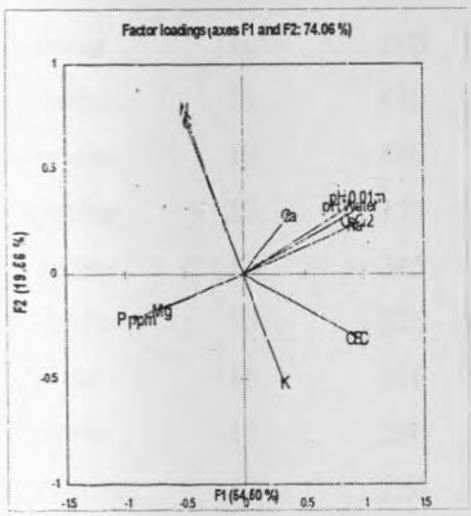
Fig. 19 a, b. Effect of soil fertility treatments on the nematode destroying fungi



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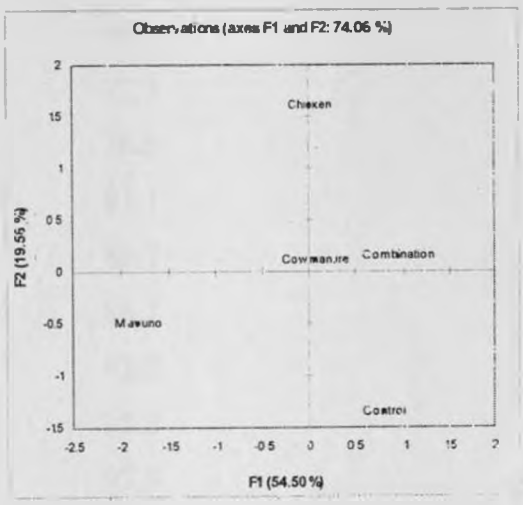

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Fig. 20 a,b. Effect of the soil fertility treatments on soil chemicals

A total of 8,871 nematode isolates were identified from the field experiment. They were grouped into 19 genera (Tabel 9). Overall, the treatment did not significantly affect the nematode community ($P \geq 0.05$).

Table 9: Nematode genera indentified in the field experiment

Nematode genera	Rank	abundance	Proportion	accumulative frequency
Eucephlobus	1	710	8.0	8.0
Plectus	2	695	7.8	15.8
Mononchus	3	680	7.7	23.5
Achromadora	4	595	6.7	30.2
Acrobeles	5	575	6.5	36.7
Alaimus	6	575	6.5	43.2
Tobrilus	7	555	6.3	49.4
Helicotylenchus	8	531	6.0	55.4
Aphelenchoides	9	525	5.9	61.3
Nygolaimus	10	490	5.5	66.9
Aphelenchus	11	480	5.4	72.3
Scutellonema	12	410	4.6	76.9
Pratylenchus	13	375	4.2	81.1
Meloidogyne	14	345	3.9	85.7
Tyelinchulus	15	325	3.7	88.7
Criconema	16	310	3.5	92.2
Trichodorus	17	280	3.2	95.3
Paratylenchus	18	220	2.5	97.8
Hemicyclophora	19	195	2.2	100.0

The occurrence of plant parasitic nematodes was significantly different ($P = 2.24 \times 10^{-4}$) across the treatments with the highest number being in the control and the least in plots treated with chicken manure. The mean of plant parasitic nematodes were 183, 112, 95.2, 90, and 79 in control, combination, mavuno plots, cow manure and chicken manure in that decreasing order. Though not significantly different, ($P \geq 0.05$) the bacterial feeders were more in plots treated with chicken manure and less in

control plots. The means recovered were 90, 141, 164, 176, and 170 in control, chicken manure, combination, mavuno and cow manure in that order. A significant effect of treatments was also observed in fungal feeding nematodes ($P = 2 \times 10^{-2}$) where high numbers were recorded in chicken manure with a mean value of 71 and least in the control with a mean value of 14. The predator nematodes were significantly affected by the treatment ($P = 8.1 \times 10^{-2}$). The highest mean (63) was recorded in cow manure and the least (31) were control and the combination (Table: 10).

Table 10: Mean value of the nematode groups per treatment

Treatment	Plant parasitic	Predators	Fungal feeders	Bacterial feeders
Chicken manure	79	57	71	176
Cow manure	90	63	20	170
combination	112	31	56	164
Control	183	31	14	90
Mavuno	95.2	52	20	141
P-Value (0.05)	0.02239	0.08121	0.01999	0.1179

The treatments with organic amendments were the richest in terms of nematode numbers. Treatments with cow manure had the highest number of nematodes while the control had the least. The cow manure had the highest diversity index (2.6) with the least being recorded in chicken manure with 2.5 (Fig. 21)

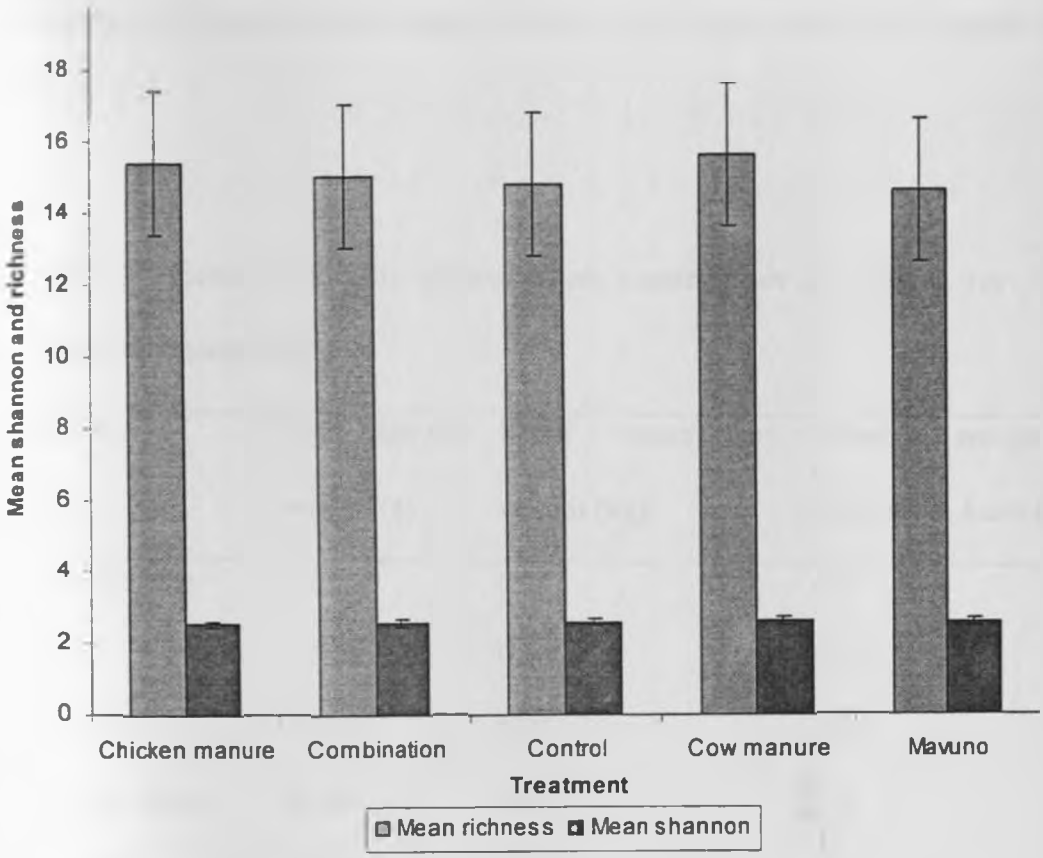

Fig. 21. The effect of soil fertility treatments on the diversity and richness of the nematode community

All the treatments were significantly different ($P \geq 0.5$) on dry shoot weight, dry root weight and tomato fruits harvested. The combination of cow and chicken manure had the heaviest mean dry shoot weight followed by mavuno, cow manure, chicken manure and least in the control. The plots with mavuno fertilizer produced the highest amount of marketable fruits with a mean weight of 7 kilograms, followed by chicken manure, combination, cow manure and then the control with mean weight of 4.9, 4.8, 3.8 and 1.6 respectively. The plots with chicken manure treatments had the highest mean root dry weight of 0.053, then combination, mavuno, cow manure and

the control with mean root dry weight of 0.0508, 0.049, 0.0476 and 0.02 respectively (Table 11).

Table 11: Effect of fertility treatments on tomato shoot dry weight, root dry weight and marketable fruits

Treatment	Mean root dry weight (g)	Mean shoot dry weight (kg)	Mean weight of marketable fruits (kg)
Chicken manure	0.053	0.94	4.9
Control	0.0200	0.54	1.6
Cow manure	0.0476	0.96	3.8
Mavuno fertilizer	0.049	1.04	7.0
Combination	0.0508	1.06	4.8
P value (0.05)	0.03704	0.02659	0.0004467

In summary, the results from this study indicate that the organic amendments stimulated the occurrence of nematode destroying fungi, changed the nematode community by reducing the plant parasitic nematodes. In addition, amendments enhanced plant growth vigor. Specifically, chicken manure alone enhances the diversity and richness of nematode destroying fungi and reduction of plant parasitic nematodes. The combination of chicken and cow manure was the best in stimulation of plant growth. Mavuno fertilizer had the highest harvestable produce although it had high number of plant parasitic nematodes

6.6 Discussion and conclusion

This study has revealed that organic amendments and especially chicken manure stimulated build-up of nematode destroying fungi, *Arthrobotrys oligospora, Harposporium angullilae*, *A. superba*, *A. dactyloides*, *Monacrosporium cionopagum*, and related nematode-destroying structures in the soil. The organic amendment supplies the needed food sources to the nematode trapping fungi hence their enhancement. This is supported by the findings of Timm *et al.*, 2001, who suggested that the increase in nematode-trapping fungi after addition of organic amendment is due to available carbon and energy from the organic amendment and nitrogen from consumed nematodes. In a related study, Jaffee, 2006, also showed that organic amendments enhanced build-up of nematode-trapping fungi *Dactylellina candidum* (Nees) though no other fungi were mentioned, and are thought to be influenced differently depending on their feeding mechanism (parasitic of saprophytic).

The fungi *Arthrobotrys oligospora* was the most enhanced in this study by the organic amendments and especially by the chicken manure. Probably compounds containing ammonia also enhance the population of nematode destroying fungi. From this study, chicken manure and then combination of chicken and cow manures stimulated the buildup of nematode destroying fungi as well as reducing the population of plant parasitic nematodes. The biological control efficacy of ammonia like the one found in chicken manure, has been shown to be equivalent to that of 1,3-D, chloropicrin, metam-sodium, cadusafos, or metam-sodium (Yucel *et al.*, 2002; Koenning *et al.*, 2003). In a related study, Jaffee, 2004, reported that *Arthrobotrys oligospora* was enhanced by large quantities of alfalfa amendments. Alfafa leaves

supplied nitrogen in the soil, which in turn increased the population of *A.oligospora* in the soil. Viaene *et al.*, 2006, reported that *A.oligospora*, which immobilizes nematodes by using mycelial traps such as non-adhesive knobs and constricting rings, could be used as a biological control agent of plant parasitic nematodes. Other species of *Arthrobotrys* have been used as biological control of plant parasitic nematodes with recommendable success (Kiewnick *et al.*, 2004). Although strong indications of nematode trapping fungi suppressing nematodes have been demonstrated in the laboratory using Petri dishes (Elshafic *et al.*, 2006), it is till not clear with field and greenhouse experiments (Jaffee and Strong, 2005, Jaffee *et al.*, 2007). However, the role of nematode destroying fungi in reducing the population of plant parasitic nematodes in the soil is not clear.

It has been demonstrated in this study that application of organic soil amendments resulted in changes in nematode community structure by increasing the abundance of free-living nematode populations and suppressing plant parasitic nematodes. Application of soil amendments is becoming a conventional practice that helps in the control of nematodes and other soil-borne diseases. Comprehensive studies like those of Koenning *et al.*, 2003, have revealed the nematicidal potential of organic products used as soil amendments. When incorporated into the soil, organic substrates undergo biologically mediated decomposition to release NH4+, formaldehyde, phenols and volatile fatty acids, among other compounds (Wang *et al.*, 2004). The involvement of soil micro-organisms in nematode control in amended soils has been confirmed by the fact that soil irradiation disrupts the nematicidal effect of these amendments (Kaskavalci, 2007). It has been established that

application of organic substrates leads to build-up of micro-organisms which serve as food substrates for free-living nematodes hence their build-up. Populations of free-living nematodes such as bacteriovores, fungivores and predators have been shown to rapidly increase following the addition of organic amendments (Akhtar and Malik, 2000; Jaffee, 2002). In addition, free-living nematodes may accelerate the decomposition of soil organic matter and increase mineralization of nitrogen and phosphorous thus triggering a chain reaction that favours their build-up (Widmer and Abawi, 2000; Kimenju *et al.*, 2004). Yucel *et al.*, 2002, is categorical that organic amendments that have high nitrogen content and release ammonia upon decomposition are more effective in nematode suppression.

In this study plants grown on soil amended with organic substrates grew and differentiated faster reaching flowering stage earlier than the control. Unlike the nematode destroying fungi and the nematode communities which were enhanced by the chicken manure, the growth of the plants was more enhanced by the combination of chicken and cow manures. The increase in growth is attributed to the release of macro - and micronutrients, plant growth regulators and stimulation of beneficial micro flora such as the mycorrhizae fungi (Kaskavalci, 2007). In the current study, the plants grown on organic amendments were taller and heavier compared to the control. Since dry weight is used to estimate productivity, (Opik *et al.*, 2005) productivity would be expected to be higher in plants treated with organic amendments. Though the inorganic fertilizers were not tested in the current study, the findings are in agreement with Widmer and Abawi, 2000, who reported that plants grown in plots receiving organic manures were always larger than those

receiving inorganic fertilizers. In a separate study, weights of tomato plants grown in the ammonia-treated soils were about six fold greater than the control. Amendments therefore represent important resource for the improvement of soil fertility because decomposed materials ultimately serve as sources of nutrients for plants and thus improve crop yields. The increase in crop vigour may partly be attributed to reduced plant-parasitic nematode populations, but nutrients availability cannot be ignored. In a previous study, decrease in populations of parasitic nematodes has been associated with increased crop yield. In turn, the decline in plant parasitic nematodes in this study could probably be attributed to the high number of nematode destroying fungi. The association between nematode-trapping fungi, organic matter, plant growth and nematodes community is complex. It would be difficult to conclude that the increase of nematode destroying fungi would lead to automatically reduction of plant parasitic nematodes hence healthy plants. Akhtar and Malik, 2000, suggested that free-living nematodes reproduce rapidly when presented with organic substrate and play an important role in recycling of plant nutrients making them available to plants. Such organic substrates again have been seen to support high numbers of nematode destroying fungi and reduced populations of plant parasitic nematodes. Therefore, low numbers of plant parasitic nematodes coupled with high numbers of nematode destroying fungi and high nutrient levels in the soil has a positive effect to plant growth. It would be impossible to attribute the performance of the crop to either of them. In order to access the contribution of nematode destroying fungi, there is need to address the quantification and efficacy methods (Jaffee, 2006). More work should

be devoted to the correlation between populations of nematode destroying fungi, plant parasitic nematodes and the actual plant yield.

This study has demonstrated the potential of organic amendments in stimulation of nematode destroying fungi for the management of plant-parasitic nematodes. The occurrence and diversity of nematode destroying fungi was associated with the decreased number of plant parasitic nematodes. Amendments cause improved plant growth and changes the nematode community structure particularly leading to decreased plant parasitic nematodes. However such alternative nematode management strategies are unlikely to be as effective and fast-acting as nematicides. Although nematicides would reduce the plant parasitic nematodes, other environmental and soil fertility issues arise. Therefore sustainable management of plant-parasitic nematodes from addition of organic amendments to the soil overrides all other considerations.

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CHAPTER SEVEN

7.1 GENERAL DISCUSSION

From the study, it is evident that all the sampled land uses differed in terms of occurrence of nematode destroying fungi, consistent with previous reports indicating that nematode destroying fungi were present in all habitats but at different densities and diversities (Nordbring-Hertz *et al.* 2002). The fungi that were isolated exhibited several mechanisms of capturing and destroying plant parasitic nematodes that included constricting rings, adhesive nets, and non-constricting rings. The structures identified are consistent with the reports of other authors (Masoomeh *et al.*, 2004; Farrell *et al.*, 2006; Yang *et al.*, 2007; Jinkui *et al., 2007*), who identified three main groups of nematode destroying fungi. The first group is the nematode trapping, then the endoparasitic fungi that attack vermiform living nematodes by using specialized structures and third, the egg-and cyst-parasitic fungi that attack these stages with their hyphal tips.

The study has also revealed that increased land use intensity resulted in increased occurrence and diversity of nematode destroying fungi. This, however, was contrary to expectation that beneficial microorganism decrease with increased intensity in land use (Vandermeer *et al.* 1998). A number of explanations can be used to account for the higher frequency of occurrence of nematode destroying fungi in the habitats that are subject to regular disturbance compared to the stable ecosystems like shrub land and indigenous forest. It is also possible that fungal tissues are fragmented and scattered in the course of farm operations, thus increasing their frequency of detection. According to Wang *et al.*, 2003, some agricultural inputs stimulate build-up of nematode trapping fungi hence the observed diversity, evenness and richeness with increased land use intensity compared to land uses which are materially unchanged by human activity (forest and shrub land). Intensive cultivation is

characterized by increased movement of soil which may result in increased spread of the microorganisms in the field. Soil disturbance, coupled with frequent changes in crop cover, subjects the soil biota to strees 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 nematode destroying fungi are able to establish and suppress other species that are poorly suited to compete effectively. The horticulture, napier and the maize/ bean land uses receives more attention in terms of inputs since they are the main source of income (horticulture and napier) and food (maize/bean) (Mutsotso *et al.*, 2005; Sylvie, 2006). Therefore agricultural practices can exert positive or negative impacts on other microorganisms in the soil (Sanchez 1997; Akhtar & Malik 2000).

Arthrobotrys oligospora was the most abundant species of nematode destroying fungi in the study area. One possible explanation in this study would be the presence of inorganic and organic inputs in the soil applied by the farmers. Jaffee, 2004, showed that organic amendments enhanced build-up of resident nematode-trapping fungi in the soil. Farrell *et al.*,2006, observed 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%. Higher soil organic matter content protects plants against nematodes by increasing soil water-holding capacity and enhancing the activity of naturally occurring biological organisms that compete with nematodes in the soil (Kaskavalci, 2007). Apart from presence of organic matter, the fungi also obtain its carbon and energy from two sources, from organic matter (saprophyte) and from trapping nematodes (parasite) making it adaptable to wide range of habitats. It is possible that members of the genus were the best adapted to the biotic and abiotic conditions prevailing in the study area.

From the study, the trapping nematode destroying fungi are strongly associated with plant parasitic nematodes while the endo parasitic nematode destroying fungi are associated with the fungal feeding nematodes. This observation is of ecological importance because the trapping fungi which are the majority are able to select the plant parasitic nematodes from the other free living nematodes. This could be explained by the fact that the plant parasitic nematodes live outside the host after hutching from the eggs (J2). This is the most destructive stage of the nematode since it leads to the attack of the plant roots by the nematodes. Unfortunately, the nematodes are at their weakest stage as they hunt for a host to feed. They are small in size and weak from hunger and are therefore easily caught by the fungi traps and adhesive mycelia. This observation was in agreement with the study conducted by Jasson and Persson, 2000, who demonstrated that the nematode size determines the possibility of its capture with the big nematodes escaping the ring traps formed by the *A.dactyloides*. Since nematodes occupy a central position in the soil food web occurring at multiple trophic levels and, therefore, have the potential to provide insights into condition of the soil food webs (Dufour *et al.*, 2003), these nematode destroying fungi too would be used as indicators of soil disturbance.

The most abundant fungi was *Arthrobotrys oligospora*. This fungus would be recommended for further study with the aim of developing it as a biological control agent. Such a study should be geared towards growth parameters of the fungus, since biological, chemical and physical factors of the soil are known to inhibit fungal growth by fungistatic compounds and is made even more complicated by crop

rotations. The ability of this fungus as a biological control agent could be improved through genetic engineering and then packaged for biological control pourposes. Apart from introduction of particular species from the genus, agricultural practices that stimulate build-up of the fungi could be identified and recommended for adoption by farmers.

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7.2 CONLUSION AND RECOMMEDATION

This is the first account of a focused study on nematode destroying fungi in Kenya. The study has confirmed that nematode destroying fungi occur in the study area and their distribution is influenced by land use and land management system. The study has indicated a potential of nematode destroying fungi that can be unlocked in the development of alternative management of plant parasitic nematodes. Land use practices that promote high populations of nematode destroying fungi in the soil especially the use of organic amendments, are recommended to the farmers.

Further study on nematode destroying fungi is recommended in this study especially on:

- interactions between nematode community and nematode destroying fungi
- formulation of nematode destroying fungi as a biological control of plant parasitic nematodes
- The mode of application of nematode destroying fungi, either to drench the soil before planting or coat the plants or parts of the plants or the seeds.

7.3 APPENDICES

7.3.1 Key to the nematode destroying fungi

1 **Endozoic parasites with assimilative hyphae within the host, fertile hyphae of limited extent passing out of the host after death, nematodes sometimes trapped on adhesive cells borne on these hyphae.....2**

1. Endozoic parasites with vegetative thalli or hyphal bodies within the host, producing motile or non-motile spores in sporangia or conidia on conidiophores; zygospores or azygospores sometimes formed.....26

1. Non-endozoic predators capturing nematodes on their hyphae by a variety of means, then invading the nematode and assimilating its body contents...33

2. Fertile hyphae aseptate, conidial branches narrow at base, swelling, and then narrowing again, recurved, bearing a single pea-pod shaped or filiform conidium.....3

2. Fertile hyphae septate.....6

3. Conidia pea-pod shaped.....4

3. Conidia filiform, curved, with a pouch-like proximal appendage.....5

4. Conidia 4.5-5.5 x 1.1-1.3μ, slightly curved, expanded at distal end, with a pointed protuberance on convex side (Fig, *2a) Euryancale obliqua* Drechsler (1955)

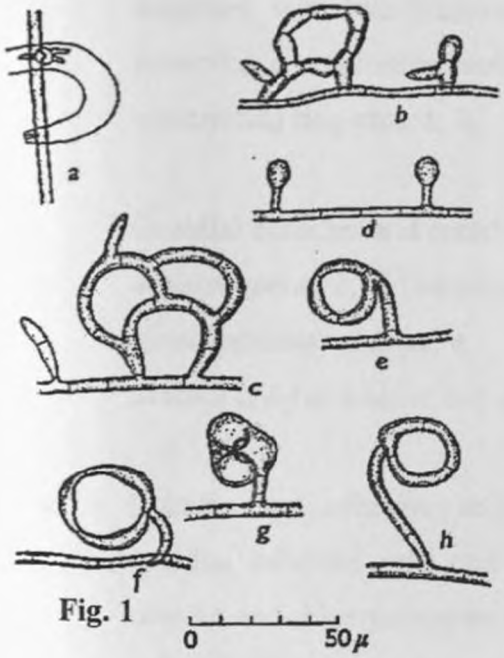

Fig. 1

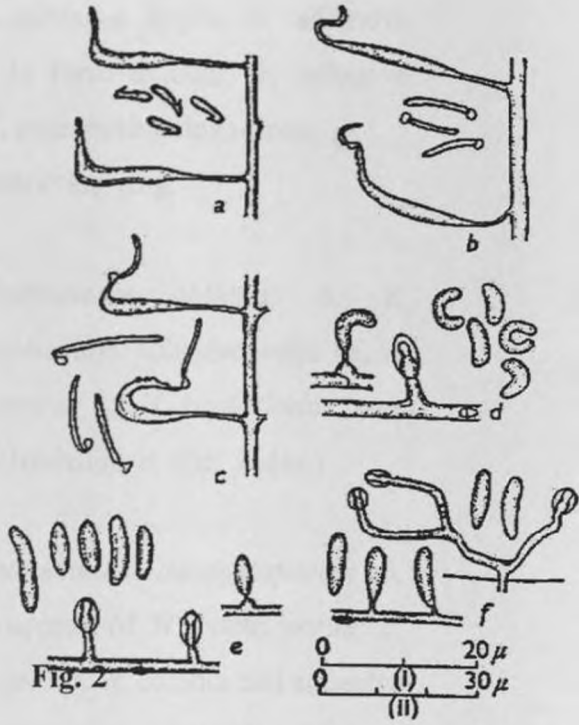

Fig. 2

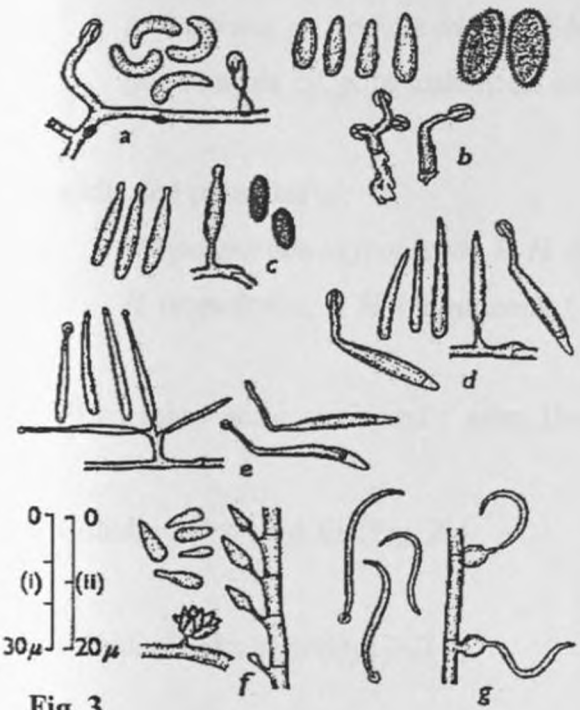

Fig. 3

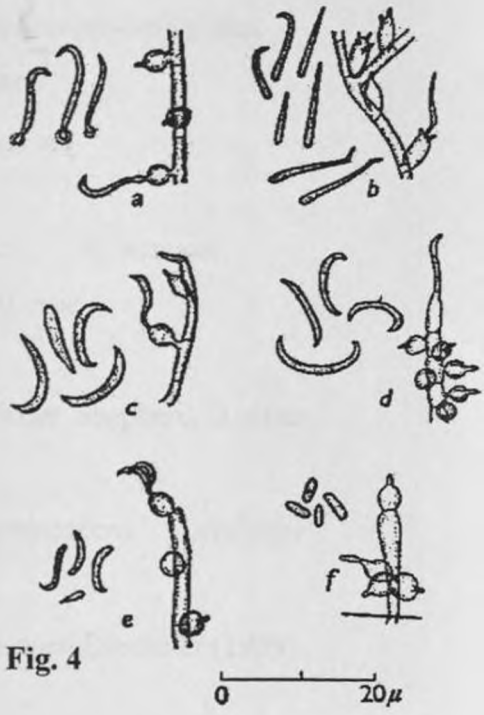

Fig. 4

Fig.1. *a,*Nematode caught on unmodified adhesive hypha; *b,* adhesive branches, with two branches joined to form a loop; *c,* adhesive networks; *d,* adhesive knobs; *e* and f, constricting rings-open; *g,* constricting ring-closed; *h,* non-constricting ring.

Fig. 2. Conidial branches and conidia of: *a, Euryancale obliqua; b, E. marsipospora; c, E. sacciospora;* conidia and adhesive cells of, *d, Nematoctonus robustus; e, N. concurrens; f, N. haptocladus. (a-c* to scale i; *d-f to* scale ii: *a-c, e, f,* after Drechsler; *d,* after Jones.)

Fig. 3. *a,* Conidia and adhesive cell of *Nematoctonlls campylosporus; b,* conidia, adhesive cells and chlamydospores of *N. pachysporus; c,* conidia and chlamydospores of *N. tylosporus; d,* conidia and adhesive cells of *N. leiosporus; e, c*onidia of *N. leptosporus; j,*conidia of *lvIeria coniospora; g* , conidia and phialides of *Harposporium helicoides. (a-e* to scale i; *f, g,* to scale ii: all after Drechsler.)

Fig. 4. Conidia and phialides of:

a, Harposporium oxycoracum; b, H. subuliforme; c, H.crassum;

d, H. anguiltulae; e, H. liltiputanum;f, H. baculiforme.

(All to same scale; *a, b* and *f* after Drechsler, *c* after Shepherd, *e* after Dixon.)

5. Conidia 7-9 x 1.2-1.6μ (Fig. *2b)* *E. marsipospora* Drechsler (1961)

5. Conidia II-I3x 0.7μ (Fig. *2C)* *E. sacciospora* Drechsler (1939)

6. Fertile hyphae with clamp connexions and elongate conidia borne singly or sometimes in groups on conical sterigmata.....7

6. Fertile hyphae lacking clamp connexions.....12

7. Fertile hyphae bearing adhesive cells on which nematodes may be capture.....8

7. Fertile hyphae lacking adhesive cells; the conidium bears a terminal adhesive knob or gives rise to a process bearing one or more adhesive knobs 9

8. Conidia elongate-cylindrical, 7-12 x 2.5-4μ, curved (Fig. *2d) Nematoctonus robustus* Jones (1964)

8. Conidia ellipsoidal, 10-23 x 3.6-5.6μ, straight or slightly curved (Fig. *2e) N. concurrens* Drechsler (1949)

8. Conidia cylindrical or elongate-ellipsoidal, 11 -18 x 3.3-4.5μ, tapering Slightly towards base (Fig. *2f) N. haptocladus* Drechsler *(1946c)*

8. Conidia elongate-ellipsoidal or cylindrical, curved, 10-13 x 2.5-4 (Fig.3a *N. ampylosporus* Drechsler (1954a)

9. Chlamydospores produced on fertile hyphae, conidia in general less than 20 μ long

9. Chlamydospores not produced, conidia in general more than 20μ long

10. Conidia elongate-ellipsoidal, 12-19 x 4.5-5.5μ, chlamydospores ovoid, 10-31 x 5.5-7.5μ, yellow, verrucose or echinulate (Fig. *3b)N. pachysporus* Drechsler (1943a)

10. Conidia fusiform 17-22 x 2.3-2.7μ, tapering distally with an expanded adhesive tip, chlamydospores ellipsoidal or obovoid, 8-1 I x 4-4.3μ, yellow, verrucose (Fig. *3c) N. tylosporus* Drechsler (1941 *b)*

11. Sterigmata unbranched, bearing a single conidium, conidia digitiform, 20-27 x 2.6-3.4μ, slightly curved, tapering distally (Fig. *3d) N. leiosporus* Drechsler (1941 *b)*

11. Sterigmata with up to four arms each bearing a conidium, conidia fusi form or digitiform, 21-28 x 1.7-2.2μ, tapering distally with an expanded adhesive tip (Fig. *3e) N. leptosporus* Drechsler (I943a)

12. Conidia borne on sterigmata, no phialides.....13

12. Conidia borne on subspherical or flask-shaped phialides, phialides frequently in whorls 14

13. Conidia conical, 4-7 x 1.8-2.5μ, basally rounded, sometimes with a small "apical swelling, conidia in a terminal cluster on each sterigma (Fig. *3f) Meria coniospora* Drechsler (1941 *b)*

14. Conidia filiform, straight, slightly curved, arcuate or pod-shaped, phialides usually sub spherical.....15

14. Conidia bacilliform, ellipsoidal, spherical, angled or rounded polyhedral, phialides usually flask-shaped.....21

15. Conidia filiform, curved, distally hooked, sometimes with a basal mucus drop following disjunction 16

15. Conidia arcuate or straight, not barbed 17

15. Conidia pea-pod shaped, barbed at one or both ends 20

16. Conidia 20-45 x 0'7-1.8μ, usually coiled in a! spiral, up to three sterigmata on each phialide (Fig. *3g) Harposporium helicoides* Drechsler (1941 *b)*

16. Conidia 15-25 x 0.7-1.4μ, f:each phialide with a single sterigma (Fig. *4a) H. oxycoracum* Drechsler (1941 *b)*

16. Conidia 12-20 x 1-1.8μ, sometimes straight, tapering distally, each phialide with 4-6 sterigmata (Fig. *4b)H. subuliforme* Drechsler (1950a)

17. Conidia arcuate.....18

17. Conidia straight or only slightly curved.....19

18. Conidia elongate, 18-22 x 2-3μ, pointed at both ends (Fig. *4c)H. crassum* Shepherd (1955)

18. Conidia elongate, 6-18 x *I-2ft,* pointed at both ends (Fig. *4d) H. anguillulae* Lohde 1874 (Karling, 1938)

18. Conidia short, 4.5-9 x 1-1.5ft, not usually pointed (Fig. *4e) H. illiPutanum* Dixon (1952)

19. Conidiophore clavate, short, up to 15ft long, conidia bacilliform, 2.5-5 x 0.7-1.5ft (Fig. *4f) H. baculiforme* Drechsler (1959)

19. Conidiophore long, up to *200ft,* conidia cylindrical, 3-5 x 0.9-1.2ft, rounded (Fig. *5a) H. sicyodes* Drechsler (1959)

20. Conidia barbed at one end, 4.5-5 x 0.8-2.1 *ft,* distally broad, and rounded (Fig.5b) *H. bysmatosporum* Drechsler (1946b)

20. Conidia barbed at both ends, 3.5-4 x 1-1.5ft (Fig. *5c) H. diceraeum* Drechsler (194 I *b)*

21. Conidia spherical, phialides in whorls.....22

21. Conidia not spherical, in terminal clusters on phialides.....23

21. Conidia *2-311* diam., in a terminal cluster on phialide (Fig. *5d) verticillium sphaerosporum* Goodey (195 I)

22. Conidia *1.3-I'7ft* diam., in a terminal cluster on phialide (Fig, *5e) Spicaria coccospora* Drechsler (194 I *b)*

23. Phialides not in whorls but occurring singly or in groups of two or more..... 24

23. Phialides in whorls.....25

24. Conidia more or less triangular in outline, 2.4-2.8 x *2.3ft* diam., distally truncate, tapering proximally (Fig. *sf) Cephalosporium balanoides* Drechsler (194 I *b)*

24. Conidia ellipsoidal or obconical, 2 x *3ft* (Fig. *6a) Acrostalagmus obovatus* Drechsler (194 I *b)*

25. Conidia bacilliform, 2-3 x *1.3-1.6ft* (Fig, *6b) A. bactrosporus* Drechsler (1941 *b)*

25. Conidia more or less triangular in outline, 3.5-4.6 x 1. 7-2.1 *ft,* distally truncate, tapering proximally (Fig, *6c)A. zeosporus* Drechsler *(1946d)*

25. Conidia rounded polyhedral, 1.3-2.1 *ft* diam" phialides elongate and

slender, tapering distally (Fig. *6d) A. goniodes* Drechsler (1942)

26. Thallus at first hyphal, later breaking up into hyphal bodies.....27

26. Parasite consisting of separate thalli from the first.....30

27. Hyphal bodies producing conidiophores which pass out of host....28

27. Hyphal bodies becoming transformed to sporangia producing zoospores.....29

28. Conidiophore distally spiral, at first aseptate, later septate, each cell bearing a single sessile conidium, conidia obovoid, 8- 11 x 6-8 *ft*, azygospores sometimes formed in place of conidia (Fig. *6e) Ivleristacrum asterospermum* Drechsler (*1940a)*

29. Zoospores uniflagellate, 40-700 formed in each sporangium, no sexual reproduction (Fig, *6f) Catenaria anguillulae* Sorokin (1876)

29. Zoospores biflagellate, reniform; adjacent hyphal bodies may conjugate, forming thick-walled zygospores (Fig. *6g) Myzocytium vermicola* (Zopf, 1888) Fischer

30. Thalli producing hyphae passing out of the host, the contents of each hypha differentiating into spores.....31

30. Thalli becoming transformed to sporangia producing non-motile spores which are ejected forcibly through papillae pushed through the host cuticle.....32

31. External hyphae 10-100 *ft* long dividing into 2-12 spores, spores cylindrical

4-9.5 x 3-4.2 *fi,* irregularly rounded, each producing a bulbous adhesive process after release (Fig. *7a) Gonimochaete horridula* Drechsler *(1946a)*

32. Spores clavate, 20-25 x *2.5-3.5Il,* straight or slightly curved, zygospores formed from conjugation of adjacent thalli (Fig, *7b) Protascus subuliformis* Dangeard (1903)

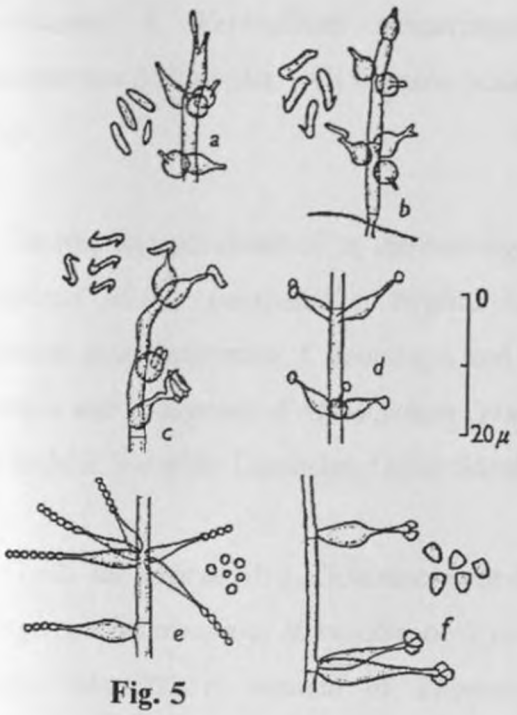

Fig. 5

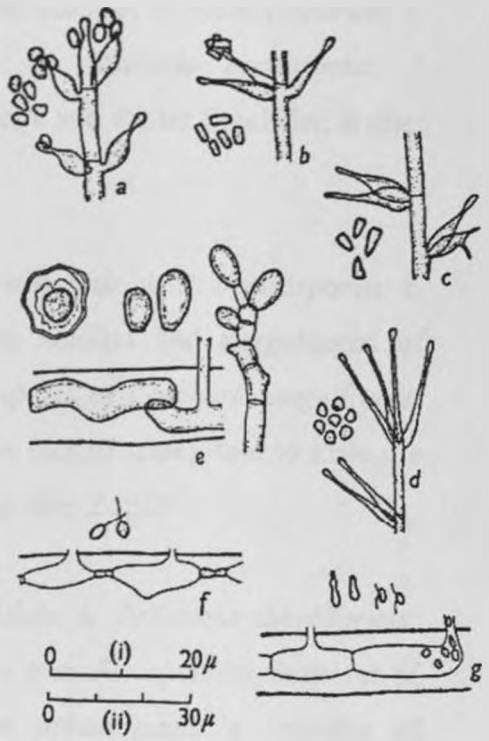

Fig. 6

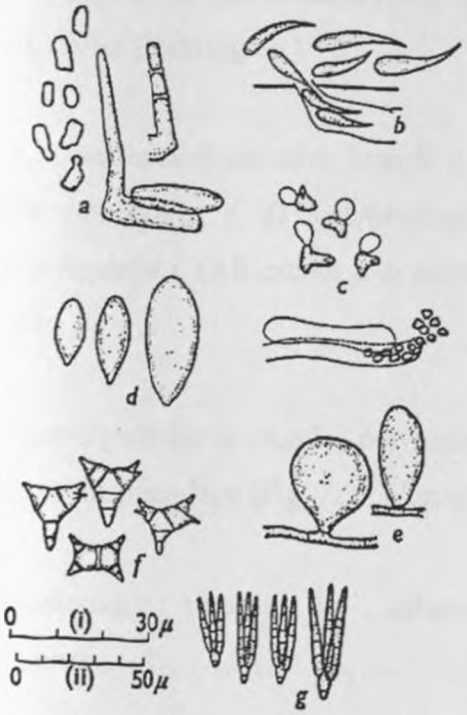

Fig. 7

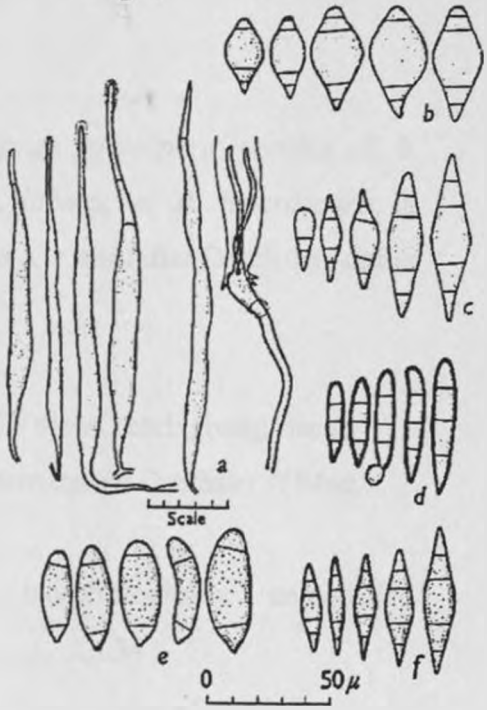

Fig. 8

Fig. 5. Conidia and phialides of: *a, Harposporium sicyodes; b, H. bysmatosporum; c, H. diceraeum; d, Verticillium sphaerosporum; e, Spicaria coccospora; f, CePhalosporium balanoides.* (All to same scale; *a-c, e* and f after Drechsler; d after Goodey.)

Fig. 6. Conidia and phialides of: *a, Acrostalagmus obovatus; b, A. bactrosporus;* c, *A. zeosporus; d, A. goniodes; e,* hyphal bodies, conidia and azygospores of *Meristacrum asterospermum; f,* sporangia and zoospores of *Catenaria anguillulae; g,* sporangia and zoospores of *Myzocytium l'ermicola* (not to scale). *(a-d* to scale i; *e* and *f* to scale ii: *a-e* after Drechsler, *f* after Sorokin, *g* after Zopf.)

Fig. 7. Thalli and spores of: *a, Gonimochaete horridula; b, Protascus subuliformis; c, Haptoglossa heterospora; d,* conidia of *Stylopage grandis; e,* chlamydospores of *Cystopage lateralis; r;* conidia of *Triposporina aphanopaga; g,* conidia of *Tridentaria implicans. (a-c* to scale i; *d-g* to scale ii: *a, c, e-g* after Drechsler, *b* after Dangeard, dafter Duddington.)

Fig. 8. *a,* Conidia and adhesive branch of *Acaulopage pectospora;* conidia of, *b, Dactylella cionopaga; c, D. gephyropaga; d,* ***D.*** *lobata; e,* ***D.*** *heterospora; f, Dactylaria haptotyla.* (All except *a* to same scale: *a-c, e* andfafter Drechsler, dafter Duddington.)

32. Spores globular or rounded polyhedral, 4'5-10# diam., each giving rise to a lobed appendage (Fig. *7c) Haptoglossa heterospora* Drechsler *(194oa)*

33. Nematodes trapped by adhesion to morphologically unmodified hyphae.....34

33. Nematodes caught in or on traps formed from morphologically modified hyphal branches.....39

34. Hyphae aseptate, yellow adhesive substance often produced rapidly by hyphae at point of contact with nematode.....35

34. Hyphae septate, conidia borne singly and terminally on erect, unbranched conidiophores 38

35. Conidia obovoid, borne singly at intervals on a simple, erect conidiophore, no sexual stage.....36

35. No conidia formed, but yellow or hyaline thick-walled chlamydospores produced in a terminal, lateral or intercalary position in or on the substratum 37

36. Globular protuberances formed on hypha at point of contact with trapped nematode, conidia obovoid, 20-45 x 13-23μ *Stylopage hadra* Drechsler (1935)

36. No globular protuberance, conidia elongate-obovoid, 20-35 x 7-18μ S. *leiohypha* Drechsler (1936)

36. No globular protuberance, conidia obovate or pyriform, 27-61 x 13-26μ *(Fig.7d)* S. *grandis* Duddington *(1955a)*

37. Chlamydospores hyaline, globose, elongate-ellipsoidal, ovoid or some what lobate, 25-50 x 10-28μ, always formed laterally on mycelial hyphae, commonly sessile but sometimes on a short pedicel (Fig. *7e) Cystopage lateralis* Drechsler (1941 *a)*

37. Chlamydospores yellowish, subspherical or elongate-ellipsoidal, 18 35 x 15-30μ, intercalary C. *intercalaris* Drechsler (1945)

37. Chlamydospores yellowish, globose or ellipsoidal, 20-30# diam., single and terminal on straight or slightly crooked branches, 2-60 X 3-6 μ, or occasionally intercalary C. *cladospora* Drechsler (1957)

38. Conidia obpyramidal, bifurcate, of up to 13 cells, 20-25μ long (Fig. 71) *Triposporina aphanopaga* Drechsler (1937)

38. Conidium furcate, trident-like, the teeth being 12-42 x 3.5-5μ, divided by 3-5 septa (Fig. *7g) Tridentaria implicans* Drechsler (1940b)

39. Hyphae aseptate, lateral branches bearing poorly differentiated terminal adhesive knobs.....40

39. Hyphae septate.....41

40. Conidia single, erect, spindle-shaped, 180-246 x 7-14μ, tapering at each end and partially embedded proximally in the substratum, often bearing a distal liquid droplet (Fig. *8a) Acaulopage peetospora* Drechsler *(1962b)*

41. Hyphal branches modified to form short, lateral adhesive organs a few cells long, sometimes linking to form simple networks, these usually two-dimensional. Conidiophores simple with a single terminal conidium.....42

41. Hyphal branches modified to form stalked or sessile adhesive, unicellular knobs, no other type of trapping organ present.....43

41. Hyphal branches modified to form stalked rings, the rings being non constricting and sometimes accompanied by stalked adhesive knobs.....50

41. Hyphal branches modified to form stalked constricting rings.....54

41. Hyphal branches anastomosing to form simple or complex two- or three dimensional networks, nematodes caught in or on these by adhesion.....63

42. Conidia broadly spindle-shaped, 35-60 x 13-21μ, rounded distally, narrowing proximally, basally truncate, 2- to 5-septate. Adhesive hyphae of 1-7 cells, constricted at the septum, adhesive hyphae sometimes linking to form two-dimensional networks (Fig. *8b) Dactylella eiollopaga* Drechsler *(1950b)*

42. Conidia turbinate, 27-46 x 16-21μ, 2-, 3- or commonly 4-septate, the median cell the largest. Adhesive hyphae frequently linking to form two-dimensional networks (Fig. *8e) D. geph)'Topaga* Drechsler (1937)

42. Conidia fusiform, 32-54 x *8-12*μ, usually 3-septate, cells O, adhesive hyphae subspherical (Fig. *8d) D. lobata* Duddington (195Ib)

43. Conidiophore usually branched near apex, conidia borne singly and terminally on each branch, adhesive knob stalked.....44

43. Conidiophore usually simple, sometimes sparingly branched near apex, single conidium borne terminally on the conidiophore or conidiophore branches. Adhesive knobs stalked or sessile.....45

44. Conidia elongate-cylindrical, 35-60 x 2.2-3.2μ usually 4-septate, distal cell globose or elongate-ellipsoidal. 1-15 conidia at conidiophore apex (Fig. *10h) Dactylaria haptospora* Drechsler *(1940b)*

44. Conidia spindle-shaped, 33-55 x *7.4-13.3*μ, tapering to a truncate base, 3- to 5- but usually 4-septate, median cell usually the largest, 2-5 conidia at conidiophore apex (Fig. *8f) D. haptotyla* Drechsler *(1950b)*

44. Conidia spindle-shaped, 32 -54 x 5.9-14'. 3μ *distally* rounded; base truncate, 3- to 5- but usually 4- septate, median cell the largest, 2-3 conidia at conidiophore apex. Resting bodies often formed by thickening of the assimilative hyphae (Fig. *9a) D. sclerohypha* Drechsler *(1950b)*

45. Adhesive knob always sessile, conidiophore simple.....46

45. Adhesive knob sometimes with a short distinct stalk or sometimes sessile or lateral, often proliferating to form short chains of adhesive cells.....47

45. Adhesive knob always stalked, conidiophore simple 48

45. Adhesive knob always stalked, conidiophore sometimes branched once or twice near apex.....49

46. Conidia spindle-shaped, 40-60 x I I-1μ, distally rounded, basally truncate, usually 4-septate, median cell the largest (Fig. *9b)Dactylella phymatopaga* Drechsler (*1954b)*

47. Conidia spindle-shaped, 35-45 x *8-14*μ distally rounded, proximally truncate, usually 4-septate (Fig. *9c) D. parvicollis* Drechsler *(1962a)*

48. Conidia ellipsoidal, 30-60 x 9-17 *ft,* distally blunted 2-,3- or usually 4-septate, median cell usually the largest (Fig. *9d) D. mammillata* Dixon (1952

48. Conidia fusiform, 24-65 x *7.5-19ft,* distally rounded, proximally attenuated, usually 4-septate, median cell the largest (Fig. *ge)D. elliPsospora* Grove (Drechsler, 1937)

49. Conidia obconical-clavate, 20-46 x 6.*5-9.5*μ, *distally* rounded, basally truncate, usually 3-septate (Fig. *9f) D. asthenopaga* Drechsler (1937)

50. Adhesive knobs not present.....51

50. Adhesive knobs present, conidiophore simple with single terminal conidium...52

50. Adhesive knobs present, conidiophore branched near apex, conidia borne singly and terminally on these branches.....53

51. Conidia elongate-fusoidal to cylindrical, 40-105 x *4-5μ*, 5- to 15-septate, conidia often giving rise to globular cells at their tips, conidiophores simple or sometimes sparingly branched near apex, conidia single and terminal on conidiophore or branches (Fig. *9g) D. leptospora* Drechsler (1937)

52. Conidia fusoid, 28-55 x *9-14μ*, distally rounded, basally truncate, 2-,3- or usually 4-septate, median cell the largest (Fig. *9h) D.lysipaga* Drechsler (1937)

53. Conidia fusiform or clavi-fusiform, 26-32 x 5.5-11.*5μ*, 4- to 6-septate, median cell usually the largest (Fig. *9i) Dactylaria candida* (Nees) SacCo (Drechsler, 1937)

54. Conidia borne in a terminal cluster at apex of conidiophore.....55

54. Conidia borne singly at the apex of a simple conidiophore.....58

55. Conidia with 1 septum.....56

55. Conidia with 2-4 septa.....57

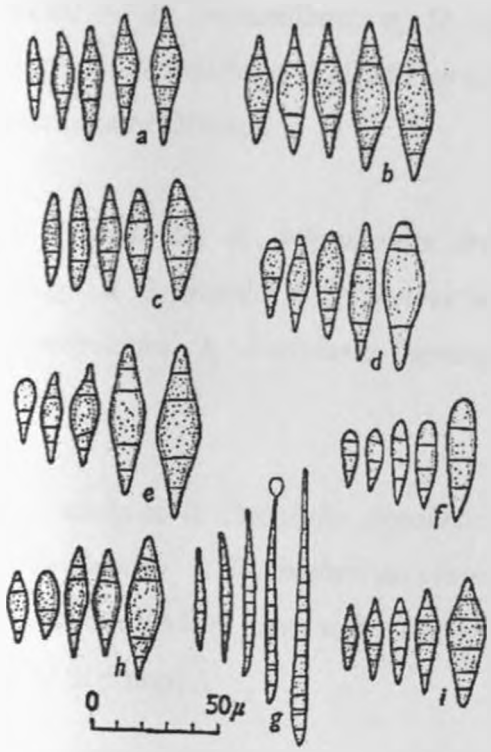

Fig. 9

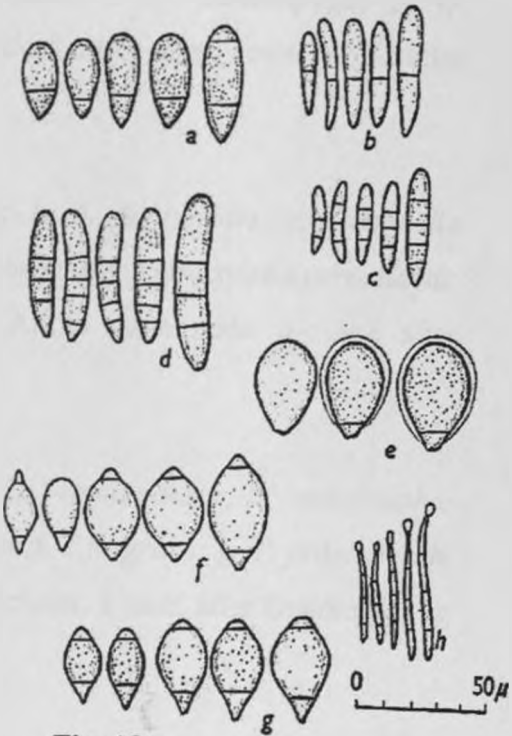

Fig. 10

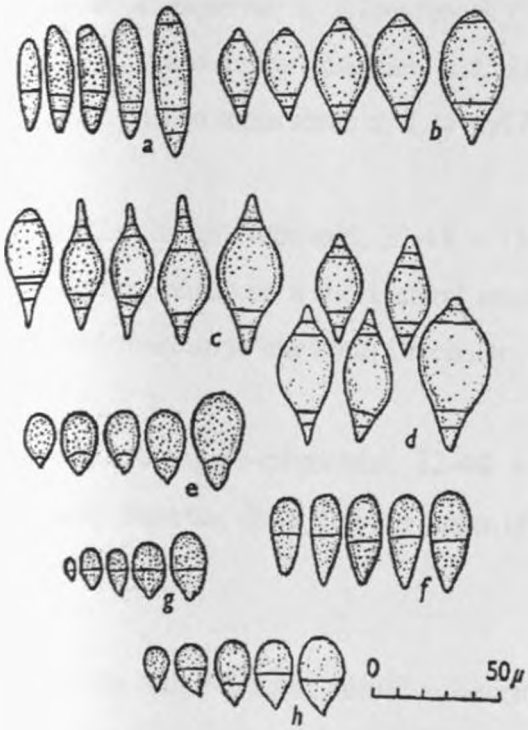

Fig. 11

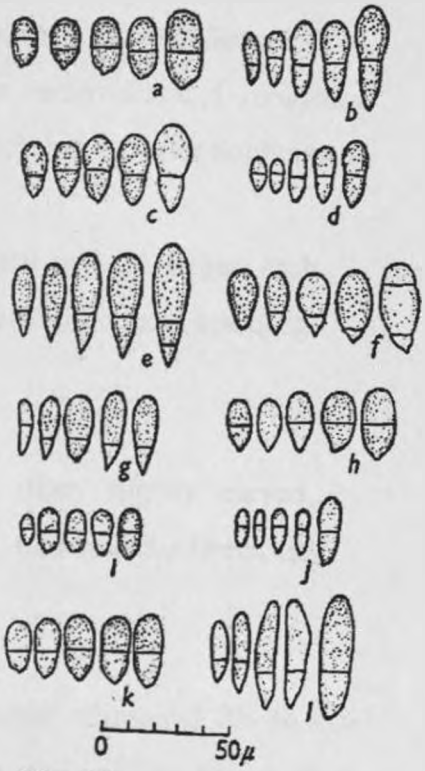

Fig. 12

Fig. g. Conidia of: *a, Dactylaria sclerohypha; b, Dactylella phymatopaga; c, D. parvicollis; ~, D. mammillata; e, D. elliPsospora; f, D. asthenopaga; g, D. leptospora; h, D. lysiPaga;* 1, *DaclJ'laria candida.* (All to same scale: *a-c,f-i* after Drechsler, *d* after Dixon.)

Fig. 10. Conidia of: *a, .4rthrohotrys aruhonia; b, A. dactyloides; c, Dactylella brochopaga; d, D. gracilis; e, Trichotheciwn polybrochum; f, Dactylella acrochaeta; g, D. doedycoides; h, Dactylaria haptospora.* (All to same scale: *a-c, e-h* after Drechsler.)

Fig. 11. Conidia of: *a, Dactylella stenohrocha; h, D. bemhicodes; c, D. coelohrocha; d, D. aPhrobrocha; e, Trichothecium cystosporium;f, T.jlagrans; g, T. pravicovi; h, T. globosporum.* (All to same scale: *a-d* after Drechsler, *e* andf after Duddington, *g* and *h* after Soprunov.)

Fig. 12. Conidia of: *a, Arthrohotrys arthrohotryoides; h, A. conoides; c, A. oligospora; d, A. superha; e, A.longispora;f, A. oviformis;g, A. doliaformis; h, A. kirghizica;* i, *A. cladodes* var. *dadodes;* j, *A. dadodes* var. *macroides; k, A. rohusta; l, A. musiformis.* (All to same scale: *a, d, i,j* and *l* after Drechsler, *e-h* after Soprunov.)

56. Conidia elongate-obovoid, 29-43 x 15-19μ, distal cell the larger, each conidium borne on a short lateral spur from the conidiophore apex(Fig. *10 a)Arthrobotrys anehonia* Drechsler *(1954b)*

56. Conidia elongate-ellipsoidal, 32-48 x 7-9.5μ, often slightly curved, basally truncate, distal cell the larger (Fig. 10 b) *A.daetyloides* Drechsler (1937)

57. Conidia with 2-4 septa, curved cylindrical to elongate-ellipsoidal, 26- 46 x 5-9μ, distally rounded, each conidium borne on a sterigma-like branch from conidiophore apex (Fig. 10 c) *Dactylella brochopaga* Drechsler (1937)

57. Conidia with 3-4 septa, elongate-ellipsoidal, 46-66 x 8-11μ slightly curved, distally rounded. tapering proximally (Fig. *10 d) Dactylaria gracilis* Duddington (1951a)

58. Conidia with 1 septum.....59

58. Conidia with 2 septa.....60

58. Conidia with mainly 3 scpta.....61

58. Conidia with mainly 4 septa.....62

59. Conidia obovoid, 35 x 24μ distal cell the larger and surrounded by a hyaline sheath *of* mucus (Fig. roe) *Trichothecium polybrochum* DrechsJer (1937)

60. Conidia turbinate, 30-42 x 13.2-22.6μ, median cell the largest, filamentous appendage on distal cell, medium protrusion from arcuate ring cells, chlamydospores yellow, often formed in conidia (Fig. *lof) Dactylella acrochaeta* Drechsler (1952)

60. Conidia turbinate, 28-39 x 15-24μ base concavely truncate, terminal knob on conidiophore, median protrusion from arcuate ring cells (Fig, log) *D. doedycoides* Drechsler *(1940b)*

60. Conidia elongate prolate-ellipsoidal, 35-47 x 13-20 μ, base truncate, smaller secondary conidia often formed, 23 -40 x *3.3-8*μ uniseptate, curved and borne in groups on branched conidiophores. Chlamydospores yellow, intercalary, median protrusion from arcuate ring cells (Fig. 8e) *D. heterospora* Drechsler *(1943b)*

61. Conidia elongate-ellipsoidal, 34-56 x 12.5-16.5μ, base truncate, I-, 2 but usually 3-septate (Fig. *11a) D. stenobrocha* Drechsler (1950b)

61. Conidia turbinate, 34-48 x 16-23μ, broadly rounded, tapering proximally to a protruded truncate base (Fig.11b) *D. bembicodes* Drechsler (1937)

61. Conidia globose-fusiform, 38-60 x 30-38μ, penultimate cell the largest *D. turkmenica* Soprunov (1958)

62. Conidia broadly spindle-shaped, 46-64 x 18-25μ, 2- to 5- but usually 4-septate, median cell the largest, distal and proximal cells protracted and tapering, arcuate cells very narrow at the septum and with a prominent elongate vacuole (Fig. 11 e) *D. coelobrocha* Drechsler (1947)

62. Conidia broadly spindle-shaped, 41-55 x 17-26μ, 3- to 4- but usually 4-septate, median cell the largest (Fig. 11 *d) D. aphrobrocha* Drechsler (1950 *b)*

63. Conidia with one septum, formed in a terminal head on branched or simple conidiophores, sometimes developed nodally along the length *of* the conidiophore.....64

63. Conidia with more than one septum 64. Conidia not truly capitate but formed in a panicle, conidia formed in basipetal succession 65

64. Conidia truly capitate, formed in close heads.....66

64. Conidia loosely capitate, borne singly on sterigma-like branches at conidiophore apex.....71

65. Conidia broadly pyriform, 25-35 x 18-24μ, distal cell much larger than the proximal, base apiculate, no chlamydospores (Fig. 11e) *Trichothecium eystosporium* Duddington (1950)

65. Conidia ovoid, 27-37 x 14-16,u, bluntly rounded at proximal end, two cells roughly equal, chlamydospores intercalary, almost spherical *24-32μ* or ellipsoidal 28-59 x 13-29μ (Fig. *11f) T. flagrans* Duddington (1950)

65. Conidia ovoid, 8-22.5 x 6.5-15.5μ tapering proximally, distal and proximal cells equal, conidiophore monopodially branched (Fig. 11g) *T. pravicovi* Soprunov (1958)

65. Conidia oblong obovate, 16-28.8 x 9.6-17.5μ, tapering proximally, base apiculate, constricted at the septum, distal cell the larger, conidia often developed in whorls (Fig. 11h) *T. globosporum* var. *globosporum* Soprunov (1958)

65. Conidia globose, 15.2-19.6 x 6-1 lμ distal cell the larger *T. g.* var. *microsporum* Soprunov (1958)

65. Conidia oblong-rounded, 18-25.5 x 9-14.5μ constricted at septum, base apiculate, distal cell the larger *T. g.* var. *roseum* Soprunov (1958)

66. Pronounced nodal development of conidia, conidia in whorls along conidiophore.....67

66. Nodal development of conidia absent, conidiophore usually branched.....70

67. Conidiophore not usually branched.....68

67. Conidiophore usually branched.....69

68. Conidia elongate-ellipsoidal, 17.1-30.2 X 10.6-16.l, μ, distally rounded, base apiculate, distal cell usually the larger, whorls often close together near conidiophore apex, chlamydospores in old cultures (Fig. *12a)Arthrobotrys arthrobotryoides* (Berl.) Lindau (Drechsler, 1944)

68. Conidia obconical, 19-42 x 8-15μ constricted at the septum, base flattened, distal cell the larger, chlamydospores yellow, globose to prolate-ellipsoidal, 18-25μ or oblong cylindrical 30--50 x 15,u (Fig. 12b) *A. conoides* Drechsler (1937)

68. Conidia obovoid, 22-32 x 12-20μ plump, constricted at the septum.base apiculate, distal cell the larger, chlamydospores yellow, cylindrical, subspherical or ellipsoidal (Fig. *12C) A. oligospora* Fresenius (1852; Drechsler, 1937)

68. Conidia elongate-obovoid or ellipsoidal, 12-23 x *6.5-9.5*μ, slightly constricted at the septum, two cells more or less equal, conidia never in more than two successive whorls, no chlamydospores (Fig. *12d) A. superba* Corda (1839; Drechsler, 1937)

68. Conidia oblong-rounded, 23-45.5 x 10.5-16.5μ, tapering proximally, constricted at the septum, distal cell the larger, base apiculate (Fig. 12e) *A. longispora* Soprunov (1958)

69. Conidia obovate, 22.5-32.5 x 10-15.5, μ constricted at the septum, distal cell the larger, base apiculate (Fig. *12f) A. oviformis* Soprunov (1958)

69. Conidia oblong-obovate, 23.5-32.5 x 9-14.5μ, constricted at the septum, distal cell much longer than the proximal (Fig. 12g) *A.dolioformis* Soprunov (1958)

69. Conidia ovoid, 18.5-24.5 x 9-12.5,u, base apiculate, distal cell the larger (Fig. 12h) *A. kirghizica* Soprunov (1958)

70. Conidia ellipsoidal or elongate-obovoid, 11-18 x *6.2-8.8, μ,* sometimes slightly constricted at the septum, distal cell equal to or slightly larger than the proximal, conidiophore apex inflated, often coralloid, no chlamydospores (Fig, 12i) *A. cladodes* var. *cladodes* Drechsler (1937)

70. Conidia elongate-ellipsoidal or elongate-obovate, 13-26 x 5-8.2μ, septum in the middle or above or below the middle of the conidium, conidiophore apex inflated, chlamydospores intercalary, made up of segments each 7-35 x *7-20μ* (Fig. 12j) *A. c.* var. *macroides* Drechsler (1944)

70. Conidia oblong-pyriform, 18-27 x *8-12μ* distal cell slightly the larger, bluntly apiculate (Fig. *12k) A.robusta* Duddington (1951 *c)*

71. Conidia ellipsoidal, 22-44 x 7.5-12.7μ slightly curved, base truncate, distal cell the larger, chlamydospores yellow, globose-ellipsoidal, *14-22μ,* (Fig. *12l) A. musiformis* Drechsler (1937)

72. Conidia borne singly at apex of conidiophore or singly on the ends of branches or spurs arising sparingly from the conidiophore.....73

72. Conidia borne in a terminal group at conidiophore apex or singly on numerous branches or spurs arising from the conidiophore, sometimes conidia developed normally.....77

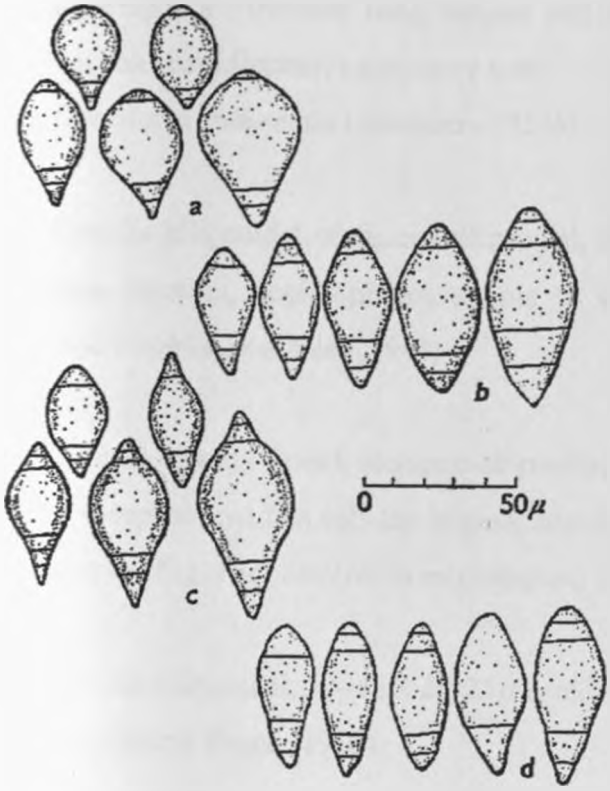

Fig. 13

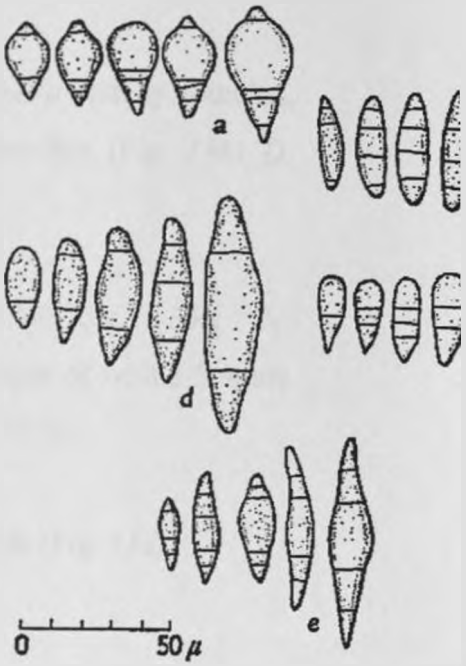

Fig. 14

Fig. 13. Conidia of: *a, Dactylaria eudermata; b, D. psychrophila; c, Dactylella megalospora; d, D. reticulata.* (All to same scale: *a-c* after Drechsler, *d* after Peach.)

Fig. 14. Conidia of: *a, Dactylaria thaumasia; b, D. polycephala; c, D. pyriformis; d, D. scaphoides; e, D. gampsospora.* (All to same scale: *a,* band *e* after Drechsler, *c* after Juniper, *d* after Peach.)

73. Conidia with mainly 3 septa.....74

73. Conidia with 1-5, mainly 3-4 septa.....75

73. Conidia with mainly 4 septa.....76

74. Conidia obovoid-turbinate, 37-55 x *21-35μ* distally rounded, proximally tapering to a truncate base, largest cell with a thick wall and prominent vacuole, conidiophores simple or with 1-4 branches (Fig. *13a) Dactylaria eudermata* Drechsler *(1950b)*

75. Conidia ellipsoidal or fusoid-ellipsoidal, 46-71 x *21-29μ* distally rounded, base truncate, conidiophores simple or with 1-2 branches (Fig. *13b) D. psychrophila* Drechsler (1944)

76. Conidia broadly fusoid, elongate-ellipsoidal or obovoid, 40-75 x *18-35μ* 2- to 4-septate, median cell the largest, conidiophores simple or with 3-5 spurs at apex (Fig. *13c) Dactylella megalospora* Drechsler (1954b)

76. Conidia ellipsoidal, 50--65 x *20-25μ* conidiophore simple (Fig. *13d) D reticulate* Peach (1950)

77. Conidiophore branched near apex.....78

77. Conidiophore unbranched, nodal development of conidia in whorls on the

77. Conidiophore unbranched, no nodal development of conidia 80

78. Conidia turbinate, 27-49 x 15-23*μ*, base truncate, 1- to 4- usually 3 septate, penultimate cell the largest, chlamydospores yellow, 18-28*μ* diameter (Fig. *14 a) Dactylaria thaumasia* Drechsler (1937)

79. Conidia fusoid-ellipsoidal, 35-46 x 8-. 5 I 2' 5 *It,* distally rounded, proximally acute, 3- to 4-septate (Fig. 14b) *D. polycephala* Drechsler (1937)

79. Conidia elongate-pyriform, 26-41 x 9-15P, distally bluntly rounded, 2- to 3-

septate, chlamydospores yellow, intercalary, globose or ellipsoidal, 20-35 x 10-25μ (Fig. *14c) D. pyriformis* Juniper (1954)

80. Conidia broadly fusiform, 26-83 x 12-1 *71l,* straight or slightly curved, 1- to 3-, usually 2-septate (Fig. *14d) D. scaphoides* Peach (1952) 80. Conidia spindle-shaped, 25-76 x 7- 16μ, slightly curved, I - to 4-, usually 4-septate, chlamydospores barrel-shaped or globose, 8-2 I x 6-17 ft (Fig. *14e) D. gampsospora* Drechsler *(1962a)*

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7.3.2 Nematode identification Key

Interactive Diagnostic Key to Plant Parasitic, Freeliving and Predaceous Nematodes
Adapted from:

An Illustrated Key to Nematodes Found in Fresh Water

- *Armen C. Tarjan (University of Florida, Lake Alfred)*
- *Robert P. Esser (Florida Department of Agriculture, Gainesville)*
- *Shih L. Chang (Environmental Protection Agency, Cincinnati, Ohio)*

Part I

Originally published in J. Water Pollution Cont. Fed. 1977. Vol 49: 2318-2337

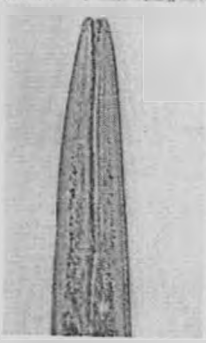
UNL Nematology Lab

1. Cephalic setae indistinct or absent .  ..2

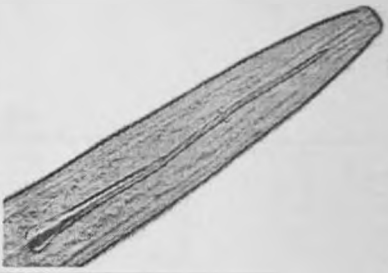
- Cephalic setae absent but setae-like head appendages present .  . 64

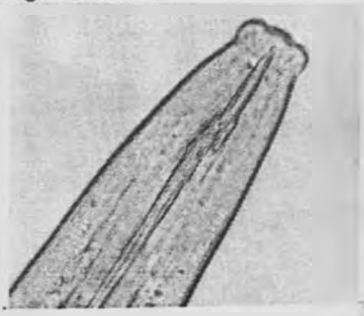
- Cephalic setae present .  ..69

2. (1) Stylet present .  .3



- Stylet absent 38

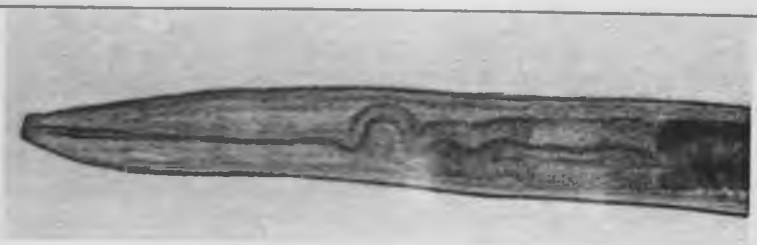
2. (2) Base of stylet knobbed or flanged .   .4



- Stylet knobs or flanges absent .. 29

3. (3) Valvate median esophageal bulb present

.5



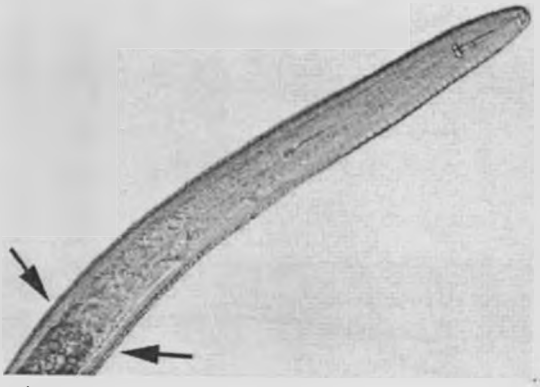
• Valvate median esophageal bulb absent . ..22

5. (4) Females eel-like . .6

• Females swollen . ..21

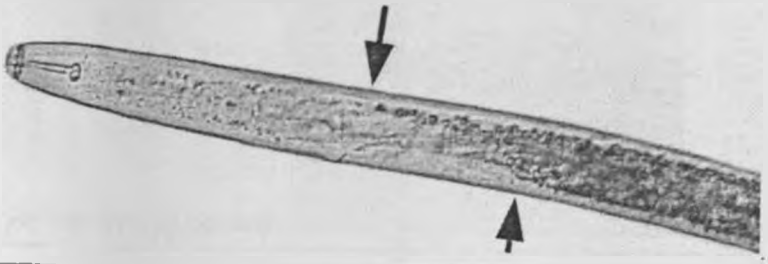
6. (5) Vulva at mid-body 7

• Vulva on lower third of body 14



7. (6) Esophagus not overlapping intestine 8

Esophagus overlapping intestine



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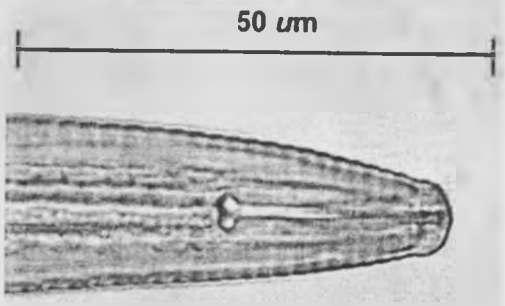
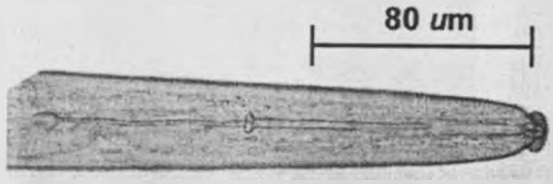
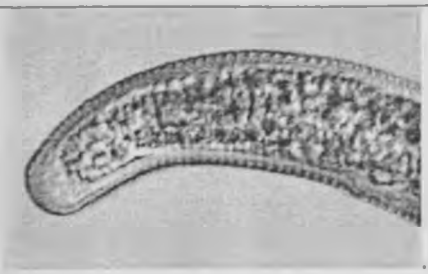


8. (7) Stylet length less than 50 microns 9



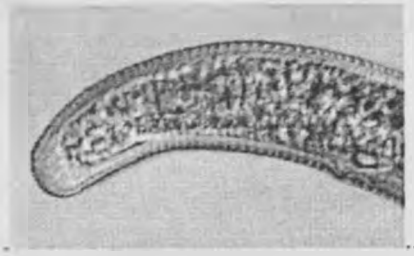
- Stylet lenght greater than 80 microns . ..... *Dolichodorus*

9. (8) Tail terminus pointed ..... *Tetylenchus*



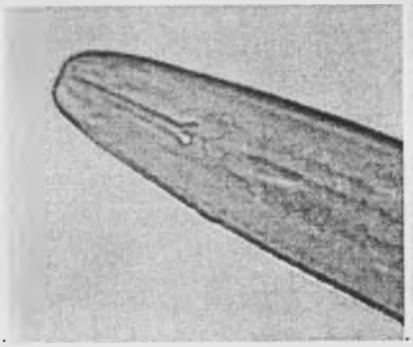
• Tail terminus not pointed 10

10. (9) Tail terminus knobbed ... *Psilenchus*

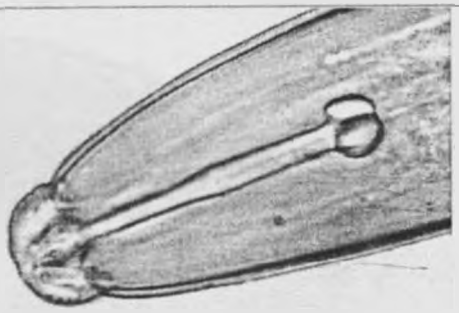


• Tail terminus never knobbed or pointed .. *Tylenchorhynchus*

11. (7) Labium offset ...12



• Labium flattened amalgamated or nearly so 13



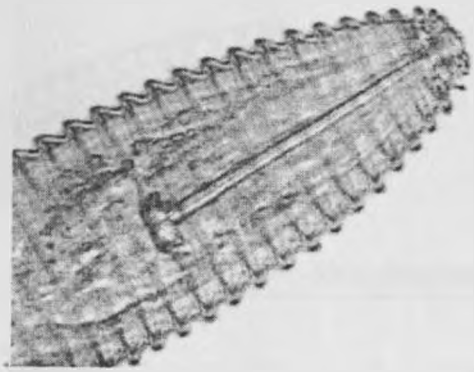
12. (11) Stylet massive, 40-50 microns long .. *Hoplolaimus*

• Stylet long and thin, greater than 90 microns long

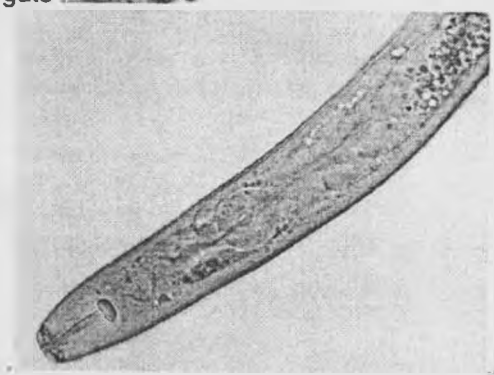
.. *Belonolaimus*

13. (11) Body 0.5-1 mm . ..... *Radopholus*

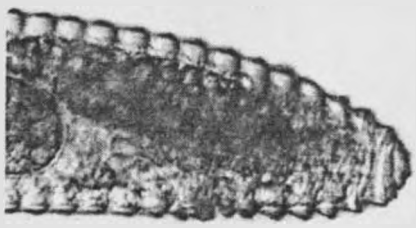
• Body 2-3 mm long ..... *Hirshmaniella*



14. (6) Cuticle heavily annulated, stylet elongate . .15



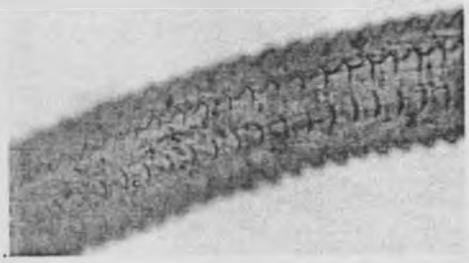
▪ Cuticle not heavily annulated, stylet short 17

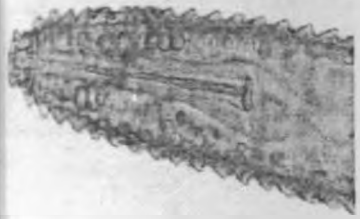


15. (14) Cuticular sheath absent . .. 16

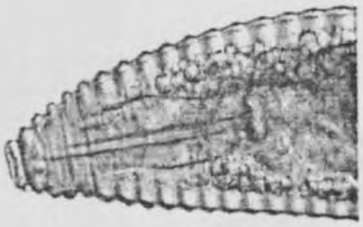
▪ Cuticular sheath present . ..... *Hemicycliophora*

16. (15) Annules with cuticular spines or scales ...





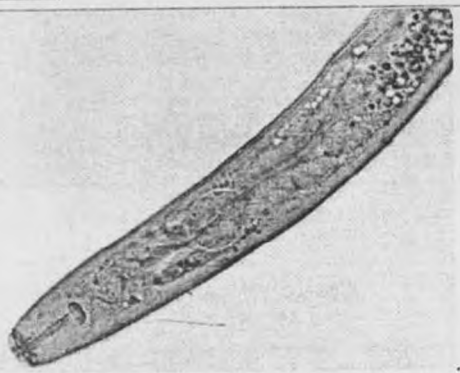
..*Criconema*



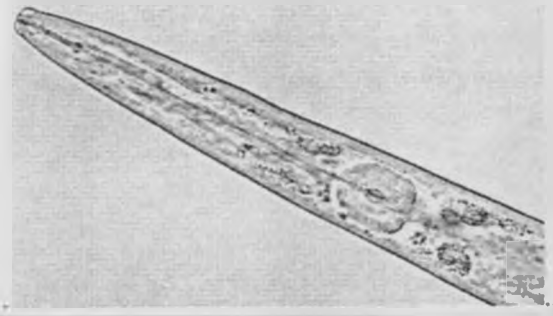
• Annules plain without spines or scales . ...*Mesocriconema*

17. (14) Body death position straight .. .18

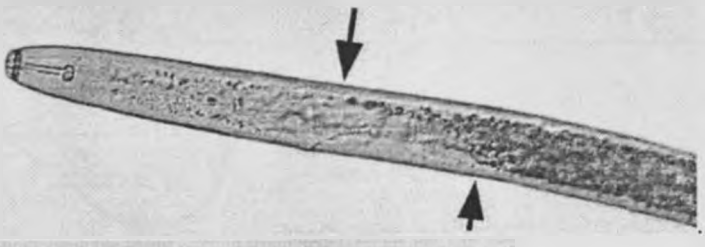
• Body death position spiral . ..... *Helicotylenchus*



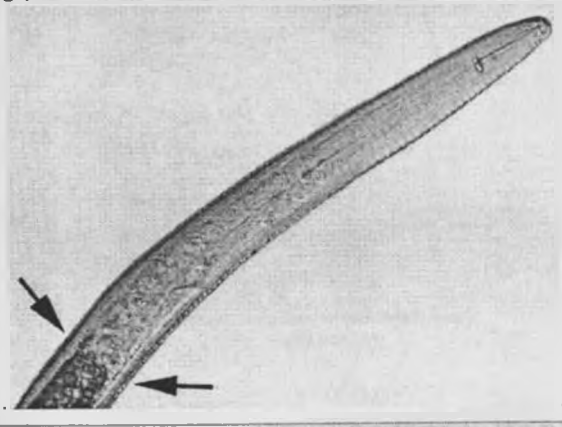
18. (17) Median esophageal bulb distinct but not pronounced 19



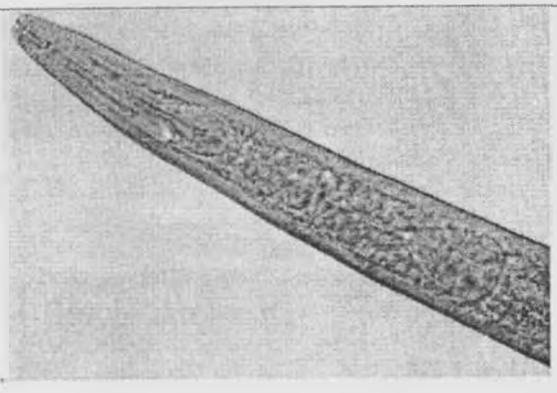
• Median esophageal bulb well-developed ... *Aphelenchoides*



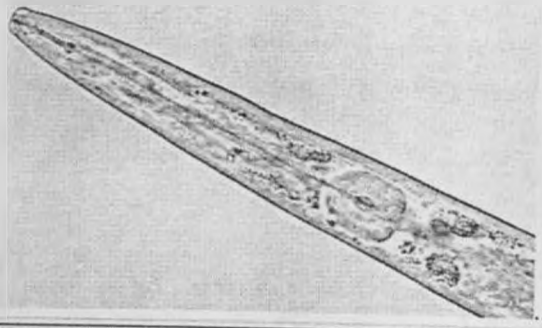
19. (18) Esophagus overlapping intestine 20

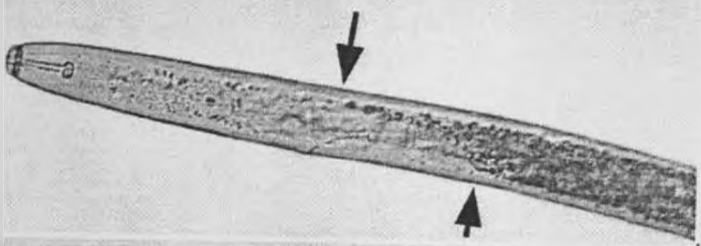


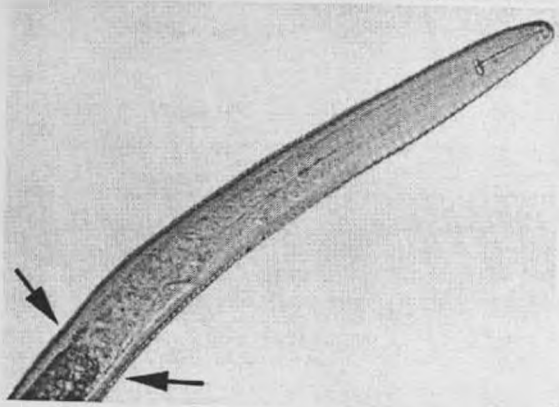
• Esophagus not overlapping intestine ..... *Tylenchus*

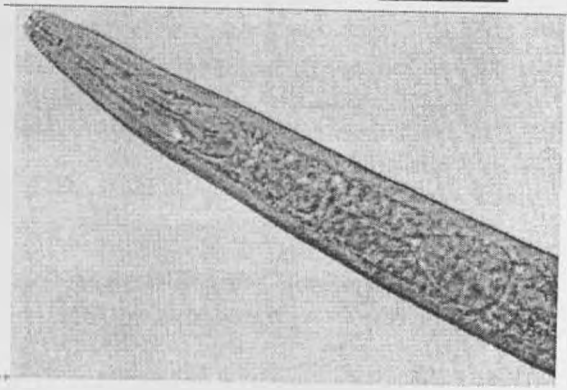


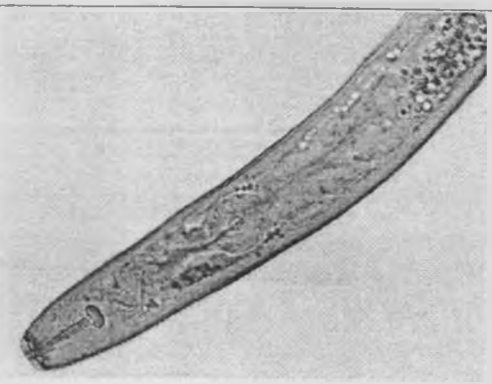
. (19) Median bulb and valves small, stylet usually weak ..
Ditylenchus

• Median esophageal bulb well-developed .  ... *Aphelenchoides*

19. (18) Esophagus overlapping intestine .  20

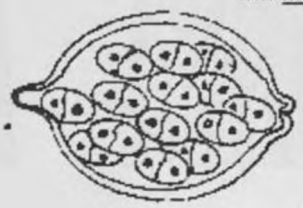
• Esophagus not overlapping intestine .  ..... *Tylenchus*

20. (19) Median bulb and valves small, stylet usually weak .. 
....*Ditylenchus*

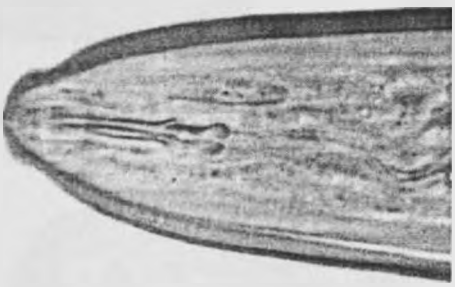


• Median bulb valves and stylet well developed, labium flattened . *Pratylenchus*

21. (5) Female body white without eggs . ..... *Meloidogyne*

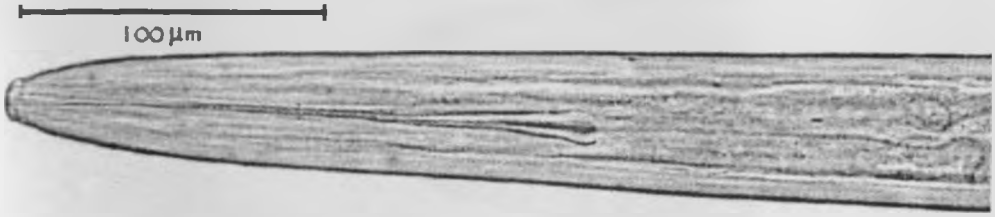


• Female body brown, usually with eggs . ..... *Heterodera*



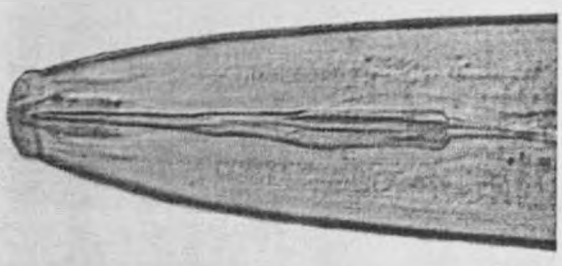
22. (4) Stylet short, less than 100 microns 23

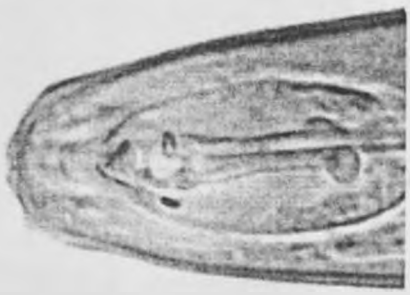
• Stylet long, greater than 100 microns



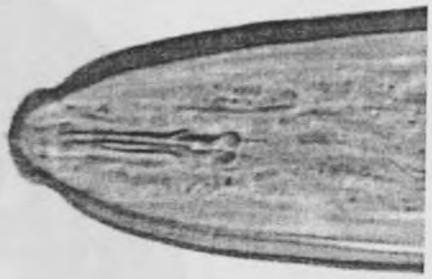
.....*Xiphinema*

23. (22) Stylet complex .  ..24

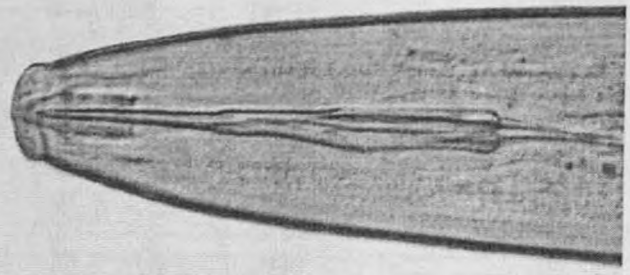
• Stylet simple .  25



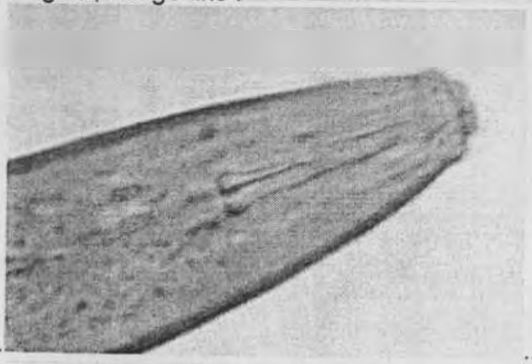
24. (23) Stylet with anterior arch-like portion . ... *Diphtherophora*



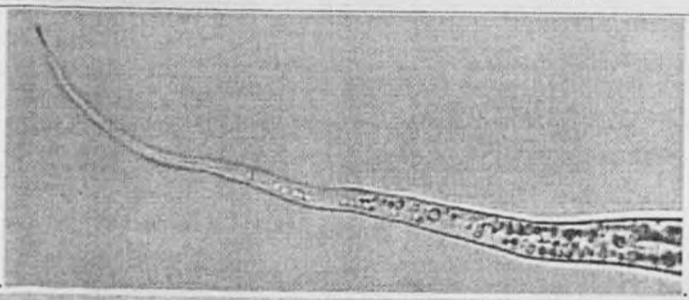
• Stylet with dorsal thickening piece . ..... *Tylencholaimellus*



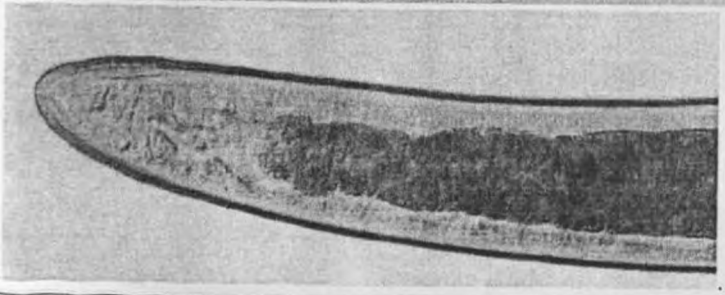
25. (23) Stylet knobs elongate, flange-like 26



• Stylet knobs round 27



26. (25) Filiform tail . ... *Aulolaimoides*

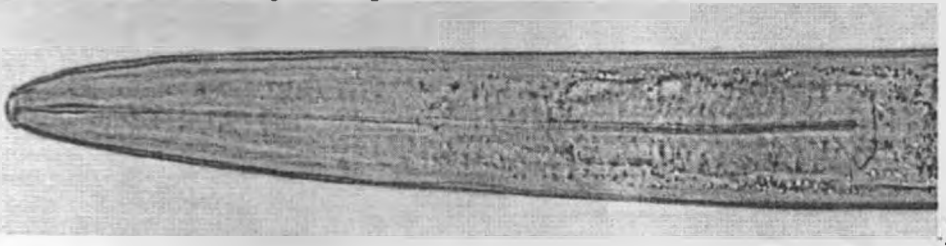


• Round tail . ..... *Enchodelus*

27. (25) Tail rounded 28

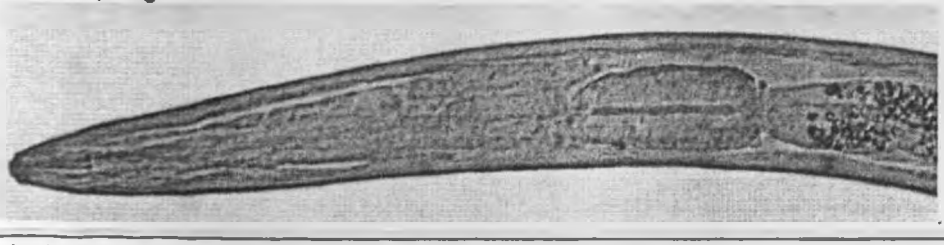
• Tail pointed ..... *Nothotylenchus*

28. (27) Basal part esophagus elongate



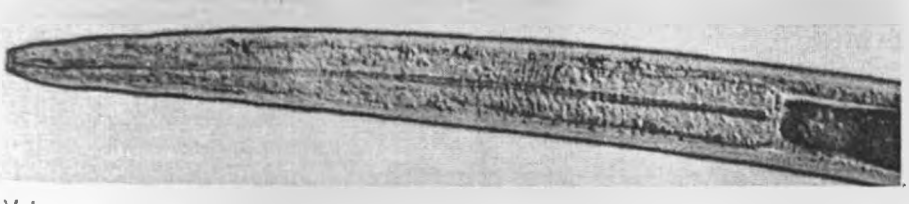
Tylencholaimus

• Basal part esophagus oval



..... *Doryllium*

29. (3) Valvate median esophageal bulb absent



.....30

• Valvate median esophageal bulb

Present 37

30. (29) Stomal walls not cuticularized 31

• Stomal walls cuticularized (*Actinolaimus*, *Metactinolaimus*, *Neoactinolaimus*, *Paractinolaimus*) .. *Actinolaiminae*

31. (30) Esophagus with basal expansions

...32

• Esophagus expanding uniformly . .. *Oionchus*

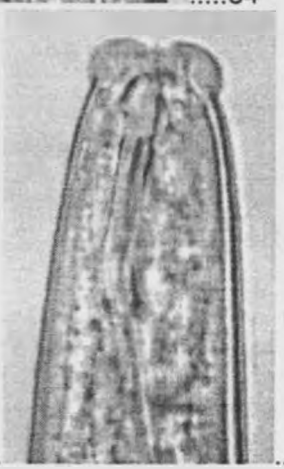
32. (31) Terminal fifth or sixth of esophagus an ovoid

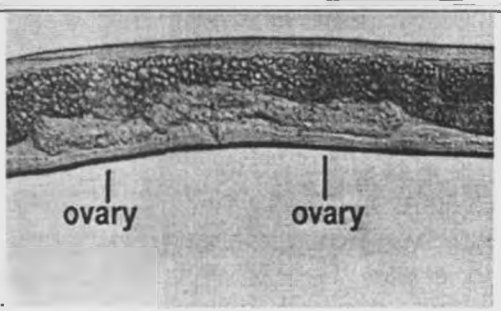
bulb ...33

• Posterior third of esophagus swollen

..36

33. (32) Stylet axial, positioned centrally .  34

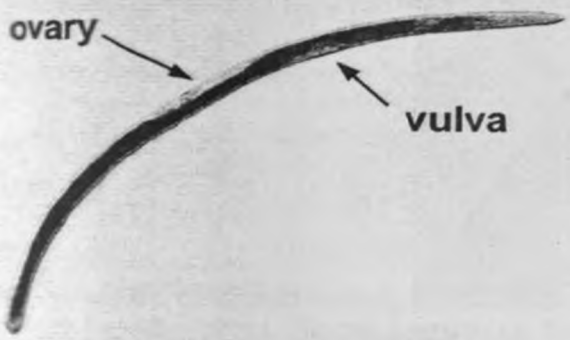
• Stylet not axial orginating from tooth in stoma wall .  ..... *Campydora*



34. (33) Gonads paired, vulva usually near mid-body .

..35

• Gonads single, posterior to vulva. Vulva usually anterior to mid-body



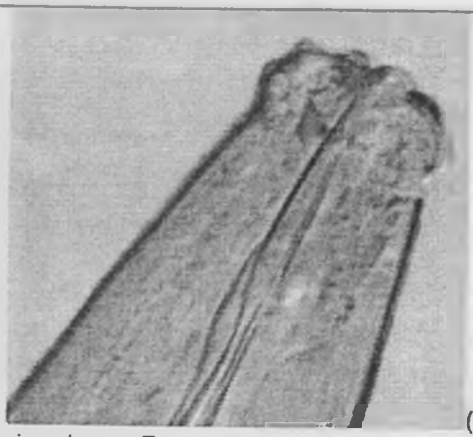
.....*Tyleptus*

35. (34) Stylet slender .

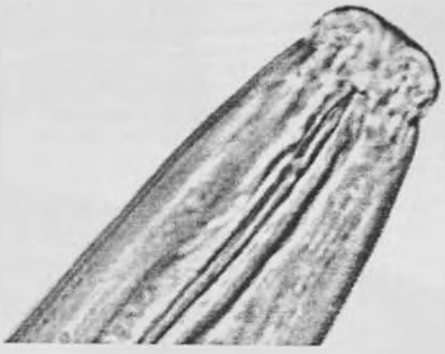
..*Leptonchus*

• Stylet not slender .

.. Dorylaimoides



36. (32) Stylet axial, positioned centrally (*Dorylaimus*, *Eudorylaimus*, *Labronema*, *Mesodorylaimus*, *Thornia*, *Laimydorus*, *Prodorylaimus*) .. Dorylaiminae

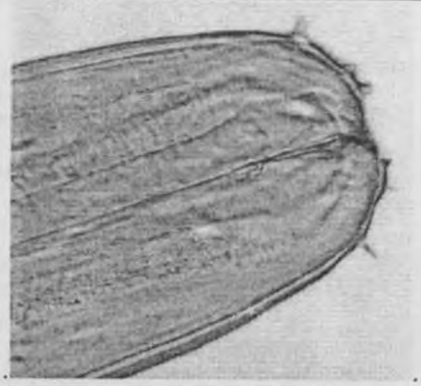


• Stylet not axial, originating from tooth in stoma wall . .. *Nygolaimus*

37. (29) Tail pointed . .. *Seinura*

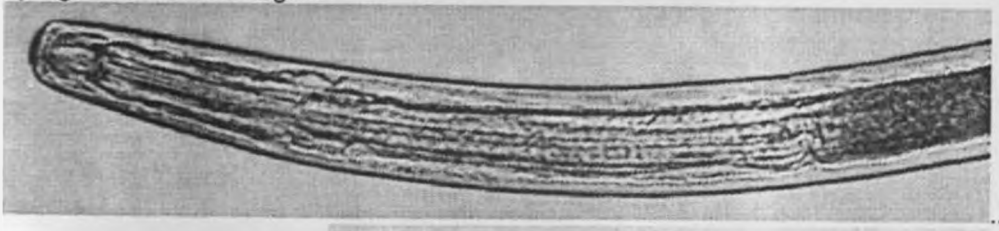
• Tail rounded . .....*Aphelenchus*

38. (2) Teeth present, prominent 39

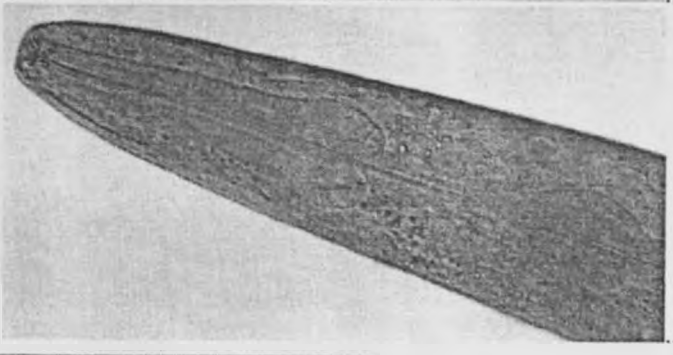


• Teeth absent, minute, or indistinct 50

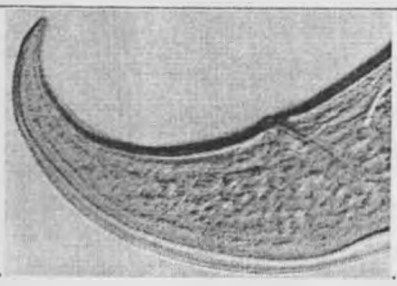
39. (38) Esophagus without mid-region expansion



.....40



• Esophagus expanded at mid-region 49

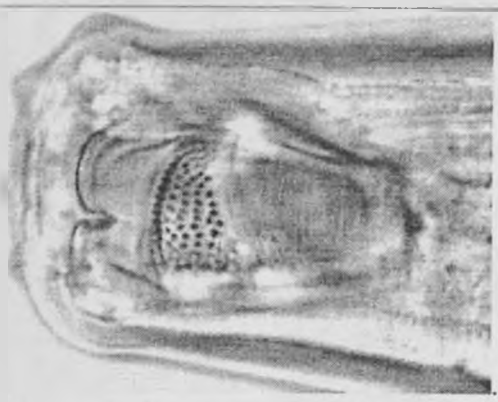


40. (39) Tail pointed or tapering. 41

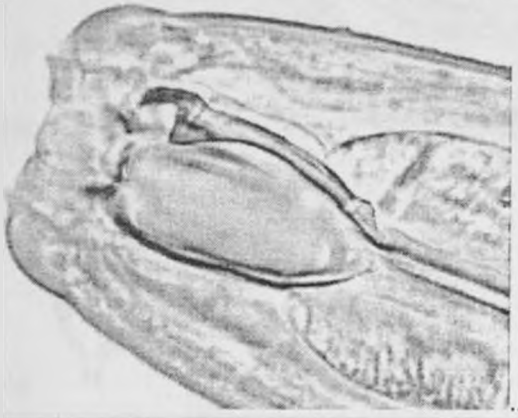
• Tail rounded 47

41. (40) Male tail without setae 42

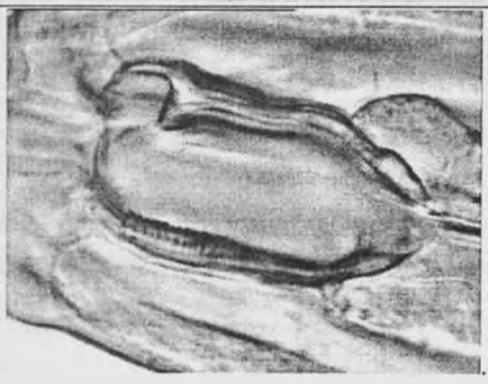
• Male tail with setae .   ..*Oncholaimus*



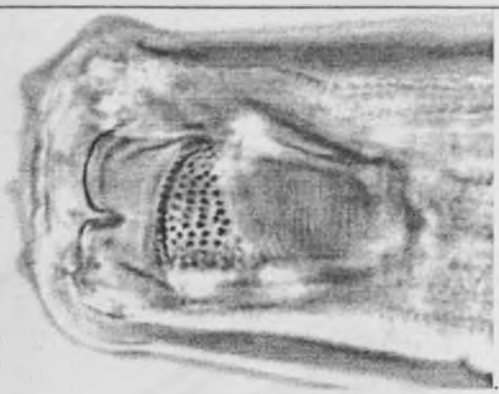
42. (41) Stoma with denticles 43



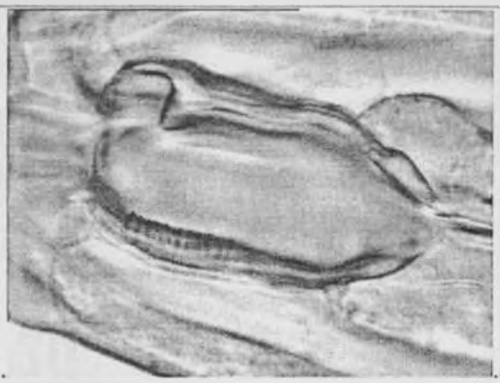
• Stoma without denticles 45



43. (42) Denticles scattered or in longitudinal rows 44

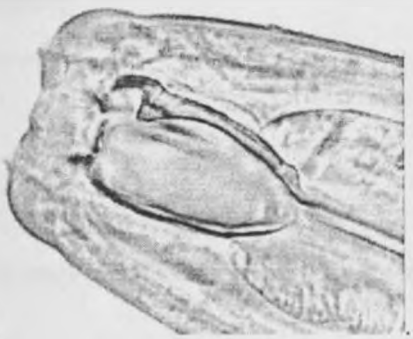


• Denticles in transverse rows . ... *Mylonchulus*



44. (43) Denticles situated on longitudinal rib of stoma . ...
Prionchulus

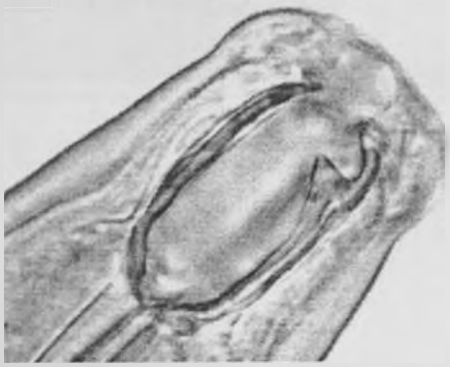
• Denticles scattered on stoma wall . ..... *Sporonchulus*



45. (42) Tooth anteriorly directed . ..pic...46

• Tooth retrorse . ..... *Anatonchus*

46. (45) Tooth in basal part of stoma .  ..... *Iotonchus*

• Tooth in anterior part of stoma  ..... *Mononchus*

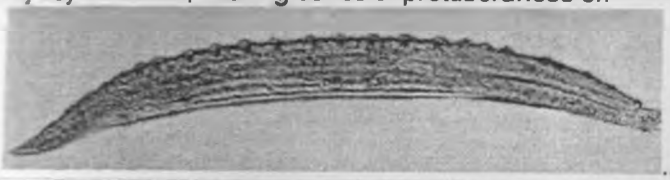
47. (40) Stoma with prominent medial or apical tooth .  48

• Stoma with small basal tooth  ..... *Bathyodontus*

48. (47) Stoma with 3 teeth, without small basal tooth, caudal glands terminal .  .. *Enoplocheilus*

▪ Stoma with large anterior & small basal tooth, caudal glands ventral .  .. *Mononchulus*

64. (1) Body symmetrical 65

• Body asymmetrical, bearing series of protuberances on side  .... *Bunonema*

65. (64) Lip appendages not elaborate 66

• Lip appendages elaborate 68

66. (65) Lateral lip appendages thorn-like directed laterally .. *Diploscapter*

• Lateral lip appendages not thorn-like or directed laterally 67

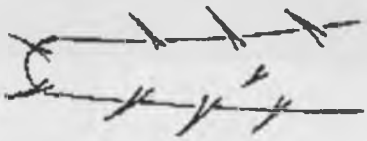
67. (66) Papillae or setae horn-like . .... *Macrolaimus*

• Lips flap-like and pointed anteriorly ..... *Teratocephalus*

68. (65) Lip appendages forked and elaborately fringed ..... *Acrobeles*

• Lip appendages membranous and wing-like ..... *Wilsonema*
.....*Tylocephalus*

69. (1) Post-cephalic setae absent 70



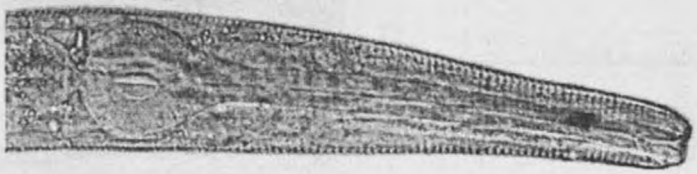
• Post-cephalic seate present (may be very faint Ex. *Tobrilus*) 92

70. (69) Stylet absent  71

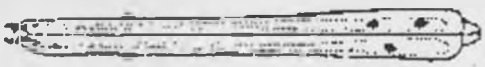
• Stylet present 91

71. (70) Teeth absent, minute or indistinct .  72

• Teeth usually present, prominent 85

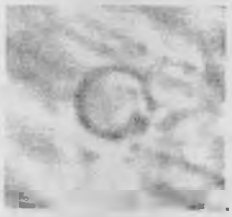


72. (71) Esophagus with basal expansions 73



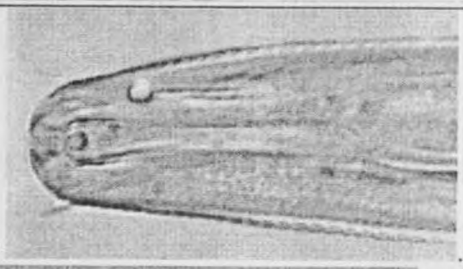
• Esophagus uniformaly cylindrical 82

73. (72) Amphids oval, spiral, or stirrup-shaped  74

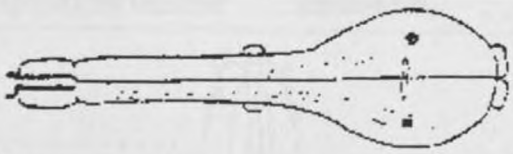
• amphids circular  ...80

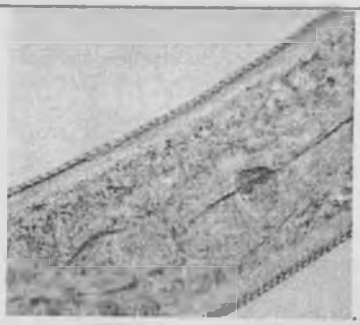
74. (73) Amphids spiral  75

• Amphids not spiral 79

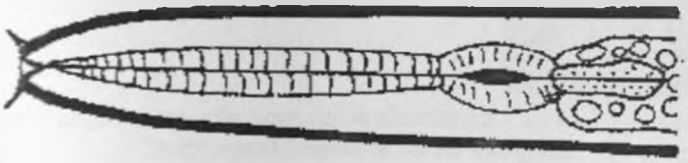
75. (74) Cuticular punctations absent  76

• Cuticular punctations present  78

76. (75) Esophageal bulb without valves ..  ...77

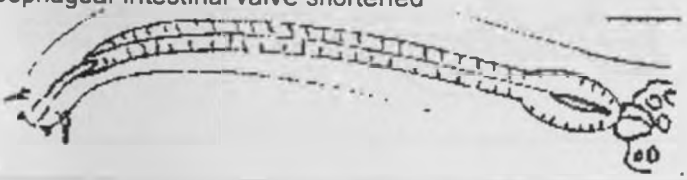
Esophageal bulb valvate  ..... *Plectus* & *Anaplectus*

77. (76) Esophageal-intestinal valve elongate



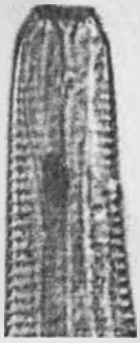
..... *Paraplectonema*

Esophageal-intestinal valve shortened



... .. *Paraphanolaimus*

78. (75) Labial region characteristically flap-like .. ... *Euteratocephalus*



Labial region not flap-like, lips bluntly rounded ..... *Ethmolaimus*

79. (74) Amphids oval ... .. *Greenenema*

Amphids stirrup-shaped .. .... *Chronogaster*

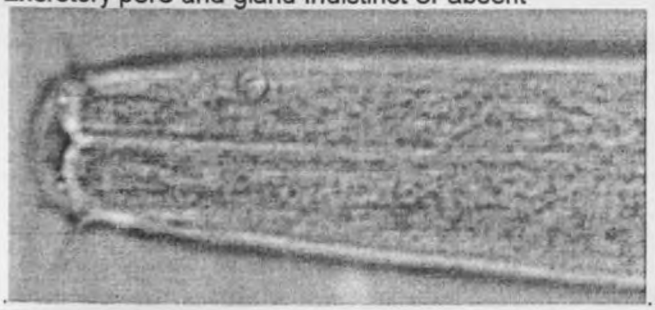
80. (73) Esophageal-intestinal valve shortened 81

Esophageal-intestinal valve elongate

... *Desmolaimus*

81. (80) Excretory pore and large excretory gland present .. *Domorganus*

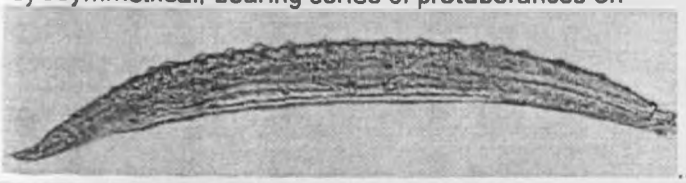
Excretory pore and gland indistinct or absent



.... Monhystera

64. (1) Body symmetrical 65

• Body asymmetrical, bearing series of protuberances on side



.... *Bunonema*

65. (64) Lip appendages not elaborate 66

• Lip appendages elaborate 68

66. (65) Lateral lip appendages thorn-like directed laterally ..

... *Diploscapter*

• Lateral lip appendages not thorn-like or directed laterally 67

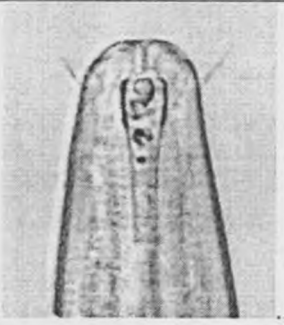
67. (66) Papillae or setae horn-like . .... *Macrolaimus*

• Lips flap-like and pointed anteriorly ..... *Teratocephalus*

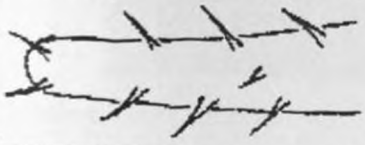
68. (65) Lip appendages forked and elaborately fringed  ..... *Acrobeles*

• Lip appendages membranous and wing-like  .....

Wilsonema .....*Tylocephalus*

69. (1) Post-cephalic setae absent  70

• Post-cephalic seate present (may be very faint Ex. *Tobrilus*)



....92

70. (69) Stylet absent  71

• Stylet present 91

71. (70) Teeth absent, minute or indistinct .  72

• Teeth usually present, prominent 85

72. (71) Esophagus with basal

expansions 73

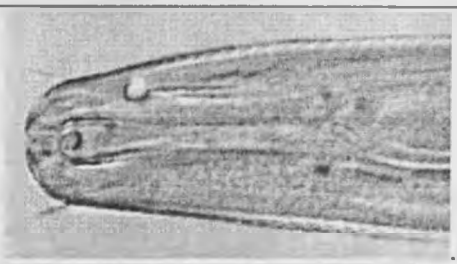
• Esophagus uniformaly cylindrical 82

73. (72) Amphids oval, spiral, or stirrup-shaped 
.....74

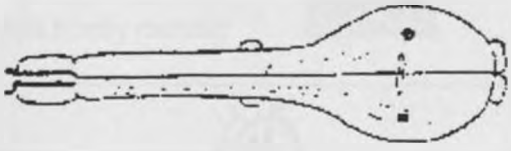
▪ amphids circular  ...80

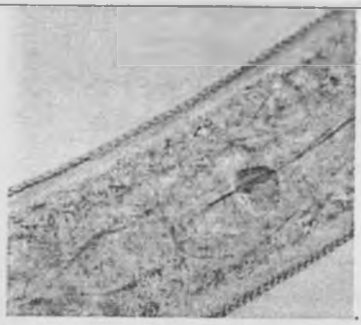
74. (73) Amphids spiral  75

• Amphids not spiral 79

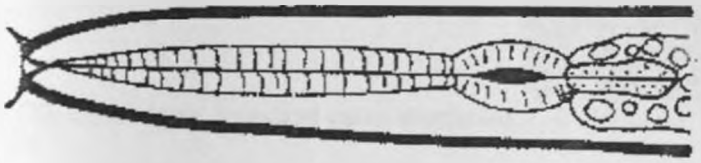
75. (74) Cuticular punctations absent  76

• Cuticular punctations present  78

76. (75) Esophageal bulb without valves ..  ...77

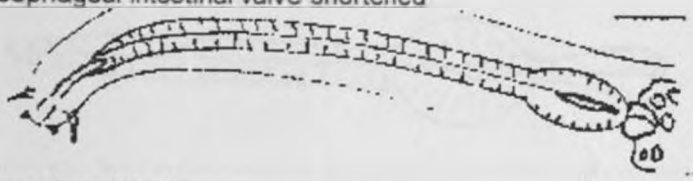
Esophageal bulb valvate  ..... *Plectus* & *Anaplectus*

77. (76) Esophageal-intestinal valve elongate



..... *Paraplectonema*

Esophageal-intestinal valve shortened



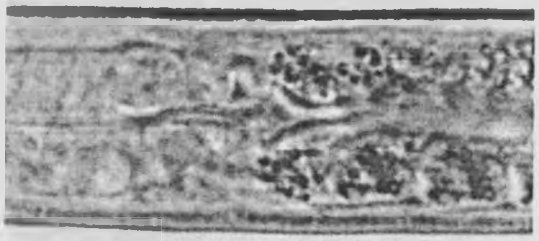
... *Paraphanolaimus*

78. (75) Labial region characteristically flap-like .. ... *Euteratocephalus*

Labial region not flap-like, lips bluntly rounded ..... *Ethmolaimus*

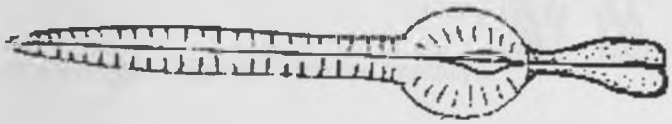
79. (74) Amphids oval ... .. *Greenenema*

Amphids stirrup-shaped .. ....
Chronogaster



80. (73) Esophageal-intestinal valve shortened 81

Esophageal-intestinal valve elongate



... *Desmolaimus*

81. (80) Excretory pore and large excretory gland present

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Excretory pore and gland indistinct or absent

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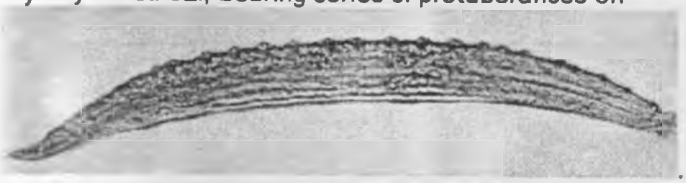
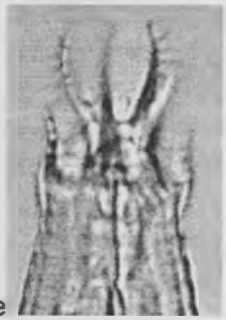
64. (1) Body symmetrical 65

- Body asymmetrical, bearing series of protuberances on side



.... *Bunonema*

65. (64) Lip appendages not elaborate 66

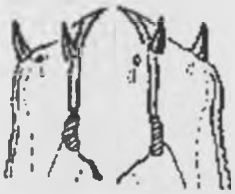


• Lip appendages elaborate 68

66. (65) Lateral lip appendages thorn-like directed laterally ..

... *Diploscapter*

• Lateral lip appendages not thorn-like or directed laterally 67



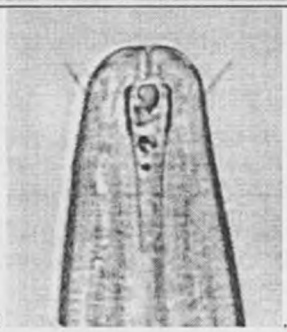
67. (66) Papillae or setae horn-like . .... *Macrolaimus*

• Lips flap-like and pointed anteriorly ..... *Teratocephalus*

68. (65) Lip appendages forked and elaborately fringed  ..... *Acrobeles*

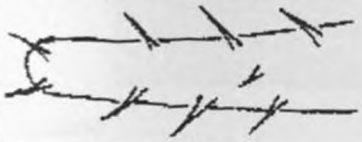
• Lip appendages membranous and wing-like  .....

Wilsonema .....*Tylocephalus*



69. (1) Post-cephalic setae absent 70

• Post-cephalic seate present (may be very faint Ex. *Tobrilus*)



....92

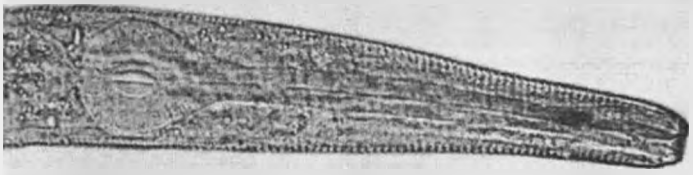
70. (69) Stylet absent  71

• Stylet present 91

71. (70) Teeth absent, minute or indistinct .  72

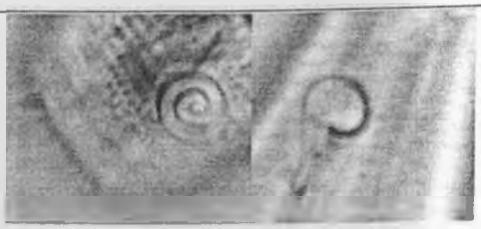
• Teeth usually present, prominent 85

72. (71) Esophagus with basal



expansions 73

• Esophagus uniformaly cylindrical 82

73. (72) Amphids oval, spiral, or stirrup-shaped 
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- amphids circular ...80

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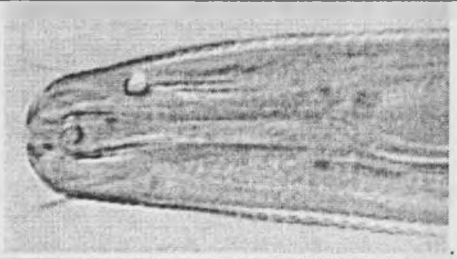
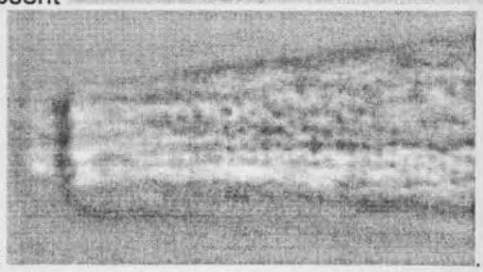
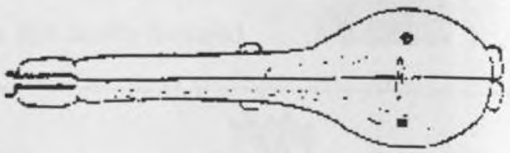
- Amphids not spiral 79



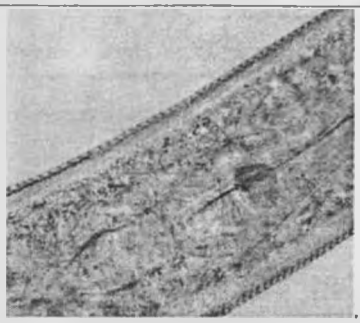
75. (74) Cuticular punctations absent 76



- Cuticular punctations present 78

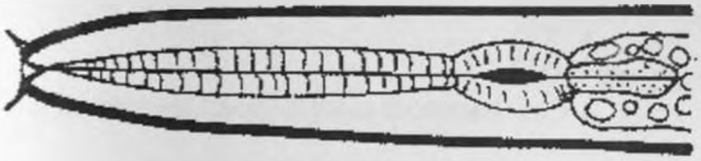


76. (75) Esophageal bulb without valves 77



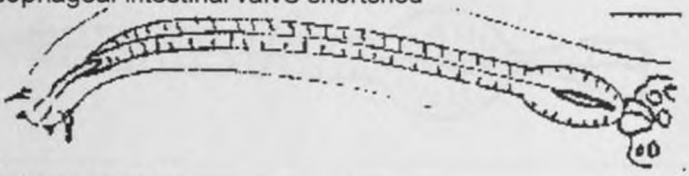
Esophageal bulb valvate ..... *Plectus* & *Anaplectus*

77. (76) Esophageal-intestinal valve elongate



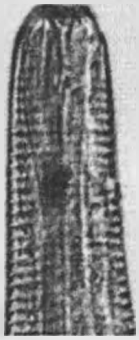
..... *Paraplectonema*

Esophageal-intestinal valve shortened



... *Paraphanolaimus*

78. (75) Labial region characteristically flap-like .. ... *Euteratocephalus*



Labial region not flap-like, lips bluntly rounded ..... *Ethmolaimus*

79. (74) Amphids oval ... .. *Greenenema*

Amphids stirrup-shaped .. ....
Chronogaster

80. (73) Esophageal-intestinal valve shortened 81

Esophageal-intestinal valve elongate

... *Desmolaimus*

81. (80) Excretory pore and large excretory gland present

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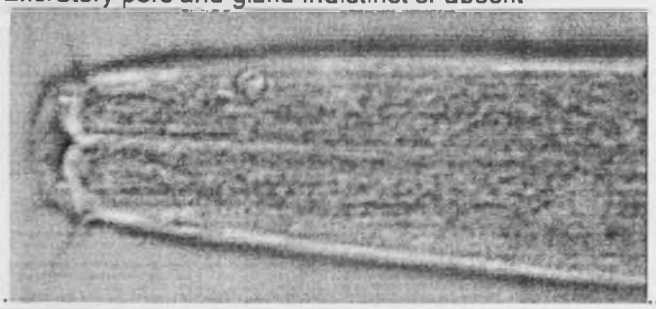
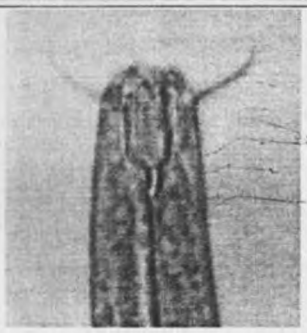
Excretory pore and gland indistinct or absent



.... Monhystera



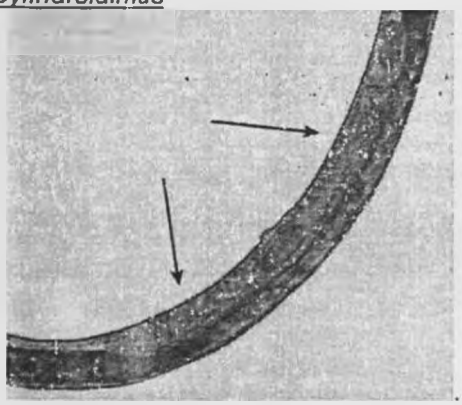
82. (72) Stoma wide and shallow, conspicuous,

tail filiform  .....
Prismatolaimus

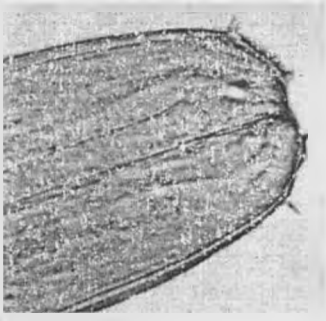
• Stoma narrow, elongate, collapsed or inconspicuous 83

83. (82) Gonad single .

.. .. *Cylindrolaimus*



• Gonads paired 84



84. (83) Amphids inconspicuous ..... *Tripyla*

• Amphids conspicuous ..... *Aphanolaimus*

85. (71) Terminal fifth or sixth of esophagus an ovoid bulb  86

• Esophagus uniformly cylindrical, stoma with massive teeth .....*Ironus*

86. (85) Cuticular punctations present 87

• Cuticular punctations absent 89

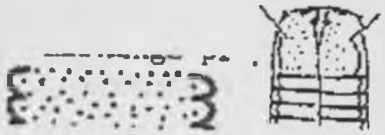
87. (86) Amphids not spiral 88

• Amphids spiral .... *Achromadora*

88. (87) Four longitudinal rows of cuticular markings present ..

. . *Chromadora*

• No longitudinal rows of cuticular markings present .. *Prochromadorella*



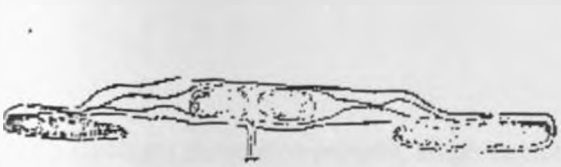
89. (86) Amphids distinct ..

..90

• Amphids indistinct .

.... *Butlerius*

90. (89) Female gonad double, amphid hook-shaped



..*Anonchus*

• Female gonad single, amphid circular .

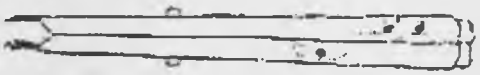
. *Monhystrella*

91. (70) Lip region annulated, not set off . .... *Atylenchus*

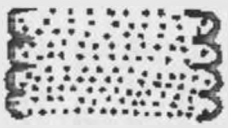
• Lip region smooth, set off . ... *Eutylenchus*

92. (69) Esophagus with basal

expansion 93

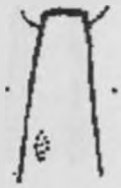


• Esophagus uniformly cylindrical 98



93. (92) Cuticular punctation present, amphids not circular 94

• Cuticular punctation absent, amphids circular.... 97



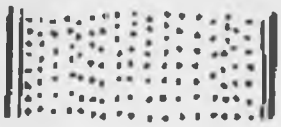
94. (93) Ocelli (eye spots) present 95

• Ocelli absent 96

95. (94) Stoma with three equal-sized teeth .. ... *Chromadorina*

• Stoma with at least one large tooth .. ... *Punctodora*

96. (94) Cuticule with lateral longitudinal rows of punctation ... *Hypodontolaimus*

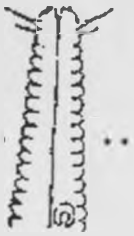


• Cuticle without lateral differentiations .. ... *Chromadorita*

97. (93) Esophageal bulb valvate .. ... *Prodesmodora*

• Esophageal bulb without valves ..... *Odontolaimus*

98. (92) Amphid anterior on body 99



• Amphid posteroirly located . .... *Bastia*

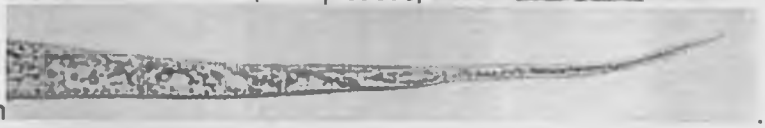
99. (98) Amphid spiral . .... *Paracyatholaimus*

• Amphid cup-shaped or obscure 100

100. (99) Stomal teeth massive .. ... *Oncholaimus*

• Stomal teeth small .. .... *Tobrilus*

82. (72) Stoma wide and shallow, conspicuous,



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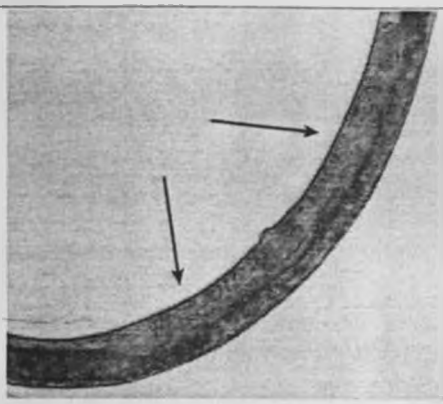
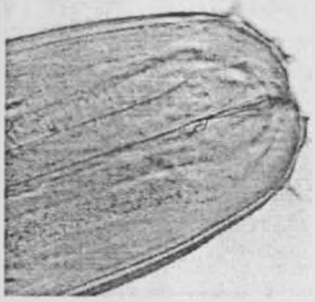
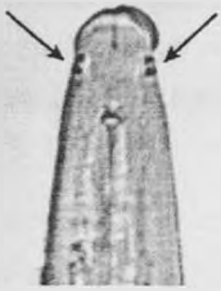
83. (82) Gonad single .

.. *Cylindrolaimus*

• Gonads paired  84

84. (83) Amphids inconspicuous  ..... *Tripyla*



• Amphids conspicuous ..... *Aphanolaimus*

85. (71) Terminal fifth or sixth of esophagus an ovoid bulb  86

• Esophagus uniformly cylindrical, stoma with massive teeth .....*Ironus*

86. (85) Cuticular punctations present  87

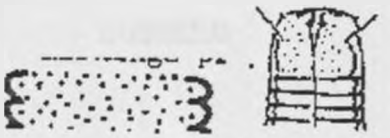
• Cuticular punctations absent .  89

87. (86) Amphids not spiral 88

• Amphids spiral ..... *Achromadora*

88. (87) Four longitudinal rows of cuticular markings present .. 

. . *Chromadora*



• Nc longitudinal rows of cuticular markings present .. ..

Prochromadorella

89. (86) Amphids distinct 90

• Amphids indistinct . .... *Butlerius*

90. (89) Female gonad double, amphid hook-shaped

..*Anonchus*

• Female gonad single, amphid circular .

. *Monhystrella*

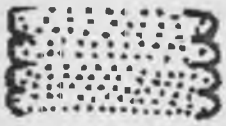
91. (70) Lip region annulated, not set off . .... *Atylenchus*

• Lip region smooth, set off . ... *Eutylenchus*

92. (69) Esophagus with basal

expansion 93

• Esophagus uniformly cylindrical 98



93. (92) Cuticular punctation present, amphids not circular 94

• Cuticular punctation absent, amphids circular... 97

94. (93) Ocelli (eye spots) present 95

• Ocelli absent 96

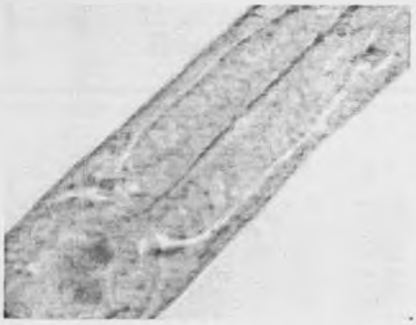
95. (94) Stoma with three equal-sized teeth .. ... *Chromadorina*

• Stoma with at least one large tooth .. ... *Punctodora*

96. (94) Cuticule with lateral longitudinal rows of punctation ... *Hypodontolaimus*

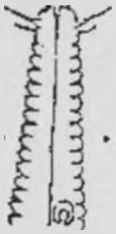
• Cuticle without lateral differentiations .. ... *Chromadorita*

97. (93) Esophageal bulb valvate .. ... *Prodesmodora*



• Esophageal bulb without valves ..... *Odontolaimus*

98. (92) Amphid anterior on body 99



• Amphid posteroirly located . .... *Bastia*

99. (98) Amphid spiral . .... *Paracyatholaimus*

• Amphid cup-shaped or obscure 100

100. (99) Stomal teeth massive .. ... *Oncholaimus*

• Stomal teeth small .. .... *Tobrilus*

7.3.3 Fertilizer nutrient composition

1. MAVUNO

Technical Specification

Planting mavuno fertilizers manufactured by Athi River Mining, Kenya is a carefully researched blend of fertilizers containing 11 nutrients in balanced proportions and is suitable for most crops and soil conditions in Kenya.

The Essential Nutrients contained in MAVUNO planting are:

Nitrogen (N½) 10%, Phosphorous (P_2O_5) 26%, Potassium (K_2O) 10%, Sulphur (SO_4) 4%, Calcium (CaO) 10%, Magnesium (MgO) 4%, and appropriate additions of other Trace Elements like: , Zinc, Copper. Molybdenum, Boron and Maganese

Benefits

Formulated for both Commercial Agriculture and Horticulture.

- Provides essential nutrients in balanced proportions.
- Accelerates the growth of Chlorophyll, which in turn increases plant growth and subsequent yields
- Reduces soil acidity and increasing Soil pH., thereby improving soil conditions
- Increase uptake and efficiency of N,P,K and other nutrients
- Increases plant resistance to disease and drought.
- In field applications Mavuno fertilizers has shown 16-40%higher yields on various crops.
- Fast acting highly efficient and very affordable.
- Convenient packages of 1kg, 10 kg, 25 kg and 50kg.

2. **Triple Superphosphate**

It is a fertilizer produced by the action of concentrated phosphoric acid on ground phosphate rock. $3Ca_3(PO_4)_2 \cdot CaF_2 + 4H_3PO_4 + 9H_2O \rightarrow 9Ca(H_2PO_4)_2 + CaF_2$

The active ingredient of the product, monocalcium phosphate, is identical to that of superphosphate, but without the presence of calcium sulfate that is formed if sulfuric acid is used instead of phosphoric acid. The phosphorus content of triple super phosphate (17 - 23% P; 44 to 52% P_2O_5) is therefore greater than that of super phosphate (7 - 9.5% P; 16 to 22% P_2O_5). Triple super phosphate was the most common phosphate (P) fertilizer in the USA until the 1960s, when ammonium phosphates became more popular. It is produced in granular and nongranular form and is used both in fertilizer blends (with potassium and nitrogen fertilizers) and by itself.

Triple superphosphate, also known as double, treble, or concentrated superphosphate, is a fertilizer material with a phosphorus content of over 40 percent, measured as phosphorus pentoxide (P_2O_5).

3. **Calcium ammonium nitrate** (CAN)

This fertilizer contains 27 % N and 20 % of ground limestone. Nitrogen is half in the nitrate form and half in the ammonial form. This results in rapid as well as permanent effect. The granulation of this fertilizer ensures a quick and exact dosing. Calcium ammonium nitrate has a form of 2 - 5 mm large of whitish till light brown colour granules. The fertilizer has excellent physico-mechanical properties and

properties for storage. Bulk density is approx. 950 - 1,000 $kg.m^{-3}$ and the angle of slope is 30°. The applications are universal. CAN is a nitrogen fertilizer applicable practically to all plants growths, and to all, even to more acid soils. This fertilizer is most frequently used for manuring of cultures during vegetation.

Safety measures:

CAN may be dangerous for human health. Avoid swallowing or contacting with mucous membranes, eyes and repeated contact with skin. Dust of the fertilizer is irritable and may cause oversensitiveness or eczemas. During manipulation it is necessary to protect the skin and eyes, eating, drinking and smoking are not allowed. After the work hands should be washed thoroughly and regeneration cream should be used. Keep out of reach of children and unauthorised persons.

Calcium Ammonium Nitrate (CAN 17) is a versatile fertilizer that contains 8.8% calcium and 17% nitrogen. The nitrogen is one-third ammonic form and two-thirds nitrate form. Calcium is a required nutrient for normal plant growth and development, and is especially critical to proper cell membrane development.

Benefits

The nitrate form of nitrogen is available immediately to plants, which makes it an excellent early-season fertilizer.

The nitrate in CAN 17 is available immediately to crops as they grow in cool soils, while the ammonic form is held in reserve until soil temperatures rise and a plant's nitrogen requirements are greater.

The calcium in CAN 17 is water soluble and readily available to plants, giving a crop the best chance at proper early-season cell wall development as well as avoiding late-season internal disorders such as blossom end rot of tomatoes, bitter pit of apples, and internal brown spot of potatoes.