NEMATODE RESPONSE TO SOIL ORGANIC AMENDMENTS IN A SEMI-ARID REGION UNDER SOYBEAN *(Glycine max)*, NAIVASHA, KENYA.

ADUKE VIOLET DOLLY ORLANDO

(B.Sc. Hons, Kenyatta University)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE MASTER OF SCIENCE IN APPLIED PARASITOLOGY DEGREE OF THE UNIVERSITY OF NAIROBI

SCHOOL OF BIOLOGICAL SCIENCES

2016

DECLARATION

I declare that this thesis is my original work and it has not been presented wholly or in part for any award in any other institution

Candidate: Aduke Violet Dolly Orlando

Signature……; Date……

Supervisors:

This thesis has been submitted with our approval as supervisors

Dr. David Odongo

School of Biological Sciences**,**

University of Nairobi, Kenya

Signature……; Date……

Prof. Sheila Okoth

School of Biological Sciences**,**

University of Nairobi, Kenya

Signature……; Date……

DEDICATION

To my daughters Abby, Wema, Tulizanna and Margaret

ACKNOWLEDGEMENTS

The realization of this study would not have been possible without the assistance of several people who contributed to the success of this work. I owe my deepest gratitude to my supervisors Prof. Sheila Okoth and Dr. David Odongo of the University Of Nairobi, School of Biological Sciences for their guidance from the conception of this work up to the end, their positive critiques, inputs, edits and encouragement. Many thanks to Ruth Vaughan, general manager Dudutech Finlay's Ltd, for allowing me to carry out the trials at their Kingfisher Farm in Naivasha. In particular am very grateful to Mr. Jack Adundo the technical manager at Dudutech for his valued support both during the field trials and in the laboratory. I thank him for sharing with me his vast knowledge and experience and for making my trials a success. Many thanks to lab technician George for dedicating his time to assist me in the lab, Edward the quality control manager for his positive inputs during the field setups and to all the people in the Dudutech laboratory and Research and Development section for their hospitality and assistance during the field and Laboratory experiments. I would also like to acknowledge Leah Njunge my fellow Msc student with whom we shared the trial plots, for the time, knowledge and many memorable moments we shared throughout the trial including the tiring road travels in over loaded 'matatus' to Dudutech and the humongous fish (Common cup) we often ate at lunch breaks with Dudutech staff by the lake shore all made this study period exciting. I am grateful to almighty God, for seeing me through this work, all glory unto him. Very special thanks to Jack my spouse for all the financial support and encouragement and to my girls for providing the necessary distraction whenever I needed it. To my parents Mr. and Mrs. Orlando, my sisters and brother for all the love and to all my friends who never stopped their prayers and encouragement throughout this journey, I am thankful.

.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

ABSTRACT

Agricultural production within the smallholder farming sector of East Africa is constrained by numerous factors including parasitic nematodes. Existing control measures involving applications of chemical nematicides are not viable in the medium to long term due to environmental concerns relating to toxic residues. There is therefore a need to develop alternative control options for integrated parasitic nematode management that will promote soil eco health and reduce parasitc nematode densities. This study evaluated population changes of soil nematodes, root galling and changes in soil properties following addition of soil organic amendments in a semi-arid region under soybean cultivation. Biochar, Vermicompost and Mycorrhizae were incorporated as organic amendments and applied as single or combined treatments using a randomised block design. *Paecilomyces lilacinus* a nematophagous fungus was used as a positive control. Nematodes were extracted from the soil using the modified Baermann extraction tray technique, identified to genera level then grouped into their trophic groups as herbivores, fungivores, predators or bacteriovores. Results at flowering stage showed no significant difference ($P>0.05$) between the treatments on predating, bacterivorous and fungivorous nematode populations. Conversely, Biochar and the untreated plots had significant high populations of parasitic nematodes ($P<0.05$).The combination of Biochar and Mycorrhizae application recorded significantly lower galling index at flowering between the treatments. At the end of the trial there were no significant differences in trophic group populations, however Biochar plots had a 40% reduction in parasitic nematode populations as compared to flowering stage populations. Vermicompost treated plots recorded significantly higher galling index and elevated amounts of extractable Phosphorous. Biochar is a viable option for use in integrated parasitic nematode control because of its potential to increase yields and reduce parasitic nematodes as was observed in this study, however more studies are needed to evaluate effects of Biochar and its interactions with Mycorrhizae on parasitic nematode densities due to their combined potential to lower parasitic nematode populations as was also observed. *Paecilomyces lilacinus* still remains a viable treatment for the control of plant parasitic nematodes.

LIST OF ABREVIATIONS

AMF	Arbuscular Mycorrhizae Fungi
ANOVA	Analysis of variance
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
J_2	Second stage juvenile
KALRO	Kenya Agricultural and Livestock Research Organisation
LSD	Least Significant Difference
MRLs	Maximum Residue Levels
NH_3^+	Ammonia
NH_4^+	Ammonium ions
PPN	Plant Parasitic Nematodes
SCN	Soybean Cyst Nematode
SDS	Sodium Deodecyle Sulphate
TAF solution	Formalin Triethanolamine solution

CHAPTER ONE

1.0 INTRODUCTION AND LITREATURE REVIEW

1.1 General Introduction.

Phyto-parasitic nematodes are a threat to several agricultural crops and can cause great yield lose threatening food security. Production of soybean is on the increase especially in Kenya due to its multipurpose utilities in both animal and human feed, medicinal values and industrial uses. Soybeans (*Glycine max*) just like the common beans (*Phaseolus vulgaris*) are susceptible to parasitic nematodes. Of economic importance are root knot nematodes *Meloidogyne sp* and cyst nematodes *Heterodera sp*, although soybeans are still prone to attack by other nematode species. Soybean Cyst Nematode (SCN) causes more than US$1 billion in yield losses annually in the United States alone. *Meloidogyne* sp can cause up to 60% loses in yield in common beans (Kimenju *et al*., 1999) especially in warm temperate regions where it has a wide distribution and high prevalence (Perry and Evans, 2009).

For many years chemical nematicides have been used to control plant nematodes effectively. Although these are effective and fast acting, they are degrading to the environment, other beneficial soil micro flora and human health (Wachira *et al*., 2009). Heavy use of pesticide has in the past resulted to deaths. For instance, in 2006, WHO reported an estimated one million people were being poisoned annually by pesticides with at least 200,000 of these cases resulting in deaths (WHO, 2006). In Kenya use of pesticides has been encouraged for increased crop yields. In 2005, approximately 7,047 metric tons of pesticides, valued at US$54 million were imported (PCPB, 2005). Due to their apparent health and environmental hazards, some of the chemical nematicides have been withdrawn or their use restricted (Thomason, 1987), for example broad spectrum pesticides methyl bromide and carbofuran. Horticultural exports are the second largest foreign exchange earner in Kenya bringing in an estimated US$300million annually (Mehrdad, 2004) and also the largest consumers of pesticides. Majority of these exports

are to the European Union countries where laws are stringent on the Maximum Residue Levels MRLs (EU Regulation 1107/2009) in exported crops.

Due to this urgency to reduce usage of chemical nematicides and to develop an integrated nematode control strategy, various studies are currently underway including the use of organic amendments. Studies have shown that organic amendments can be used to reduce parasitic nematode populations to levels below damage thresholds. Their effects being indirectly attributed to stimulation of other soil microbes that release nematicidal substances (McSorley and Gallager, 1995; Oka *et al*., 2002). Organic amendments derived from livestock manure, sewage wastes and different composts have been reported to have an effect on plant parasitic nematodes and free living micro flora (Renco *et al*., 2012; D'Addabbo *et al*., 2011; Akhtar and Malik 2000). Biochar is an organic amendment that is currently being promoted as a soil additive and helps in carbon sequestration and improved soils. There is limited information on the use of Biochar in parasitic soil nematode management and less still, its effect on other nematode trophic groups when applied in combination with other amendments namely Vermicompost and Mycorrhizae in Kenya. The aim of this study was to evaluate the response of plant nematodes to Biochar, Vermicompost and Mycorrhizae organic amendments application in a semi-arid area under soybeans, Naivasha Nakuru County, Kenya.

1.2 Literature review

1.2.1 Soybean production and its economic importance in Kenya

Drought is a major hindering factor in agricultural production especially in arid and semi-arid parts of Kenya which constitute 80% of agro ecological zones. Soybean is a drought tolerant crop with potential of improved productivity (Mathu *et al*., 2010, Chianu *et al*., 2008). It is therefore suitable for areas with rainfall of 300 to 1200mm annually and 0 -2200m altitude.

In Kenya, soybean crop (*Glycine max*) is not as largely cultivated as the common bean (*Phaseolas vulgaris* L.). Kenya's soybean production is estimated to be about 0.1% of total output in Africa. Nigeria is the largest producer accounting for 50% of Africa's total output (Chianu *et al*., 2008). Soybean production in Kenya is about 5,000 tonnes per annum which is below the demand of 50,000 to 100,000 tonnes imported annually by soybean processors in Kenya (Karuga and Gachanja, 2004). It is mainly produced in parts of the Rift valley, Central, Eastern, Nyanza and mostly in Western Kenya which accounts for 50% of its total production (Chianu *et al*., 2008).

Soybean is becoming an increasingly important crop globally. Under Vision 2030 (Government of Kenya, 2007) soybean was earmarked as one of the crops that would contribute to agricultural economic growth because of its health and industrial usages. In Kenya, soybean is used to produce vegetable cooking oil and in the manufacture of animal feeds where it amounts to almost 60% of most livestock feeds (Chianu *et al*., 2008). Due to its low cholesterol and high protein levels of 40% unlike other legumes with 20% protein or less (Greenberg and Hartung, 1998), soybean is considered heart "friendly". It is also rich in essential minerals and vitamins (Liu, 1997), making it a very important nutritional component of the diet especially in vulnerable groups like infants below the age of five, expectant and lactating mothers, immune compromised individuals and the elderly. Value added products such as soy sauce; beverages, snacks and milk are also important to health and are a good source of income as they promote cottage industries and employment.

Soybean being a leguminous crop can be intercropped with maize to increase soil fertility through nitrogen fixation (Sanginga *et al*., 2003). This nitrogen fixing potential of soybean has been reported to increase maize yields by up to 25% (Chianu, 2008). When intercropped with cereal crops like maize, soybean can slow down the build up of pests, diseases and weeds leading to reduced pesticide use which in turn reduces the impact of chemicals to the

environment by reducing contamination to water sources and the associated effects namely high energy input and CO_2 emissions (Mahasi *et al*., 2011).

1.2.2 Nematodes attacking soybean

Soybean is susceptible to parasitic nematodes, of economic importance being the root knot nematodes *Meloidogyne* sp (Ngundo and Taylor, 1974) and cyst nematodes *Heterodera* sp. Most nematodes can be observed only with magnification, but the adult females and cysts of *Heterodera glycines* also referred to as the soybean cyst nematode (SCN) are visible to the unaided eye. Other species that attack soybean include reniform nematodes (*Rotylenchulus reniformis*) that feed on and cause severe root necrosis, lesion nematodes (*Pratylenchus* sp.), burrowing nematodes (*Radopholus* sp) that cause toppling of plants especially in bananas, and sting nematodes (*Belonolaimus longicaudatus*) that feed on root tips.

1.2.3 Economic importance of nematodes

Parasitic nematodes migrate from the soil to the roots of host plants where they use a specialised mouth piece (stylet) to pierce plant cells to establish source of nutrients for sustainability. Heavy infestations by nematodes can reduce the uptake of essential nutrients from the soil to the rest of the plant. Under such circumstances yields are reduced due to impaired nutrient and water uptake caused by distorted and reduced roots.

Root rot initiated by burrowing parasites *Radopholus similis, Longidorus* sp (pin nematode), *Trichodorus* sp, *Paratrichodorus* sp (stubby-root nematode) and *Xiphinema* sp (dagger nematode), is aggravated by invasion of fungal pathogens for example *Cylindrocarpon musae* and *Rhizoctonia* sp which can increase damage to roots. *Radopholus similis* is also known to increase the infectivity of the fungus *Fusarium solani* and the bacteria *Xanthomonas* sp in plants (Aragaki *et al*., 1984; Luc *et al*., 2005).

Root knot nematodes *Meloidogyne* sp and cyst nematodes *(Heterodera* and *Globodera* sp*)* are widely distributed and more prevalent in warm temperate regions (Perry and Evans, 2009) like Kenya. These species can attack a wide range of horticultural and field crops, even forest trees (Ibrahim and Traboulsi, 2009) with loses due to root knot nematodes ranging from 18 to 30% for water melon, 24 to 38% for tomatoes and 25% or more for potatoes (www.infonet-biovision.org). Above ground symptoms of nematode disease may not be visible but in severe cases affected plants appear as yellow patches and might be confused with nutrient deficiency symptoms. Nematodes are therefore sometimes often overlooked by some farmers as serious pests and yet they have negative economic impacts to agriculture (Bridge, 1996).

1.2.4 Nematode management

Management of parasitic plant nematodes has so far involved the use of various methods namely biological, cultural and chemical control or integration of two or more methods. Biological control methods such as the use of resistant cultivars are cheap to farmers. Recently scientists have identified an area on chromosome 18 called Rhg1 (for resistance to *Heterodera glycines* 4) the location that is the main source of soybean cyst nematode resistance and have been able to increase the expression rate of the resistant genes to increase resistance effect (Cook *et al*.,2012; Kandoth *et al*., 2011 and Liu *et al*.,2012). A major drawback to use of resistant cultivars is the prolonged period of time it takes to breed and screen for resistant varieties, furthermore it is difficult to develop a plant that is resistant to all parasitic nematodes.

Use of natural predators' for example fungi and bacteria, have been reported in various studies to be effective and a promising control method for parasitic nematodes. Mycorrhizal fungi (Castillo *et al*., 2006) compete with nematodes for nutrients therefore retarding the parasites growth. The fungi *Paecilomyces lilacinus* (Kienwick and Sikora, 2003) and *Trichoderma atroviride* (Darago *et al*., 2013) have nematicidal effects of destroying nematode

eggs. Non-pathogenic strains of *Fusarium oxysporum* (Bancy *et al*., 2014) are also able to inhibit or kill juveniles. The bacterium *Pseudomonas* has also been reported to be effective in the bio control of nematodes by killing juveniles and adults by producing lethal hydrogen cyanide (Gallager and Manoil, 2001; Imran *et al*., 2006). Use of naturally predating microorganisms is hindered by the cost and difficulty in production of large amounts for commercial use whereas for small scale farmers buying cost is a challenge. Microbials are also readily inactivated by environmental factors such as sunlight; rain and wind, making them effective only for a short while (Thacker, 2002).

Crop rotation is another method of biological control where nematode host plants are alternated with non hosts. This method is only effective when a farmer has a wide choice of crops to grow, enough land space for rotation and if the problematic nematode does not survive in crypto biotic stage for a long time in the soil or does not have a wide range of hosts, but in most cases nematodes will have more than one host especially *Meloidogyne sp* (Thomason and Caswell, 1987).

Organic amendments are materials derieved from plant or animal materials that can be added to soils to improve their chemical and physical properties. Organic amendments from various sources have been reported to reduce nematode populations therefore making this method a potential strategy in the management of parasitic nematodes, although other studies have also reported no change or an increase in nematode populations when organic amendments are applied in various crops (McSorley and Gallager, 1995; Kimpinski *et al*., 2003). Nematicidal effects of these amendments have been attributed to several factors including increase in facultative parasites due to their richness in organic matter and release of toxic substances during decomposition (Sikora, 1992; Oka *et al.*, 2007). Although soil organic amendments can be effective and have additional benefits like increased plant immunity to diseases, they can be

bulky because large quantities are needed especially in large farms but may also be easily prepared in small farm holdings (Rodriguez-Kabana, 1986).

Other methods currently on trial include soil solarisation and heat treatment. Increasing temperatures by solarization can be of use in controlling nematodes (FAO, 1991; Gaur and Perry, 1991b), but this is only possible during long uninterupted periods of sunshine. Solarisation reduces nematode populations and competition from the soil micro flora, enabling biological control agents to proliferate (Sikora, 1992).

1.2.5 Nematode life cycle

Plant parasitic nematodes are round; non-segmented obligate worms that complete their life cycle in the soil environment in the presence of a host plant. They have six stages within their life cycle, an egg, four juvenile stages and an adult stage. With each stage separated by a moulting phase (Fig.1). For most plant parasitic nematodes, the first moult occurs in the egg which hatches into the second-stage juvenile (J_2) that penetrates the root tissue. Most parasitic nematodes are dioecious while some species are monoecious with the females producing both eggs and sperms. Egg production and length of lifecycle varies and depends on nematode species, host plant and the soil environment.

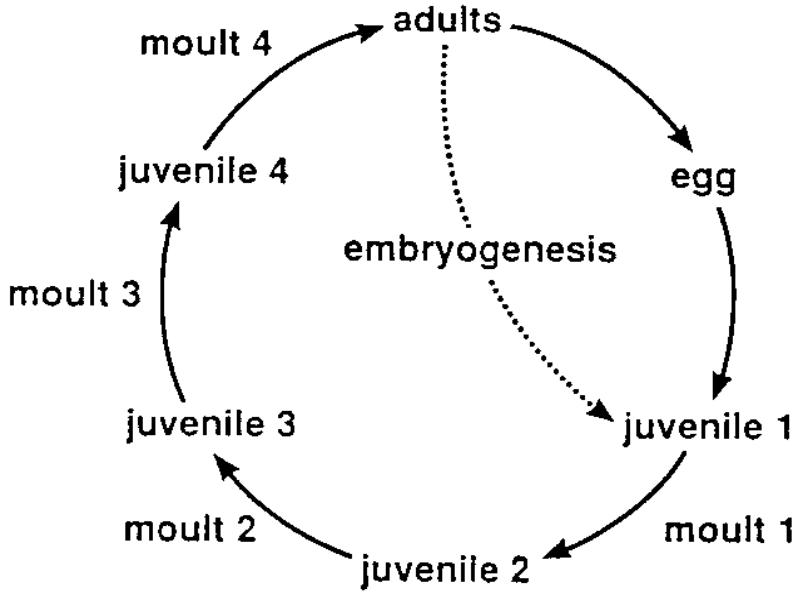


Figure 1.1: Generalised life cycle of nematodes (O'Brien and Stirling, 1991.)

1.2.6 Environmental factors influencing nematode survival

Soil abiotic factors have a marked effect on nematodes and their host plants. Factors that are stressful to the host plants can increase nematode populations as the host plant loses its tolerance or immunity against nematodes. Soil moisture, structure, texture and temperature are the major physical and environmental factors affecting nematode survival (Lambert and Baker, 2002). Water acts as a medium for active migration that enables infection of host plants by Plant parasitic nematodes (Sultan and Ferris, 1991). Plant nematodes may also multiply in wet soils. However, water logged soils prevent aeration and kills nematodes. Moisture is an important factor in prevention of nematode egg desiccation as it acts like a lubricant. Soil texture influences nematode populations, highly porous and loose soils support high nematode populations because of high aeration and mobility (Sultan and Ferris 1991, Norton 1979).

Small pore sizes of soil e.g. in clay soils may hinder nematode movement especially for the migratory endoparasites while fine sand and sandy loams offer a better medium (Olabiyi *et al.,* 2009). Most nematodes are active, lay eggs and complete their life cycles at temperatures between 25-30˚C on the upper soil horizon in warm temperate regions (Norton 1979, Perry and Evans 2009).

1.2.7 Plant-nematode inter relationships

Plant parasitic nematodes feed on plant tissues using an oral stylet with which they pierce and inject enzymes into the plant cells and ingest the cell contents. Parasitic nematodes are either ectoparasites, feeding on plant tissues from outside or endoparasites feeding inside the plant tissues. Ectoparasitic nematodes include *Helicotylenchus* sp (spiral nematodes), *Paratylenchus* sp (pin nematode) and *Tylenchorhynchus* sp (stunt nematode). Adult nematodes that move freely in or within plant tissues are said to be migratory while those that are permanently immobile are referred to as sedentary. Sedentary endoparasitic nematodes establish a specialised feeding site within the plant tissue cells. They include root knot nematodes of *Meloidogyne* sp, cyst forming nematodes *Heterodera* sp and reniforms *Rotylenchus* sp. Migratory endoparasites include *Pratylenchus* sp (lesion nematodes) and *Radopholus* sp (burrowing nematodes). Root knot and cyst nematodes cause root galling and distortion of roots thereby affecting nitrogen fixation by Rhizobium especially in leguminous plants like soybeans, and infection by Mycorrhizae that assist in mineral uptake (Lambert and Baker, 2002). Adult and later stage juveniles of endoparasites are shielded from predators and pesticides that might be present in the soil whereas ectoparasitic nematodes are exposed.

1.2.8 Organic amendments in nematode management

The use of organic amendments in the form of manure, compost, stabilised bio solids and plant extracts is on the increase and has been attributed to their ability to decrease parasitic nematode populations and disease intensity on plants (Chen *et al.*, 2000). Application of soil organic amendments is not only beneficial to nematode management but also to plant growth and productivity (Oka *et al.,* 2007, Orisajo *et al.,* 2008). The nematicidal effects of organic amendments act directly or indirectly on parasitic nematodes. Changes in the soil physical and chemical properties can improve plant health making the plants to be more tolerant to nematode and other pathogenic attacks. McSorley and Gallager (1995a) showed that despite high populations of *Meloidogyne incognita*, crop yields were high in vegetable crops with addition of yard waste manure.

Organic amendments have the ability to not only stimulate beneficial and free living nematodes, but also other important micro flora around the plant rhizosphere that are antagonistic to parasitic nematodes (Renco and Kovacik, 2012; Pakeerathan *et al.,* 2009). Extracts from various plants such as neem and *Tagetes* sp have been shown to have toxic effects on nematodes (Hooks *et al.,* 2010). Studies by Atungwu *et al.,* (2009) on the effect of neem leaf powder in soybean showed a reduction in root galling and nematode reproduction. Neem extracts were also shown to enhance the performance of other organic amendments when used in combination (Oka *et al.,* 2007).

Decomposing plant residues and other organic amendments release compounds or by products such as nitrogen and organic acids that may have nematicidal effects (Oka, 2010; Thoden *et al.,* 2011). Ammonia is a major by-product of organic decomposition and source of nitrogen. Studies by Mian and Rodriguez-Kabana (1982c) showed that galling by *Meloidogyne arenaria* decreased as percentage nitrogen increased when 15 different organic amendments were used, and that nematicidal activity did not usually occur in a higher Carbon: Nitrogen ratio

(C:N) of more than 20 due to slow decomposition and low levels of ammonia. A lower C: N has however been reported to have phototoxic effects (Rodriguez-Kabana *et al.,* 1986). Nematode suppression may also be achieved by organic products that increase the soil pH as ammonia is easily ionised to NH_4^+ in lower soil pH than in higher pH (Oka *et al.,* 2007; Zasada, 2005). A wide variety of soil organisms consisting of parasitic nematode antagonists like nematofungus, bacterivorous and other non parasitic nematodes are stimulated when organic matter is added into the soil (Akhtar and Malik, 2000; Oka, 2010) and may contribute towards parasitic nematode reduction. However it is always difficult to single out a direct cause and effect in the nematode reduction as the reduction is always a combination of many effects (Stirling, 1991).

1.2.8.1 Vermicompost

Vermicompost is an organic fertiliser produced by microbial composition of organic wastes through the activity of earth worms. Processing of organic waste through composting produces a more stable product with reduced environmental risks suitable for application in the field (Laczano *et al.,* 2008). It is richer in organic carbon from increased organic matter and other nutrients like Sodium (N), Potassium (K) and Phosphorous (P) (Manivannan *et al.,* 2009). Application of vermicompost in soil influences its physical, chemical and biochemical properties. Vermicompost is highly porous well aerated and have increased water holding capacity (Romina *et al.*,2011) it can be produced from various agricultural wastes which otherwise may have no economic value. Research findings on Vermicompost have shown nematode inhibitory effects. Different combinations of Vermicompost from buffalo dung and gram bran with different bio pesticides have significant effect on control of parasitic nematodes (Akhtar and Mohamood, 2004).

1.2.8.2 Biochar

Biochar is charcoal used for soil application and is produced when organic material is heat in high temperatures under low or no oxygen, a process known as pyrolysis. Biochar application to soils has an effect on the soils physical properties by increasing its pore size (Chan *et al.,* 2007) therefore improving water holding capacity. Some types of soils especially sandy soils experience excessive drainage and therefore addition of Biochar to such soils helps in reduction of nutrient leaching (Lehmann *et al.*, 2003). In soils that are too compact like clay, Biochar application reduces compatibility and makes the soil more aerated, with the created spaces providing protective habitats for other microbes (Kolb, 2007) some of which are antagonistic to nematodes. Improved soil aeration also stimulates mycorrhizal fungi abundance that increases plant productivity (Nishio, 1991). Biochar can therefore be used to improve crop production and soil quality (Blackwell *et al.,* 2009). Application of Biochar can reduce fertilizer application to soil since it has potential of staying in the soil for very long period. Biochar has been recommended as a long term method for carbon sequestration in soils and therefore a tool in carbon emission reduction. In addition, Biochar increases organic matter in soil that promotes accumulation of microbes that are antagonistic to parasitic nematodes (Zhang *et al.,* 2012).

1.2.8.3 Mycorrhizae

Mycorrhizae association is a mutual relationship between a fungus and the host plant. Arbuscular Mycorrhizae fungi (AMF) are obligatory biotrophs benefitting from the photosynthetically produced carbon from the host plant whereas the plant in return, benefits from increased mineral uptake by the fungal mycelium in places its roots are not able to reach(Smith and Read, 2008). Endomycorrhizae are found in many plant species (Peterson *et al.*, 2004) including soybeans and they provide protection to host plant against soil pathogens (Smith and Read, 2008). The symbiotic association of AMF and host plants have been shown to reduce

fungal pathogenic damage to host plants (Tahat *et al*., 2010) as well as nematode severity (Smith and Read, 1997). Bio control effects of AMF have reportedly been attributed to changes in root system morphology and enhanced nutrient uptake when host plant is infected by the fungus. AMF therefore appears to enhance plants tolerance by improved growth and compensates for the functional and biomass loss caused by nematodes and other pathogens (Cordier *et al.,* 1996). AMF may increase or decrease populations and activity of microbial functional groups, therefore an increase in nematode antagonist populations due to AMF will lower parasitic nematode populations. Competition for space in the root system and carbon compounds between AMF and other organisms could probably cause suppression to nematodes and other parasitic organisms (Smith and Read, 1997). AMF symbiotic interactions thicken the root cuticle and cell walls making it difficult for nematodes to penetrate them using their stylets and the J_2 eventually dies (Villenaye *et al.,* 2003). Studies the effect of AMF (Glomeromycota fungi) on coffee, showed a decrease in meloidogyne populations, an increase in phosphorous above shoot content and an increase in root and shoot biomass (Raul *et al.,* 2013).

Several factors determine the efficacy of AMF as a disease control agent. Most importantly are the soil moisture, soil physical and chemical properties, mycorrhizae species, inoculation time and the potential of pathogens in the soil (Singh *et al.,* 2000).

1.2.8.4 *Paecilomyces lilacinus*

This is a hyphomycete fungus that produces branched and septate hyphae and has been known to infect eggs of nematodes. The vegetative hypha enters the nematode matrix or egg cysts and proliferate producing conidiophores that infect egg masses and first stage juveniles (J_1) within the eggs. However, it has also been reported to infect mobile stages of nematodes, and was shown to control the mobile *Radopholus similis* in bananas (David and Zorilla, 1985). Adult females become infected when the hyphae gets into their reproductive organs (Jatala and

Bokanjel, 1979) with the infection resulting into reduced egg masses; egg hatching and juvenile populations (Morgan and Rodriqeuz, 1984). It has a wide distribution in agricultural soils and has been explored as a biological control for parasitic nematodes. Effective use of *Paecilomyces lilacinus* as a biological control agent depends on several factors, level of concentration, virulence, method of application and environmental factors such as soil type (physical and chemical properties), organic matter content in soil and susceptibility of host crop. Pre-planting application together with seed treatments and post planting drenching, recorded increased yields and reduced number of galling per root in greenhouse experiments using susceptible tomato varieties (Kienwick and Sikora, 2004).

1.3 Problem statement

Nematode infestation is a major constraint and production limiting factors in Sulmac area, Naivasha, Kenya (ITC Sulmac area soil report, 2001). This has led to the use of chemicals/fumigants resulting to higher levels of chemicals in the soil (ITC Sulmac area soil report, 2001) that are detrimental to both plant and human health. Sulmac area is dominated by sandy top soil that has low water holding capacity and nutrient retention capabilities. Studies have shown that plant parasitic nematodes vary in soils of different textural classes with soils of higher sand percentages typical of semi-arid areas in Kenya having an increased abundance of nematode species (Olabayi *et al.*, 2009). Nematodes are often overlooked by some farmers as serious pests and yet they have negative economic impacts to agricultural production as the costs of nematicides are sometimes out of reach for small farm holders (Bridge, 1996).

1.4 Justification

Nematodes if not managed, can cause great yield loses which can hinder farmers from realising their full farm potential and can lead to huge economic loses, food insecurity and malnutrition. Soy beans are of economic importance to Kenya as they can be used as an intercrop with maize by small holder farmers. Parasitic nematodes and especially root knot nematodes are a major limiting factor to bean farming in Kenya and can cause up to 60% lose on yields (Ngundo and Taylor, 1974).

For many years, plant parasitic nematodes have been controlled by chemical nematicides which although effective and fast acting, are detrimental to the environment and human health (Wachira *et al*., 2009). There is need for alternative methods that are eco-friendly, readily available, low on cost and can improve soil quality and plant health. The use of organic amendments in nematode management is important because of their environmental safety. Usage of organic amendments also promotes the economical usage of crop wastes which otherwise have no economic value and reduction of amounts of chemical pesticide inputs.

1.5 Objectives

1.5.1 General objective

The aim of this study was to assess nematode response to addition of organic amendments in soil under soybeans.

1.5.2 Specific objectives

1. To evaluate the effects of Biochar, Mycorrhizae and Vermicompost organic amendments on soil population densities of plant parasitic and non parasitic nematodes.
2. To evaluate effects of organic amendments on root galling of soybeans by plant parasitic nematodes.

3. To evaluate the effect of organic amendments on the soil macro and micro nutrients, pH and texture.

1.6 Hypothesis

Organic amendments Biochar, Mycorrhizae and Vermicompost have an influence on nematode populations, root galling and soil properties.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Study site

This research study was conducted under field conditions at Finlay's kingfisher farm located on the southern part of Lake Naivasha, Sulmac area in Eastern Rift valley at an altitude of 1890 meters. It is approximately 3 Kilometres from the shores of Lake Naivasha, approximately 20 Kilometres from Naivasha town and 100 Kilometres North-West from Nairobi the capital city of Kenya (Fig.2.1). The area lies between latitudes 0˚ 49' 45" and 0˚ 50' 30" South and longitudes 36˚ 20' 15" and 36˚ 20' 45" East. Daily temperatures and rainfall range from 7.3 - 22.7 ˚C and 156-1134 millimetres per month. The long rain season starts from March to May and the short rain season is between October and November. The major economic activities in Naivasha include sedentary agriculture, ranching and horticultural farming mainly for export. Other economic activities in the area include fishing, wildlife conservation, electricity generation and tourism. Naivasha district is 3,035 km^2 with a population of 376,243 (Kenya population census data 2009). Majority of the people living in Naivasha are immigrants who work in the commercial horticultural farms.

The soil type in the study area is classified as gravely sandy loam/loamy, sand to loam soil. The topsoil is predominantly sandy and has a negative effect on the water holding and nutrient retention capacity. Due to semi-arid weather conditions of the area, proper irrigation is therefore necessary. The major factor hindering agricultural production in this area is nematode infection; this has lead to the use of chemicals/fumigants leading to higher levels of chemicals in the soil (Atkilt and Rossiter, 2001).

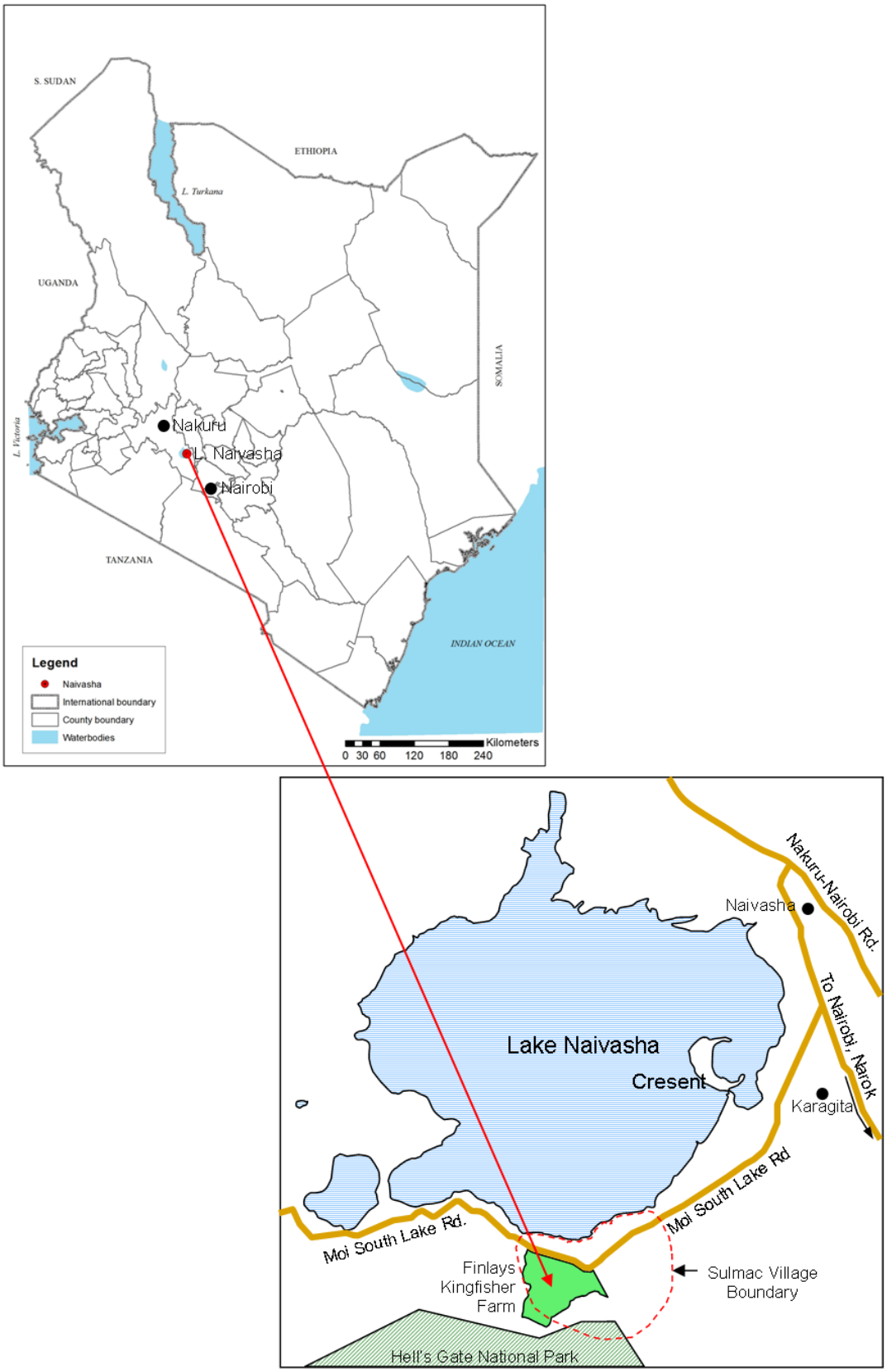


Figure 2.1: Map of study site, Sulmac area indicating the location of Kingfisher farm (Shaded green). (Courtesy Department of Geography, University Of Nairobi)

2.2. Study design

The experiment was carried out in a randomised block design with three replicates. Each triplicate plot measured 3×2 m^2 with a spacing of 1m between each block (Fig.2.2). Three main organic treatments were used in this trial namely Biochar, Vermicompost and Mycorrhizae. Treatments were applied as single treatment, a combination of each two and as a combination of all the three amendments. Mytech (*Paecilomyces lilacinus)* a nematofungus was used as a positive control.

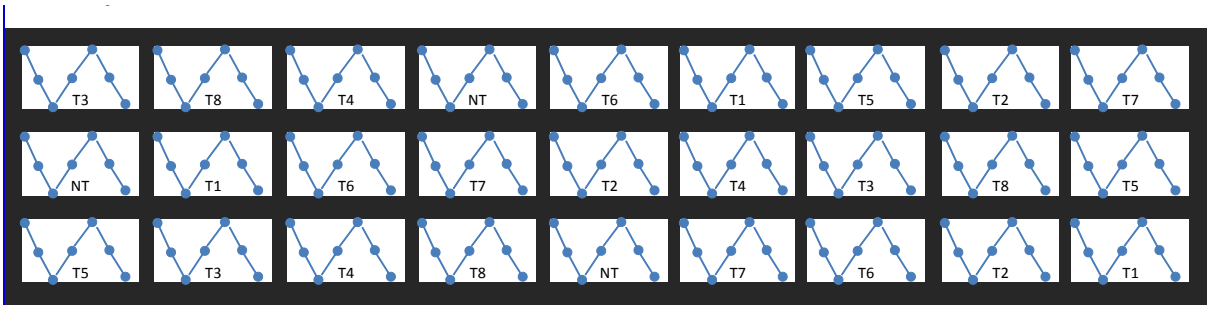


Figure 2.2: Experimental plot layout

Rectangle blocks 3×2 metres

Block paths 1 metre wide

Nematode sampling points

T_1 Biochar B

T_2 Vermicompost V

T_3 Mycorrhizae M

T_4 Biochar + Vermicompost BV

T_5 Biochar + Mycorrhizae BM

T_6 Vermicompost + Mycorrhizae MV

T_7 Biochar+ Vermicompost+ Mycorrhizae BVM

T_8 Positive control Mytech (*Paecillomyces Lilacinus*) MY

NT Untreated control

2.2.1 Seed variety and agronomic practice

Two seeds of Nyalla variety best suited for semi-arid conditions (KARLO) were planted 2 cm deep per hole with an intra spacing of 15 cm and interspacing of 30 cm. The crops were under irrigation when necessary due to the semi-arid type of climate in Naivasha and weeding was done on need basis. There was no application of pesticides throughout the trial.

2.2.2 Treatment dosage

A pre-prepared mixture of roots, mycelium and spores of Glomeromycota fungi (*Glomus mosseae, Glomus etunicatum, Glomus claroideum and Glomus intraradices*) going by commercial name Rhizatech were used as AMF inoculums and applied at a rate of 60L/ha, (Table 2.1) Pre-prepared Vermicompost (Vermitech) composited by *Eisenia foetida* (red worm) was applied at a rate of 2000kg/ha, Mytech (*Paecilomyces lilacinus*) at the rate of 250g/ha in 1000 litres one week before planting and repeated 4, 6 and 8 weeks after germination by drenching. Due to the hydrophobic nature of *Paecilomyces lilacinus,* Sodium Dodecyle Sulphate (SDS) was used to dispense the Mytech treatment at 0.02 % of total water of dispensation. Biochar was applied at 2000kg/ha.

Table 2.1: Dosage of organic material used to amend soil before soybean planting.

Treatments	Source	Recommended Doses	Dose/plot
1.Vermicompost (Vermitech)	Vegetable and flower wastes	2000kg/ha	6.75kg/plot
2. Biochar	Forest wastes	2000kg/ha	6.75kg/plot
3. Mytech (Positive control)	*Paecilomyces lilacinus* strain	250g/ha in 1000ltrs	0.15g/plot in 22.5 litres
4. Mycorrhizae (Rhizatech)	Propagules of Arbuscular Mycorrhizal Fungi	60L/ha	2250ml/plot or 2250g/plot

2.2.3 Soil sampling to assess nematode population densities

A baseline survey across the trial field was done before harrowing to determine nematode population and diversity in the field. Nematodes were sampled again at 75% flowering and at harvest in order to determine changes in nematode diversity and populations per each treatment plot after organic amendments. Using a soil auger each soil sample was collected using a zigzag pattern 15 cm deep from the rhizosphere of ten sampled plants per plot at flowering and harvest. The soil cores at each stage were then homogenised per plot to form a composite sample. From each composite sample, soil clods were broken, plant debris removed and the soil well mixed. 500 grams was then put in clearly marked plastic bags and placed in a cool box before being transported to the laboratory for nematode extraction. The ten sampled plants were used for galling assessment.

2.2.4 Nematode extraction and bioassay from soil samples

The modified Baermann tray technique (Hooper *et al.*, 2005) that relies on the motility of nematodes was used to extract the plant parasites from the soil.Sample soil of 200cc was placed in an extraction sieve laced with a paper serviette and the extraction sieves placed in an extraction tray (Fig. 2.3). Distilled water was then added to the extraction tray to a level just below the base of the extraction sieve to allow wetting of the soil. The set up was left for 48 h in a dark cabinet at laboratory temperature following which the sieves with the soil were carefully removed and discarded and the water in the extraction tray concentrated by passing it through a 90 µm sieve to remove soil particles and then a 25 µm sieve to retain the nematodes. The sieved nematode suspension was then backwashed into universal bottles and left to settle for a day in a cold room. The nematode suspension was further reduced to 50 ml by sucking excess water using a pipette.

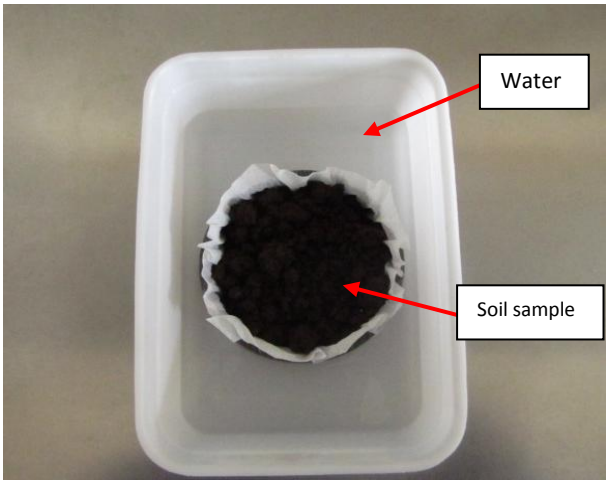


Figure 2.3: Apparatus used to extraction of nematodes from soil using the modified Baermann tray technique.

2.2.5 Nematode identification and enumeration.

The 50 ml nematode suspension was gently shaken and 2 ml transferred to a counting dish with counting grids for identification and enumeration. Nematode identification and counts were performed at x100-150 magnification under a stereo microscope. Counting was repeated twice using a tally counter and the average was used to calculate nematode numbers present in the suspension and expressed as nematodes per 200 cc of soil. Nematodes were identified to genus level using a revised version of Mai and Mullin (1996), Geraert and Raski, (1987) identification keys and grouped by their trophic levels as either bacteria feeding nematodes (bacteriovores), fungi feeding nematodes (fungivores), plant parasitic nematodes (herbivores) and nematodes predating on insects and other nematodes (predators) as previously described (Bongers and Bongers, 1998).

2.2.6 Nematode preservation

For long term storage, the nematodes were preserved in double TAF fixative prepared from 7 ml formalin (40% formaldehyde), 2 ml triethanolamine and 91 ml distilled water (Hooper, 1970). The nematode suspension was left to settle and suspension reduced to 4 ml. The TAF fixative was heated to 70-75˚C and an equal amount of 4 ml immediately added to the nematode suspension. The heating effect of the fixative worked to kill and fix the nematodes simultaneously.

2.3 Assessment of root galling index

A total of 10 root plants were randomly selected per plot at 75% flowering and at harvest. Using a trowel the plants were carefully uprooted so as not to damage the root system. The roots were then washed in water, mopped and galls observed percentage root galling determined. Root galling was assessed using the 0-10 galling index (Bridge and Page, 1980)

where 0 = no galls, 1 = 10% of the root system galled, 2 = 20% of the root system galled, 3 = 30% of the root system galled, 4 = 40% of the root system galled, 5 = 50% of the root system galled, knotting on parts of main root system and 10 = all the root system severely knotted, no root system. The number of galls developed on the soybean roots was an indication of the severity of root infection.

2.4 Soil physical and chemical properties.

The chemical and physical properties of soil Vermicompost and Biochar before trial and at the end of the trial were analysed. A composite sample (500 grams) from 23 sampling points within the trial field before treatment and from each treated plot at the end of the trial was obtained and subjected to physical and chemical analysis at University of Nairobi's College of Agriculture and Veterinary Studies (CAVs) department of crop science and plant protection. The parameters analysed included soil texture, moisture content, soil pH, organic matter content, and macro nutrients (total and available Nitrogen (N), Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Phosphorous (P) and amount of Organic Carbon (OC)). The chemical analysis of soil before and after amendment application was analysed as follows: Particle size was determined by hydrometer method and expressed in percentage of silt (%SL), clay (%CL) and sand (%SA). Moisture content was measured gravimetrically after drying in an oven at 105˚c for 48 hr. Soil pH was measured in 1:2.5 water solutions with a glass electrode pH meter and organic carbon by wet oxidation using the Walkley Black method (Walkley and Black, 1935). The amount of exchangeable Na and K, were determined by flame photometry using a flame photometer, Ca and Mg by atomic absorption spectrophotometry (Gallaher *et al*., 1975). Kjedahl method (Bremner, 1996) was used to measure total N of the soil and available P by Mehlich method (Mehlic, 1984) of ascorbic acid and blue colour.

2.4.1 Yields and yield attributes of soybean.

At harvest, the roots, shoot, pod weight and dry seeds of 10 plants selected in a zig zag pattern per plot were weighed after drying to constant weight. The yields were compared against the nematode populations, galling indices as well as the physical and chemical properties of soil.

2.5 Data collection and analyses

Nematode counts were recorded as nematodes per 200cc of soil. ANOVA was used to determine the differences among organic amendment treatments. Means were separated using Least Significant Difference (LSD) at 5% level of probability and differences with $P < 0.05$ were considered as statistically significant. All statistical analyses were performed by IBM SPSS statistical software version 20.

CHAPTER THREE

3.0 RESULTS

3.1 Nematode populations at pre-planting

Baseline survey of soil nematodes prior to organic amendment showed that bacterivorous nematodes were more populous with a mean of 985 per 200cc of soil compared to the other trophic nematode groups with the least numerous with a mean of 10 being predating nematodes (Fig.3.1)

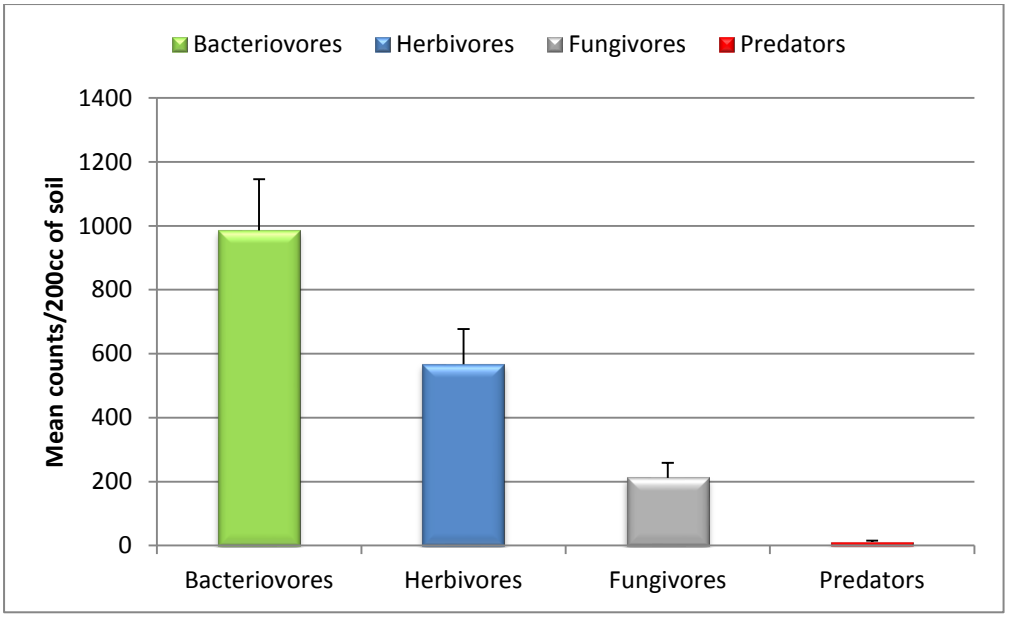
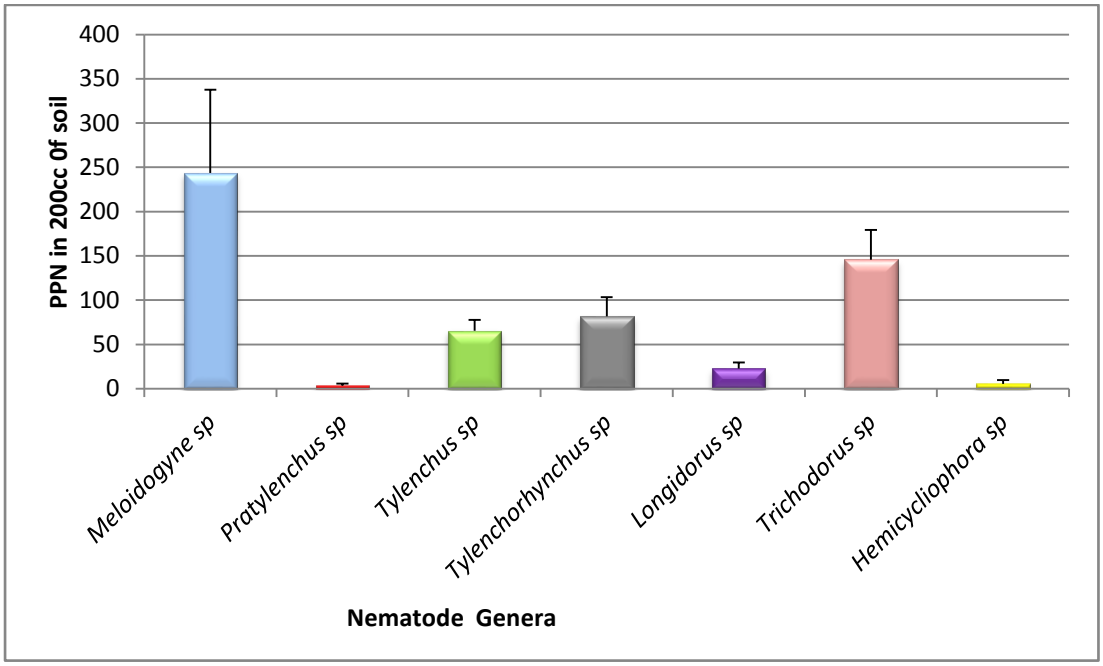


Figure 3.1: Soil nematode population densities based on their trophic group levels at pre-planting.

A total of 7 genera of plant parasitic nematodes were found in the soil at pre-planting with the most predominant being *Meloidogyne* sp with a mean of 243.47 followed by *Trichodorus* sp 145. The least recorded nematode genera were *Pratylenchus* sp and *Hemicyliophora* sp with means of 3.26 and 5.43 respectively (Fig. 3.2).



PPN= Plant Parasitic Nematodes

Figure 3.2: Genera and diversity of plant parasitic nematodes at pre-planting densities and genera diversity.

3.2 Nematode populations at flowering

Herbivore population density significantly ($p<0.05$) varied between the organic amendments ($F(8, 18) = 3.027$, $p=0.028$). LSD post hoc analysis further revealed a significantly higher herbivore mean count in Biochar applied alone and untreated plots in comparison to other treatments. However there was no significant difference between Biochar treated plots and the untreated control plots (Table 3.1). Field plots with Biochar amendment recorded the highest parasitic nematode counts while, the lowest counts were observed in Mytech (positive control) and in the combination of Mycorrhizae+Vermicompost plots respectively (Table 2). There was no significant difference between Vermicompost, Mycorrhizae, Biochar+Vermicompost, Biochar+Mycorrhizae, Mytech positive control plot, Biochar+Vermicompost+Mycorrhizae and untreated control plots on parasitic nematode populations at flowering.

Fungivores, and predator mean densities did not differ significantly (P>0.05) at the flowering stage. However, relatively higher fungivore populations were observed in the combination of Mycorrhizae+Vermicompost treated plots. Untreated plots recorded higher predator densities than the treated plots at this stage (Table 3.1). Bacteriovore populations did not differ significantly (P>0.05) between the treatments but numerically higher bacteriovore populations were observed in Biochar and Vermicompost treated plots whereas low numbers were observed in Mytech (positive control) and untreated control plots respectively.

Table 3.1: Nematode trophic group mean populations at flowering stage of soybeans plots treated with different organic amendments.

	NEMATODE MEAN POPULATIONS AT FLOWERING STAGE OF SOYBEANS			
TREATMENT	**FUNGIVORES**	**BACTERIOVORES**	**PREDATORS**	**HERBIVORES**
B	166.67	1816.67	133.33	1333.33[a]
V	233.33	1583.33	133.33	466.67[b]
M	200.00	933.33	66.67	683.33[b]
BV	33.33	1050.00	16.67	650.00 [b]
BM	200.00	1550.00	216.67	566.67 [b]
MV	300.00	866.67	83.33	350.00 [b]
BVM	133.33	1316.67	83.33	716.67 [b]
MY	133.33	700.00	100.00	366.67 [b]
NT	283.33	850.00	266.67	966.67[a]
LSD(P=0.05)	**0.811**	**0.080**	**0.721**	**0.028**

B=Biochar, V=Vermicompost, M=Mycorrhizae, BV=Biochar+Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=NoTreatment, BVM=Biochar+Vermicompost+Mycorrhizae. Mean numbers with different letter superscripts in the same column differ significantly at P < 0.05

3.3 Nematode populations at harvest

Following harvest, the nematode populations of the four trophic groups did not differ significantly between the eight treatment plots. Herbivore populations were numerically higher in Biochar treated plots and lowest in the positive control plots treated with Mytech (*Paecilomyces lilacinus*). Biochar+Mycorrhizae treated plots had the lowest bacteriovores populations at harvest. Biochar in combination with Vermicompost treated plots on the other hand had the highest populations of bacteriovores, fungivores, and predators (Table 3.2).

Table 3.2: Nematode trophic group mean populations at harvest stage of soybeans plots treated with different organic amendments.

NEMATODE MEAN POPULATIONS AT HARVEST STAGE OF SOYBEANS

TREATMENT	FUNGIVORES	BACTERIOVORES	PREDATORS	HERBIVORES
B	150.00	1275.00	41.68	791.67
V	150.00	1458.33	75.00	708.33
M	133.33	1500.00	50.00	500.00
BV	400.00	2333.33	108.33	575.00
BM	91.67	675.00	16.67	458.33
MV	141.67	1450.00	66.67	691.67
BVM	75.00	1233.33	50.00	696.12
MY	125.00	1533.33	50.00	450.00
NT	283.33	1041.67	66.67	675.00
LSD(P=0.05)	**0.272**	**0.569**	**0.461**	**0.389**

B=Biochar, V=Vermicompost, M=Mycorrhizae, BV=Biochar+Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=NoTreatment, BVM=Biochar+Vermicompost+Mycorrhizae.

A total of 12 genera of parasitic nematodes were found present at the end of the field trial. The genus *Trichodorus* sp was the most dominant across all treatments followed by *Tylenchus* sp and *Xiphinema* sp., while *Paratylenchus* sp and *Radopholus* sp were the least dominant (Table 3.3). *Paratylenchus* sp was only found in Mycorrhizae treated plots while, *Xiphinema* sp and *Dorylaimus* sp were only observed in the untreated and Mycorrhizae+Vermicompost treated plots respectively. Nematodes belonging to the genera *Trichodorus, Meloidogyne, Tylenchus* and *Tylenchorhynchus* were found distributed across all the field plots. At the end of the field trial experiment, *Meloidogyne* sp populations occurred at lower levels across all the plots compared to the numbers observed at pre-planting, while the *Trichodorus* sp numbers were observed to have increased.

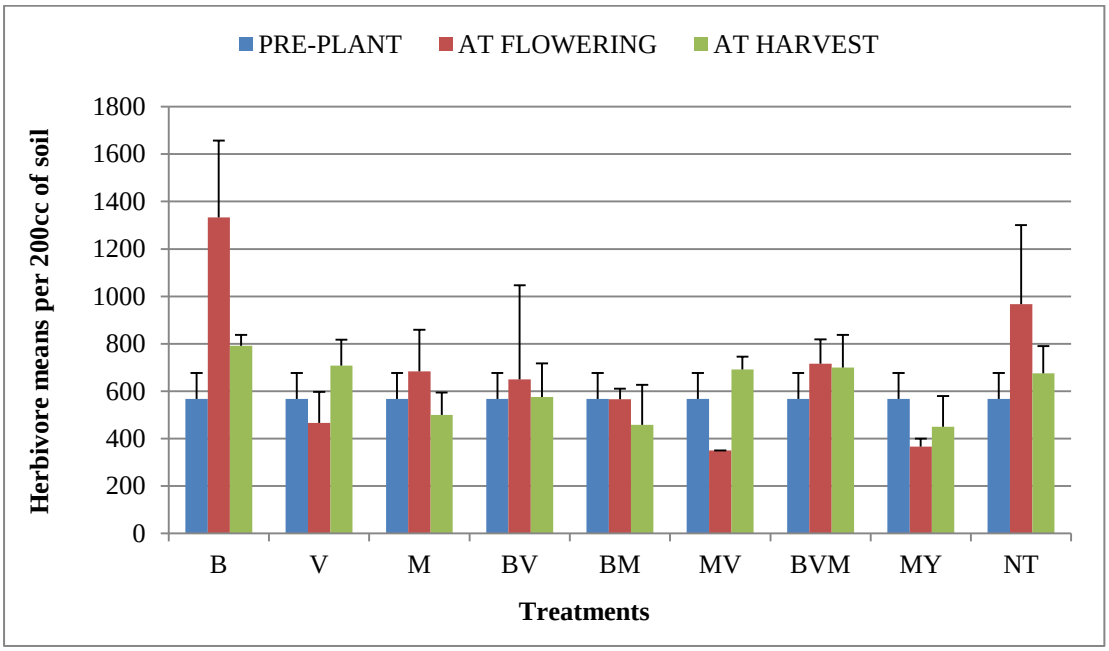
Table 1.3: Variations in abundance and nematode genera of plant parasitic nematode between organic amendments at the end of trial.

Genus	**Organic Amendments**								
	B	BM	BV	BVM	M	MV	MY	NT	V
Endoparasites									
Meloidogyne	83.33[a)]	16.67	41.67	16.68	24.99	24.99	24.99	24.99	75
Pratylenchus	-	16.67	-	-	-	-	24.99	-	8.34
Ectoparasites									
Tylenchus	75.00	83.34	116.67	191.67	91.68	166.68	150	91.65	141.66
Helicotylenchus	66.67	133.34	-	108.33	33.33	99.99	16.68	41.67	16.68
Tylenchorhynchus	66.67	50.01	41.67	66.66	50.01	91.68	108.33	116.67	58.22
Dorylaimus	-[b)]	-	-	-	-	8.34	-	-	-
Trichodorus	**466.67**	**150**	**375**	**304.44**	**249.99**	**258.33**	**125.01**	**391.68**	**366.66**
Longidorus	8.33	-	-	-	33.33	41.67	-	-	24.99
Radopholus	8.33	-	-	-	-	-	-	-	-
Criconemella	16.67	8.33	-	8.34	8.34	-	-	-	16.68
Xiphenema	-	-	-	-	-	-	-	8.34	-
Paratylenchus	-	-	-	-	8.34	-	-	-	-

[a)] Mean, [b)] Not found, B= Biochar, V= Vermicompost, M= Mycorrhizae, BV= Biochar+Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=No Treatment, BVM Biochar+Vermicompost+Mycorrhizae.

3.4 Nematode population trends during the growing season

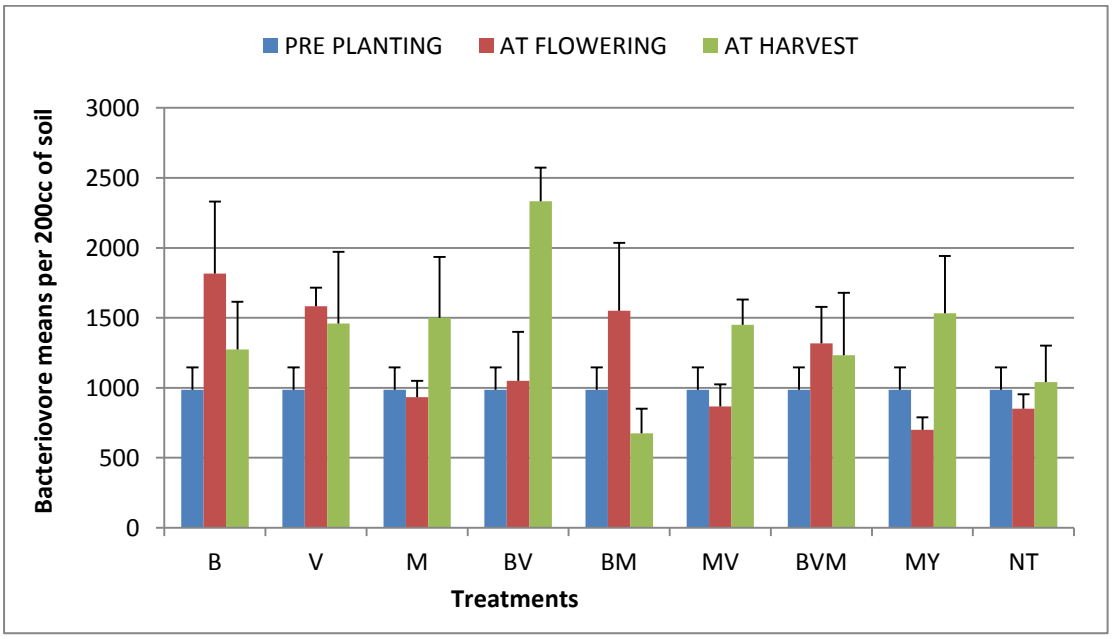
The highest population mean 1333.33 (Figure 3.3) of herbivores at flowering recorded with Biochar treatment declined to 791.67 at harvest representing a 40.58% reduction followed by a 26.79% reduction in Mycorrhizae and 19.22% in Biochar+Mycorrhizae treatments. Biochar, Vermicompost, Mycorrhizae+Vermicompost, triple combination of Biochar, Vermicompost and Mycorrhizae and the untreated control showed increased herbivore populations whereas Biochar+Vermicompost relatively maintained the same herbivore populations as at pre-planting with a slight increase at flowering. Vermicompost and Mycorrhizae+Vermicompost showed a different trend whereby a reduction in the herbivore population density at flowering was followed by an increase at the end of the trial. Biochar had the highest herbivore population densities at the end of the season (Fig. 3.3).



B=Biochar, V=Vermicompost, M=Mycorrhizae, BV=Biochar+Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=No Treatment, BVM= Biochar+Vermicompost+Mycorrhizae. Error bars denote standard error of mean

Figure 3.3: Population variations of plant parasitic nematodes through during the growing season of soybeans treated with different organic amendments.

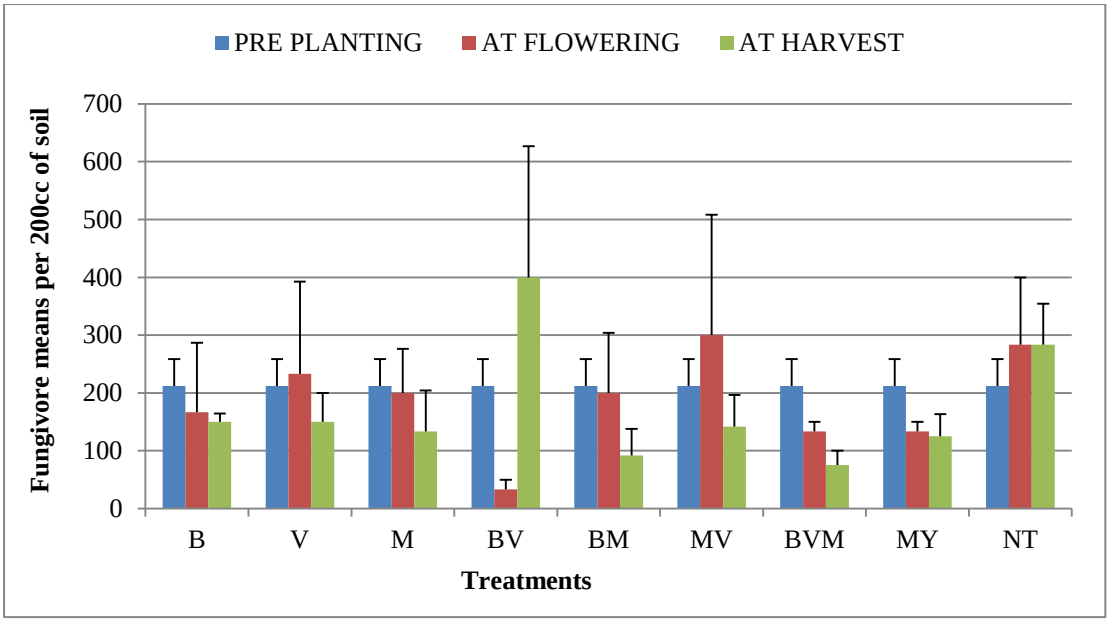
The combination of Biochar and Vermicompost treatments showed a 54.99% increase of bacteriovores population at harvest compared to flowering stage whereas the populations in the untreated control plots did not have significant changes throughout the growing season with means (Figure 3.4).



B= Biochar, V= Vermicompost, M= Mycorrhizae, BV= Biochar+Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=No Treatment, BVM= Biochar+Vermicompost+Mycorrhizae. Error bars denote standard error of mean

Figure 3.4: Population variations of nematodes bacteriovores through the growing season of soybeans treated with different organic amendments.

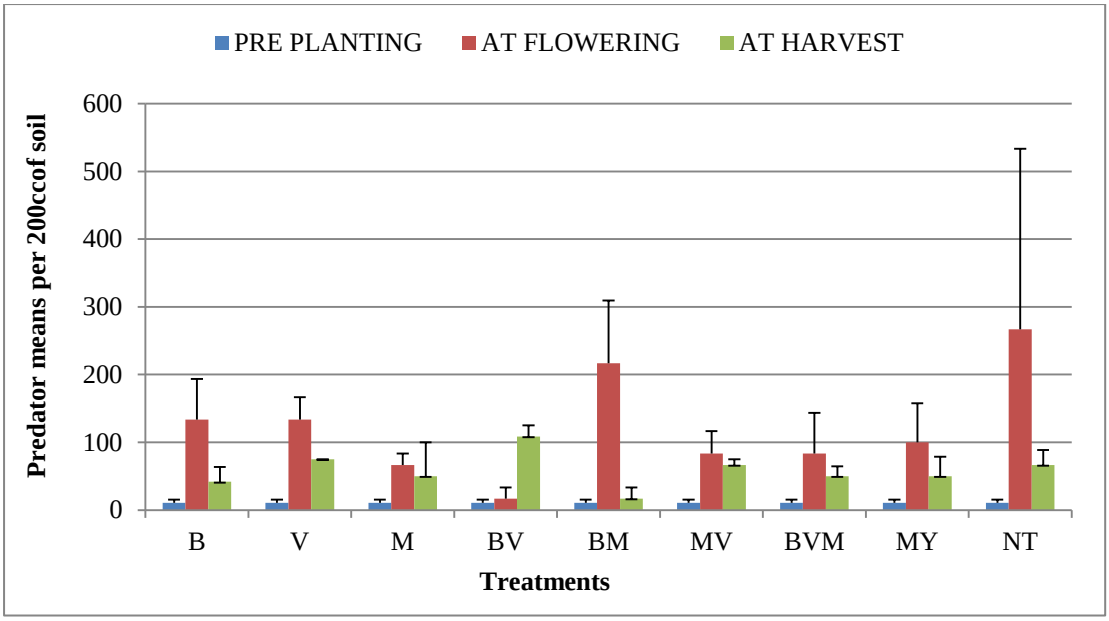
Fungivore populations declined through the season in Biochar, Mycorrhizae, Biochar+Mycorrhizae, Biochar+Vermicompost+Mycorrhizae and in the Mytech positive control plots. Biochar and Vermicompost combination had the highest fungivore populations at the end of the season (Fig.3.5).



B=Biochar, V=Vermicompost, M=Mycorrhizae, BV=Biochar+Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=NoTreatment, BVM=Biochar+Vermicompost+Mycorrhizae

Figure 3.5: Population variations trend of fungivores through the growing season of soybeans treated with different organic amendments.

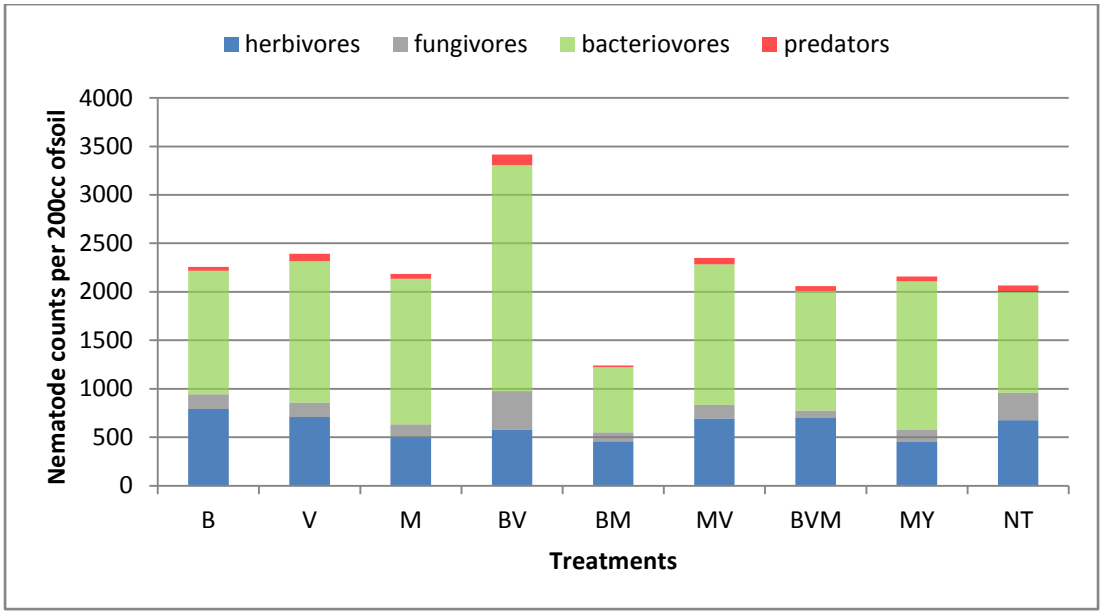
Predator populations were increased across the treatments throughout the season except for Biochar+Mycorrhizae treated plots that recorded elevated population densities at flowering and a significant drop at harvest (Fig. 3.6). Planting stage had no effect on the four nematode trophic group populations.



B=Biochar, V=Vermicompost, M=Mycorrhizae, BV=Biochar+Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=No Treatment, BVM =Biochar+Vermicompost+Mycorrhizae. Error bars denote standard error of mean

Figure 3.6: Population variations of predators through the growing season of soybeans treated with different organic amendments.

There was no significant difference in total nematode abundance at harvest ($p>0.05$) between the organic amendments. The combined application of Biochar and Vermicompost treated plots had the highest total nematode populations whereas the combination of Biochar and Mycorrhizae recorded the lowest abundance (Fig. 3.7).



B=Biochar, V=Vermicompost, M=Mycorrhizae, BV=Biochar+Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=No Treatment, BVM Biochar+Vermicompost+Mycorrhizae. Error bars denote standard error of mean.

Figure 3.7: Response of the total nematode and trophic group abundance to organic amendments.

3.5 Effect of organic amendments on root galling indices of soy bean

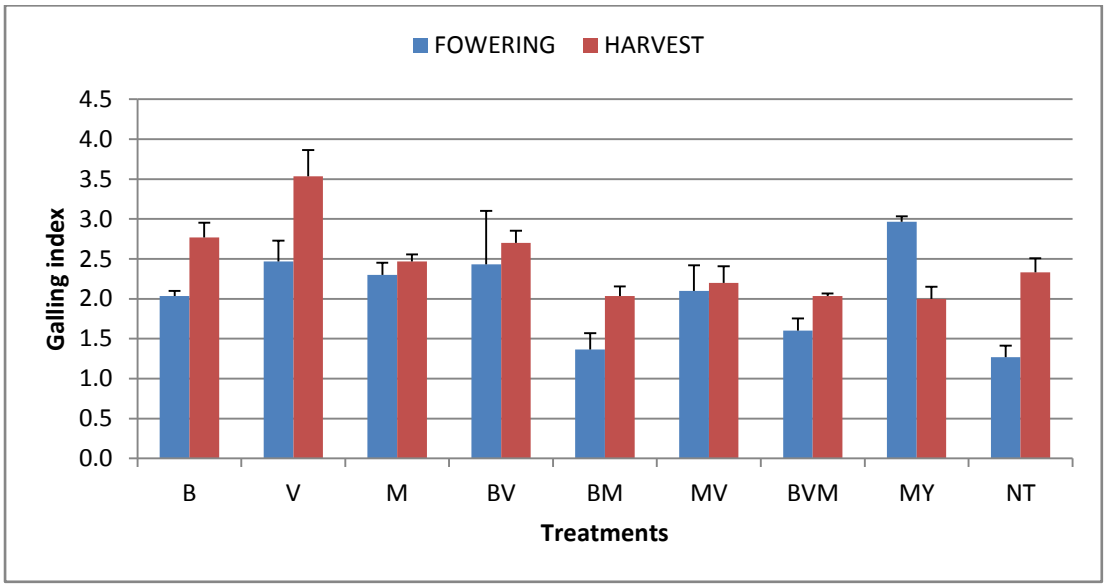
3.5.1Galling index

Galling indices at flowering were significantly different between the plots (F (8, 18) = 4.197, P=0.007). LSD post hoc analysis revealed significant differences in galling indices between Biochar, combination of Biochar+Vermicompost+Mycorrhizae, Biochar+Mycorrhizae, Mycorrhizae+Vermicompost and Mytech positive control. Mytech plots recorded the highest mean galling index of 3.0 whereas the untreated plots recorded the least galling index of 1.3 (Fig. 3.8).

Significant difference between treatment groups was also observed in the root galling index at harvest (F (8, 18) =7.702, P=0.000). LSD post hoc test revealed significant differences

in galling index between Vermicompost, Biochar, Biochar+Vermicompost and the positive control Mytech. The lowest galling index was observed in Mytech positive control group at 2 whereas Vermicompost recorded the highest galling index of 3.53 (Fig. 3.8).

Root galling indices increased across the eight treatments throughout the trial with significant increase in galling between flowering and harvest time observed in Biochar, Vermicompost, Biochar+Vermicompost+Mycorrhizae, Biochar+Mycorrhizae, and the untreated control plots. There was a significant reduction in galling with the positive control Mytech. Mycorrhizae, Biochar+Vermicompost and Mycorrhizae+Vermicompost had no significant differences in galling between flowering and harvest period (Fig. 3.8).



B= Biochar, V= Vermicompost, M= Mycorrhizae, BV= Biochar +Vermicompost, BM=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, MY=Mytech, NT=No Treatment, BVM =Biochar+Vermicompost+Mycorrhizae. Error bars denote standard error of mean.

Figure 3.8: Soybean galling index trend during the growing season.

3.6 Effect of organic amendments on soil physical and chemical properties at harvest

Baseline soil analysis of the trial site classified the soil type as loamy sandy with a 10.8%, 77.12%, 12% clay, sand and silt respectively. The soil had low organic carbon of 1.5%. Carbon and Nitrogen constituted 0.84 and 0.51% of its dry weight. The soil was basic with a pH of 7.38 (Table 3.4). At harvest, Vermicompost had higher organic matter content of 23.59% than Biochar at 11.78%.

Table 3.4: Pre planting soil chemical and physical properties of soil, Vermicompost and Biochar.

Characteristics	**Soil**	**Vermicompost**	**Biochar**
(% dry weight)			
C	0.84	13.18	6.58
N	0.51	1.54	1.33
C:N	1.65:1	8.5:1	4.95:1
(cmol/kg)			
K	1.95	40	2
Ca	4	35.5	8.84
Mg	0.7	7.83	0.67
Na	0.3	0.5	0.4
(µg/Kg dry weight)			
P	159	23.59	11.78
Fe	109	29	34
Cu	1.4	0.6	1.1
Mn	44	141	11
Zn	24.5	20.3	1.8
Moisture (%)	7.29	18.59	17.56
pH(1:2.5 Water solution)	7.38	8.5	9.15
Organic matter (%)	1.5	23.59	11.78
Clay (%)	10.88	-	-
Sand (%)	77.12	-	-
Silt (%)	12	-	-
Soil classification	Loamy Sandy	-	-

At the end of the trial, soil pH across the treatments was relatively maintained at basic pH as before amendment application across the eight treatments. However other soil properties varied between the treatments compared to the levels observed during the pre-planting period. The blend of Mycorrhizae+Vermicompost and Biochar+Vermicompost+Mycorrhizae recorded the most moisture content at 13.6% and13.5% (Table 3.5) almost double the amount before amendment application. Organic matter percentage was highest in Biochar + Vermicompost +Mycorrhizae treated plots at 1.93% with no significant difference from pre amended soil of 1.5%. Mycorrhizae amended plots showed no change in organic matter content.

Vermicompost, Biochar+Vermicompost and Biochar amended plots recorded the highest carbon to nitrogen ratio of 10.5:1, 10.3:1 and 10:1 respectively showing an increase in Carbon content and a decrease in Nitrogen amounts. Mycorrhizae and Mycorrhizae+ Vermicompost amended plots had the least carbon to nitrogen ratio at 7.7:1 and 6.8:1.The changes in amounts of exchangeable ions Calcium, Magnesium, Potassium and Sodium were negligible across the treatments. Vermicompost, Biochar and the combination of Biochar+Vermicompost+Mycorrhizae amended plots recorded the highest amounts of Phosphorous at 262, 250 and 250µg/kg respectively. Mycorrhizae and Mytech (positive control) recorded the least amounts at 215 and 216µg/kg. Phosphorous amounts increased across all the amended plots as compared to pre amendment amounts of 159µg/kg.

Biochar, Vermicompost and Biochar+Vermicompost recorded the lowest amounts of ammonium ions NH_4^+ at 15.77, 17.15 and 17.15µ/kg as compared to the other treatments. Biochar+Mycorrhizae had the highest amounts of ammonium ions. Mycorrhizae amended plots had the highest amounts of ammonia ions NH_3^+ while Vermicompost had the lowest amounts.

Table 3.5: Soil physical and chemical parameters after organic amendment at harvest.

		.....%.....						.....Exchangeable.....				.....Available Elements.....		
No.	Treatment	OM	MC	Total N	Total C	pH	C:N	Ca	Mg	K	Na	P	$N\text{-}NO_4^-$	$N\text{-}NO_3^-$
								µg/kg				µg/kg		
1	M	1.54	11.09	0.112	0.86	7.17	7.7:1	3.7	0.81	1.2	0.40	215	27.38	33.16
2	B	1.77	13.29	0.098	0.99	7.46	10.1:1	3.8	0.82	1.3	0.30	250	15.77	31.56
3	MV	1.7	13.6	0.14	0.95	7.36	6.8:1	4.1	0.55	1.6	0.45	247	24.19	25.4
4	MY	1.61	12.98	0.098	0.9	7.14	9.2:1	3.4	0.85	1.3	0.50	216	23.14	20.71
5	BV	1.84	12.56	0.1	1.03	7.41	10.3:1	3.7	0.83	1.3	0.40	243	17.15	23.28
6	BVM	1.93	13.50	0.112	1.08	7.4	9.6:1	3.9	0.86	1.4	0.40	250	18.16	27.85
7	NT	1.61	11.53	0.098	0.9	7	9.2:1	3.1	0.78	1.2	0.40	245	28.46	23.51
8	BM	1.63	13.20	0.112	0.91	7.28	8.1:1	3.2	0.55	1.2	0.40	236	32.96	23.19
9	V	1.84	12.54	0.098	1.03	7.36	10.5:1	3.9	0.76	1.5	0.40	262	17.15	17.15

*pH 1:2.5 water solution, MC Moisture Content, OM Organic Matter, BVM Biochar+Vermicompost+Mycorrhizae, B Biochar, MY Mytech, Vermicompost V, M Mycorrhizae, BV Biochar+Vermicompost, BM Biochar+Mycorrhizae, MV Mycorrhizae+Vermicompost, NT=No Treatment.

3.6.1 Yield attributes of soybeans post harvest

There was significant difference between groups in dry seed weight as determined by one-way ANOVA (F (8, 18) =4.613, p=0.003). LSD post hoc test revealed significant variations in dry seed weight from plants between Biochar and Biochar+Vermicompost, Biochar+Mycorrhizae, Mycorrhizae+Vermicompost, No Treatment and Mycorrhizae amended plots. Likewise dry seed weight means from Vermicompost amended plots varied significantly from Mycorrhizae, Mycorrhizae+Vermicompost and the untreated plots. Post harvest dry seed weight from positive control plots treated with Mytech (*Paecilomyces lilacinus*) was significantly different from Mycorrhizae, Biochar+Mycorrhizae, Mycorrhizae+Vermicompost and the untreated plots. The heaviest dry seed weight mean was observed in Biochar and Mytech amended plots at 0.17 and 0.15 kilograms respectively (Table 3.6). The lowest dry seed weight mean was observed in Mycorrhizae+Vermicompost, Mycorrhizae and untreated plots at 0.06 kilograms each.

There was no significant difference in the stover and shoot weight among the eight treatments, however plants from Biochar+Mycorrhizae and Mycorrhizae amended plots recorded the heaviest shoots at 1.77 and 1.62 kilograms respectively while the lowest shoot weight was observed in plots amended with the triple combined application of Biochar+Vermicompost+Mycorrhizae and the untreated plots at 1.01 and 1 kilograms respectively.

When shoot and root weights were combined (Stover weight), the same observation as for shoot weight was observed, with heavier stover weights of 1.9 and 1.77 kilograms being recorded in Biochar+Mycorrhizae and Mycorrhizae treated plots respectively. Biochar+Vermicompost+Mycorrhizae, Mytech and Biochar+Vermicompost recorded the least weights of 1.15, 1.18 and 1.18 kgs respectively.

Table 3.6: Effect of Organic amendments on soybean yield attributes at harvest.

Treatment	Root Weight (kg)	Shoot Weight (kg)	Stover Weight (kg)	Dry seed Weight (kg)	Pod Weight (kg)
B	0.14	1.42	1.56	0.17	1.04
V	0.15	1.3	1.45	0.14	0.76
M	0.15	1.62	1.77	0.06	0.61
BV	0.1	1.08	1.18	0.09	0.57
BM	0.13	1.77	1.9	0.09	0.62
MV	0.14	1.06	1.2	0.06	0.56
BVM	0.14	1.01	1.15	0.12	0.76
MY	0.15	1.04	1.18	0.15	0.9
NT	0.15	1	1.22	0.06	0.5
LSD(P=0.05)	**0.600**	**0.467**	**0.514**	**0.006**	**0.178**

B=Bio char V=Vermicompost=Mycorrhizae, BV=Biochar+Vermicompost, M=Biochar+Mycorrhizae, MV=Mycorrhizae+Vermicompost, BVM=Biochar+Vermicompost+Mycorrhizae, MY=Mytech (positive control), NT=No Treatment (Negative control).

3.6.2 Variation of nematode populations with soil chemical properties

The mean population of parasitic nematodes (herbivores) was found to correlate with phosphorous. There was a significant positive correlation between herbivore densities and phosphorous ($P = 0.007$, $r(7) = 0.822$) amounts in the soil across the eight treatments (Fig. 3.9). Similarly there was a significant positive correlation between phosphorous and organic matter content in the soils ($P=0.037$, $r(7) =0.696$) (Fig. 3.10). Conversely there was no significant correlation ($P>0.05$) between total Carbon C, Nitrogen N, Organic Matter OM,

Ammonium ions NH_4^+, Ammonia ions NH_3^+, Potassium and Magnesium and the four trophic groups of nematodes.

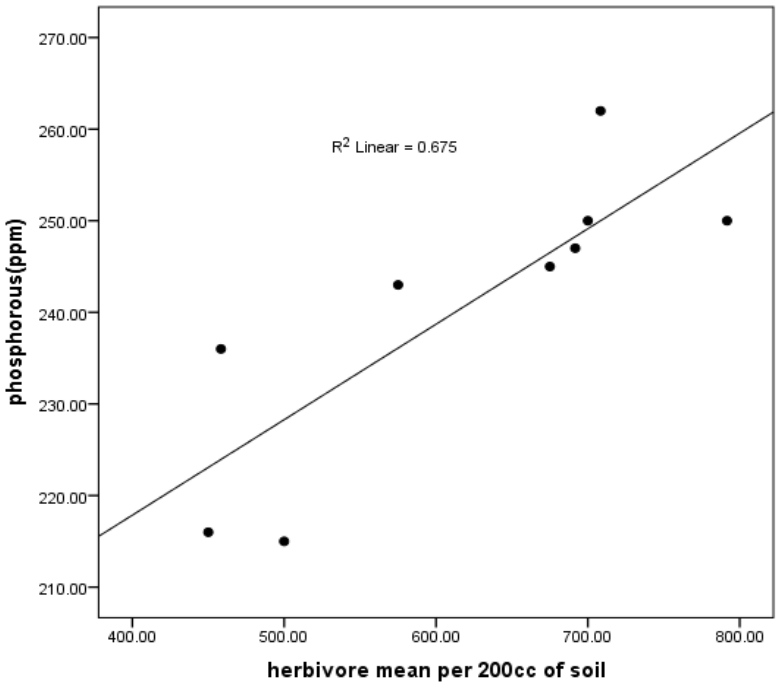


Figure 1.9: Relationship between phosphorous and plant parasitic nematodes (herbivores).

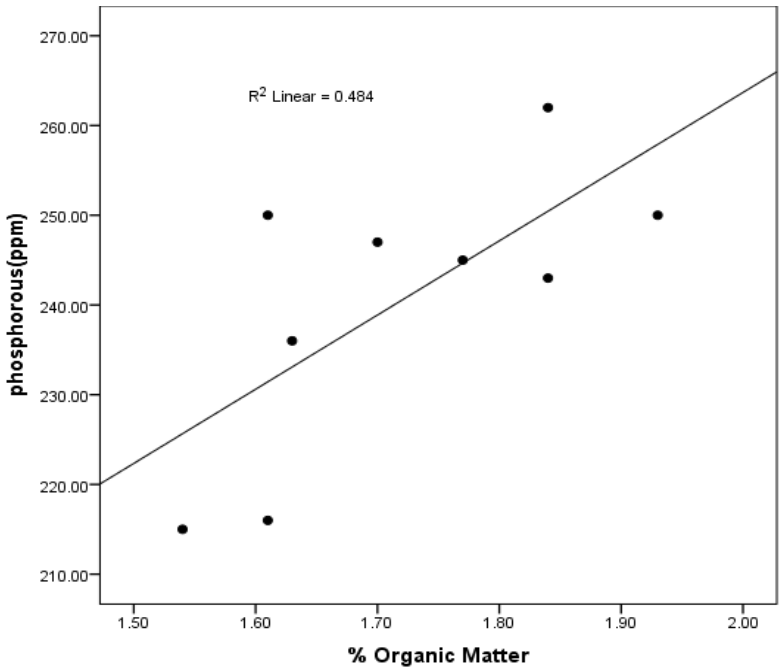


Figure 3.10: Relationship between phosphorous and percentage organic matter in the soil under organic amendments.

CHAPTER FOUR

4.0 DISCUSSION

Application of organic amendments has in the past been recognized in the management of parasitic nematodes and improvement of soil health. Due to their apparent environmental nontoxic benefits they have been considered in integrated nematode management with inorganic amendments. Although many studies have shown reduced parasitic nematode populations with the use of organic amendments, others have shown no change or increased populations with the effectiveness of these amendments depending mostly on starting material (Renco and Kovasik, 2012).

4.1 Effect of organic amendments on nematode populations and galling index

In this study, the application of organic amendments Biochar, Vermicompost and Mycorrhizae applied as single or blended applications influenced populations of parasitic and non parasitic nematodes. Increased populations of bacteriovores and fungivores were observed with the combined application of Biochar and Vermicompost, Biochar and Mycorrhizae and single application of Mycorrhizae and Vermicompost organic amendments. Biochar and Vermicompost increased populations of bacteriovores and fungivores in the soil since they are both highly porous and increased the soil surface area of the soil upon application. Similar studies have shown that addition of Vermicompost and other organic amendments increases porosity and overall microbial activity in organic matter and have long been considered in improvement of soil health. Porosity and increased surface area offers refuge to other microbes in the soil especially beneficial bacteria and fungi therefore increasing the food base and numbers of bacteriovores and fungivores in the soil (Neher, 2001; Edward, 1998; Mahmood *et al.,* 2003,). Increased Bacteria and fungi feeding

nematodes in the soil was an indication of improved soil organic matter with Vermicompost and Biochar amendments.

Although herbivore populations did not differ significantly between the organic treatments at the end of the trial, the numerical decrease that was observed in Biochar+Mycorrhizae, Biochar and *Paecilomyces lilacinus* (positive control) treated plots at the end of the trial compared to flowering stage shows the potential of Biochar in reducing parasitic nematode populations both as a single application or when combined with Mycorrhizae. This is because lower parasitic nematode populations were maintained with this combination, however since this study was a one season field experiment further experiments are needed to ascertain this. In a similar study Zhang *et al.,* 2013 in an experiment of Biochar effects on soil nematofauna in a wheat field, reported a decrease in plant parasitic nematodes with addition of different rates of Biochar compared to no Biochar application. The combination of Biochar and Mycorrhizae had almost same effects on parasitic nematodes as the positive control *Pecilomyces lilacinus*. *Pecilomyces lilacinus* has been shown to decrease parasitic nematode populations of juvenile counts and eggs (Rodriguez *et al*., 1984) where the conidia of this fungus was able to penetrate and destroy various stages of developing eggs of *Meloidogyne arenaria*.

Decline in populations of *Meloidogyne* sp, a root knot nematode at the end of the trial across all treatment plots and especially in Mycorrhizae, Biochar+Mycorrhizae and Biochar+Vermicompost+Mycorrhizae could be attributed to these organic amendments directly or indirectly by either competition by Mycorrhizae for root colonisation or increased microbial antagonists in the soil by Biochar and Vermicompost. This is supported by a previous study by Shreenivasa *et al*., (2007) who reported that the Mycorrhizae *Glomus fasciculatum* significantly reduced root knot nematodes in tomatoes. In addition, a study of plant fungi and nematode suppression by Arbuscular Mycorrhizae (Rillig, 2011) showed that

nitrogen fixing dicotyledons like soybeans were better protected against nematode pathogens by Arbuscular Mycorrhizal fungi than non-nitrogen fixing plants. In view of this study, Biochar and Vermicompost could be having potential for facilitating mycorrhizal colonisation because Mycorrhizae seemed to have greater effects when blended especially with Biochar and also with Vermicompost.

Higher galling indices were observed in Biochar and Vermicompost amended plots. This is attributed to the elevated densities of root knot nematodes of *Meloidogyne* sp recorded in these two plots when compared with the other treatment plots although in general there was a decline in *Meloidogyne* sp across the treatment plots compared to pre plant populations. Root galling however increased across all the eight treatments throughout the trial. At the end of the trial, soil analysis showed increase in populations of *Trichodorus* sp populations across all the organic amended plots including the positive control suggesting that this stubby root nematode is a menace in this particular region and these particular organic amendments probably did not have an effect on this particular genus of nematodes. Overall there was no significant effect on total nematode abundance across the organic amendments. The effects of organic amendments were more observed in the individual trophic groups showing that probably short term application of organic amendments may not be enough to significantly have an effect on nematode food web. Similar results were observed with Biochar application in a wheat field where there was no significant effects on total nematode abundance with short term application of Biochar (Zhang *et al.*, 2013).

4.2 Effect of organic amendments on soil physical and chemical properties

Biochar and Vermicompost organic amendments application increased phosphorous, carbon and organic content of the soil that in turn positively influenced parasitic and beneficial nematode overall population densities. It was further observed that plant parasitic

nematode densities were higher in Biochar+Vermicompost+Mycorrhizae, Vermicompost and Biochar amended plots whereas they were lower in Mytech and Mycorrhizae plots that had lower phosphorous, carbon and organic matter content. Previous studies have also shown that Vermicompost application increases Phosphorous concentrations. A study conducted by Devliger and Verstraete (1997) found a significant increase in Phosphorous in Vermicompost amended soils attributing this to increase in enzymatic activity of phosphatases from earthworms. As also observed in this study, increase in phosphorous in both Vermicompost and Biochar plots increased populations of plant parasitic nematodes.

Organic matter in this study was also shown to positively influence carbon and phosphorous amounts in the soil. Biochar+Vermicompost+Mycorrhizae, Vermicompost and Biochar+Vermicompost amended plots recorded increased organic matter and in turn also elevated amounts of Carbon and Phosphorous in the soil. Biochar and Vermicompost also exhibited a slightly higher Carbon to Nitrogen ratios due to the increase in Carbon amounts. Carbon is of great importance to soil micro-flora as it serves as a source of energy and this explains the increased nematode populations and especially of bacteriovores and fungivores as observed in the treatment combination of Biochar and Vermicompost. Nitrogen amounts on the other hand reduced across all the plots including the untreated control plots when compared with amounts before planting. This perhaps is because soybean being a leguminous plant and rich in protein (Greeberg and Harting 1998) will therefore require sufficient amounts of nitrogen. Most of the nitrogen from the soil was therefore used during the plants growth. Free living nematodes however play important roles in soil nutrient cycling. Nematode excretion may contribute up to 19% of soluble Nitrogen in soil (Neher, 2001). This nitrogen is available to plants as either NH_4^+ or NH_3^+.Increased NH_3^+ in the soil due to nitrification by nitrifying bacteria is toxic to plant parasitic nematodes.

From this study, a combination of Vermicompost and Biochar treated plots showed increased numbers of both fungivores and bacteriovores compared to other treatments which can be beneficial to soybean as this might increase nitrogen availability to the soil. Contrary to studies by Rodriguez-Kabana (1986) that nematicidal activity was increased in C:N of below 20, in this study, herbivore populations were not significantly lowered apart from Biochar, even though all treatments had C:N of below 20.

Biochar and Vermicompost have an effect on soil pore size, are highly porous and well aerated therefore improving water capacity of soils (Chan *et al.*, 2001), this compares with this study where Biochar and Biochar+Vermicompost+Mycorrhizae amended plots had a slightly higher moisture content percentage than the other treatments. Moisture and soil porosity is important for nematode movement and fastens decomposition of organic matter. Decomposition of organic matter releases toxic wastes like NH_3^+ that can be nematicidal to plant parasitic nematodes. Although the Soil structure class remained as sandy loamy across all the treatment plots, the ability of Biochar to be able retain moisture in the soil means it can be applied in semiarid areas to decrease moisture loss and nutrient leaching.

The soils in this study were relatively maintained at neutral pH. Soils from Biochar+Mycorrhizae, Mycorrhizae and the untreated control plots had elevated amounts of ammonium ions but whereas the first two treatments showed lower levels of plant parasitic nematodes at harvest the untreated plots had higher densities. Decomposing organic matter release toxic compounds like Ammonia gas which is toxic to nematodes. Ammonia is easily transformed to ammonium ions (NH_4^+) in soils with low pH (Oka *et al.*, 2007; Zasada, 2005).

4.3 Effect of organic amendments on soybean yields and biomass

Biochar and Vermicompost treated plots despite having greater galling indexes and parasitic nematode densities when compared to the positive control Mytech, recorded significantly heavier dry seed weight mean at harvest. Biochar, Vermicompost and their blend with Mycorrhizae gave a 100% increase in dry seed yield over the untreated control. These yield benefits can be attributed to increased nutrients in the soil by the organic amendments. It is of great importance that improved yields can still be achieved even in the presence of increased parasitic nematode populations in the soil. Organic amendments have been reported to improve plant tolerance to nematode damage and in turn promote better yields (Mc Sorley and Gallager 1995b; Oka *et al.,* 2007; Orisajo *et al.,* 2008). Although there was no significant differences in stover weight (dry shoot plus root biomass) amongst the treatments, Biochar+Mycorrhizae and Mycorrhizae treated plots recorded the heaviest stover weights compared to the positive control Mytech and the untreated plots. This can be attributed to increased organic matter and phosphorous in the soils of Biochar amended plots that upon decomposition released nutrients whose uptake was enhanced by Mycorrhizae. Mycorrhizae especially enhance uptake of phosphorous an important mineral that promotes growth. Phosphorous is removed from the soil and located in the above ground (non-seed) vegetative plant parts. Stover weight is of importance as soybean leaves and roots provide good feed for sheep and goats (Dugje *et al.,* 2009) therefore creating other usages for the plant. Improved soil aeration due to Biochar also stimulates mycorrhizal fungi abundance that increases Mycorrhizae intracellular growth that enhances plant productivity Nishio, (1991) and Zhang *et al.,* (2010) also showed that addition of Biochar produced from wheat straw increased soil organic Carbon and Nitrogen thereby improving fertility.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Nematode populations respond differently to application of different organic amendments and soil chemical characteristics. Among the organic amendments tested, Biochar application significantly increased bacteriovores. Biochar increased parasitic nematode populations at mid-season which dropped significantly at the end of the trial indicating a potential of Biochar to reduce parasitic nematodes and the potential of Biochar to reduce parasitic nematodes can be enhanced by its combination with Mycorrhizae as it was evident that the combination of Biochar and Mycorrhizae as well as Mytech (*Paecilomyces lilacinus*) showed lower parasitic nematode densities.

In this study, increased organic matter in Bio char and Vermicompost increased phosphorus amounts in the soil which in turn showed increased parasitic nematodes in the soil. Biochar despite showing significant high levels of parasitic nematodes at flowering produced better seed yields.

Meloidogyne sp (root knot nematode) decreased across all the trial fields. It is evident that continued use of the organic amendments will result in control levels for root knot nematodes. As much as root knot nematodes decreased across all the treatments, higher levels in Biochar and Vermicompost resulted in higher root galling indices. The lesion nematode of the genera *Trichodorus* that cause stubby roots in crops is a major problem in this site as populations were significantly increased across all the treatments.

5.2 Recommendations

1. Biochar is a viable option for use in integrated plant nematode management because of its potential to decrease parasitic nematode populations and increase yields**.**
2. *Paecilomyces lilacinus* still remains a viable treatment for the control of plant parasitic nematodes.
3. Increased Phosphorous amounts in the soil due to Bio char and Vermicompost positively influenced plant parasitic nematode populations, further studies are needed to establish its relationship to parasitic nematode ecology and physiological requirements.
4. Combination effects of organic amendments against plant parasitic nematodes especially the combination of Biochar and Mycorrhizae that showed reduced parasitic nematode populations is highly recommended.
5. Since *Meloidogyne sp* populations were reduced across all the treatment plots after organic amendment application, further studies to assess the effect of these organic amendments on this species are recommended.
6. Further studies are needed to determine long term effects of organic amendments on soil nematofauna and their depletion rates.

6.0 REFERENCES

Akhtar, M. and Mahamood, I. 2004. Organic Soil Amendment in relation of Nematode management with Particular reference to India. *Journal of Integrated Pest Management,* 1:201-205.

Akhtar, M. and Malik, A. 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: A review. *Bio resource Technology,* 74:35–47.

Aragaki, M., Apt, W.J., Kunimoto, R.K., Ko, W.H. and J.Y. Uchida. 1984. Nature and control of anthurium decline. *Plant Disease,* 68: 509-511.

Atkilt, G. and Rossiter, G. 2001. Soil Investigations on the Sulmac Farm, Naivasha, Kenya by the *International Institute for Aerospace Survey & Earth Sciences* (ITC) Technical Report ITC, Soil Science Division Enschede, the Netherlands.

Atungwu, J.J., Ademola, A.A. and Aiyelaagbe, O.O. 2009. Evaluation of organic materials for inhibition of nematode reproduction in soybean. *African crop science*, 17:167-173.

Bancy, W., Losenge, T., Esther, K., Daniel., C. and Thomas, D. 2014.Non–pathogenic *Fusarium oxysporum* endophytes provide field control of nematodes, improving yield of bananas (*Musa* sp). *Biological Control*, 74; 82-88.

Bird, D.M and Kaloshian, I. 2003. Are roots special? Nematodes have their say. *Physiological and Molecular and Plant Pathology*, 62:115-123.

Blackwell, P., Reithmuller, G. and Collins, M. 2009. Biochar applications to soil. In Bio char for environmental management. Earthscan, London; Sterling, VA, pp 207-226.

Bongers, T. and Bongers, M. 1998. Functional diversity of nematodes. *Applied Soil Ecolology*, 10:239-251.

Bremner, JM. 1996. Nitrogen-total. In: DL Sparks. Methods of soil analysis. Part 3. Chemical Methods. Madison, MI: ASA and SSSA.1085–1086.

Bridge, J. (1996). Nematode management in sustainable agriculture. *Annual Review of Pathology*, **34**: 201-225.

Bridge, J. And Page, S.L.J. 1980. Estimation of root-knot nematode infestation levels on roots using a rating chart. *Tropical Pest Management*, 26:296-298

Calvet, C., Pinochet, J. and Hernández-Dorrego, A. 2001. "Field Microplot Performance of the Peach Almond Hybrid GF- 677 after Inoculation with Arbuscular Mycorrhizal Fungi in a Replant Soil Infested with Root-Know Nematodes." *Mycorrhizae*, 10:295-300.

Chan, K.Y., Van Zwieten, L. Meszaros, I. Downie, A. and S. Joseph. 2007. Agronomic values of green waste biochar as a soil amendment. *Australian Journal of Soil Research*, 45:629-634.

Chen, J., Abawi, G.S. and Zuckerman, B.M.2000. Efficacy of *Bacillus thuringiensis*, *Paecilomyces marquandii* and *Streptomyces constaricanus* with and without organic amendment against *Meloidogyne hapla* infecting lettuce. *Journal of Nematology*, 32: 70-77.

Chianu Jonas, N., Vanlauwe, B., Mahasi, J. M., Katungi, E., Akech, C., Mairura, F. S., Chianu Justina, N. and Sanginga, N. 2008. Soybean situation and outlook analysis:The case of Kenya. *TSBF CIAT, AFNET*.68 pp

Cook, D., Lee, T., Guo, X., Melito, S., Wang, K., Bayless, A., Wang, J., Hughes, T., Willis, D., Clemente, T., Diers, B., Jiang, J., Hudson, M., & Bent, A. 2012. Copy Number Variation of Multiple Genes at Rhg1 Mediates Nematode Resistance in Soybean *Science*, DOI: 10.1126/science.1228746

Cordier.C., Gianinazzi. S. and V. Gianinnazzi-pearson, 1996. Colonization patterns of root tissues by Phytophthora nicotiana var. Parasitica related to reduced disease in mycorrhizal tomato. *Plant soil*, 185:223-232.

D'Addabbo, T., Papajova´, I., Sasanelli, N., Radicci, V., and Renc̆o, M. 2011. Suppression of root-knot nematodes in potting mixes amended with different composted biowastes. *Helminthologia* 48:278–287.

Darago, A.,Szabo, M.,Hracks, K.,Takacs, A., Nagy, P.I. 2013. Invitro investigations on the biological control of Xiphinema index with *Trichoderma* sp. *Helminthologia*, 50:132-137.

Davide, R.G. and R, A. Zorilla, 1983. Evaluation of a fungus, paecilomyces lilacinus (Thom.) Samson, for the biological control of the potato cyst nematode *Globodera rostochiensis* as compared with some nematicides. *Phillippine Agriculturist*, 66:397-404.

Davide, R.G. and R.A. Zorilla, 1985. Evaluation of a fungus Paecilomyces lilacinus for biological control of root knot nematodes Meloidogyne incognita on okra as compared with nematicide Isazofos. *Philippine Agriculturist* 68:493-500.

Devliegher, W. and Verstraete, W.1997. The Effects of Lumbricus Terrestris on Soil in Relation to Plant Growth: Effects of Nutrient Enrichment processes and Gut Associated processes .*Soil Biology and Biochemistry*, 29:341-346.

Downie, A., A. Crosky and P. Munroe 2009. Physical properties of biochar. In Biochar for for environmental management: science and technology Eds. J. Lehmann and Joseph, S. Earthscan, London; Sterling, VA, pp. 13-32.

Dugje, I.Y., Omoigui, L.O., F. Ekeleme, R. Bandyopadhyay, P. Lava Kumar, and A.Y. Kamara, 2009. Farmers'Guide to Soybean Production in Northern Nigeria. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria

FAO. 1991. *Soil solarization.* FAO Plant Production and Protection Paper. No. 109. By J.E. DeVay, J.J.Stapleton and C.L. Elmore. Rome.

FAO. 1992.Plant Nematode Problems and their Control in the Near East Region Karachi, Pakistan 22-26 November 1992. FAO Plant Production and Protection Paper. No.144

Gallagher, L.A. and Manoil, C. 2001. *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. *Bacteriology,* 183:07-14.

Gallaher, R.N., and Weldon, C. Futral, J.1975. An aluminium block digester for plant and soil analysis. *Soil Science,* 39: 803-806.

Gaur, H.S. & Perry, R.N. 1991b. The use of soil solarisation for control of plant-parasitic nematodes. *Nematological Abstracts,* 60: 153-167.

Geraert, E. and Raski, D.J. 1987. A reappraisal of Tylenchina (Nemata) 3. The fam Tylenchidae Orley,1980. *Revue de Nematologie,* 10:143-161.

Government of Kenya 2007, The Kenya Vision 2030, transforming national development, Nairobi, Government Printer.

Greenberg, P. and H.N. Hartung. 1998. The whole soy cookbook: 175 delicious, nutritious, easy-to-prepare recipes featuring tofu, tempeh, and various forms of nature's healthiest bean. Three Rivers Press, New York.

Hooks, C. R. R., Wang, K.-H., Ploeg, A., and McSorley, R. 2010. Using marigold (Tagetes sp.) as a cover crop to protect crops from plant-parasitic nematodes. *Applied Soil Ecology,* 46:307–320.

Hooper, D.J, J. Hallmann and S.A Subbotin, 2005. Methods for Extraction, Processing and detection of Plant and Soil Nematodes.In:Plant parasitic Nematodes in Subtropical and Tropical Agriculture, Luc, M., R.A Sikora and J. Bridge (Eds). CAB International Publisher, UK., pp:53-86

Hooper, D.J. 1970. Handling, fixing, staining and mounting nematodes. In: *Laboratory Methods for Work with Plant and Soil Nematodes*. Southey, J.F. (Ed.), pp. 39-54. Ministry of Agriculture, Fisheries and Food Technical Bulletin 2. London, Her Majesty's Stationery Office.

Hooper, D.J. 1990. Extraction and processing of plant and soil nematodes. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. Luc, M., Sikora, R.A. and Bridge, J. (Eds.), pp. 45-68. CAB International, Wallingford.

Ibrahim, S.K. and Traboulsi,A.F. 2009.The impact of solarisation integrated with plant bio fermentation on root knot nematodes. *Lebanese science* journal. Vol 10, No. 2.

Imran, A., Siddiqui, S., Shahid Shaukat, Imtiaz Hussain Sheikh, and Aly Khan, 2006. Role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. *Microbiology and Biotechnology*, 22:641-650.

Kandoth, P., Ithal, N., Recknor, J., Maier, T., Nettleton, D., Baum, T., & Mitchum, M. 2011. The Soybean Rhg1 Locus for Resistance to the Soybean Cyst Nematode Heterodera glycines regulates the Expression of a Large Number of Stress- and Defense-Related Genes in Degenerating Feeding Cells. *Plant Physiology*,155: 1960-1975

Karuga, S. And Gachanja, J. 2004. Soybeans development study: Final report prepared by market economies development for Japan International cooperation (JICA), Nairobi, Kenya.

Kienwick, S. And Sikora, R. A. 2003. Efficacy of *paeciliomyces lilacinus* (strain 251) for the control of root rot nematodes. *Applied Biological Science*, 68:123-68.

Kiewnick, S. and Sikora RA. 2004. Optimizing the efficacy of Paecilomyces lilacinus (strain 251) for the control of root-knot nematodes. *Applied Biological Science,* 69 :373-80.

Kimenju, J.W., Karanja, N.K. and Macharia, I.1999. Plant parasitic nematodes associated with common bean in Kenya and the effect of *Meloidogyne* infection on bean nodulation. *African Crop Science Journal,* 7:503-510.

Kimpinski, J., Gallant, C. E., Henry, R., Macleod, J. A., Sanderson, J. B., and Sturz, A. V. 2003. Effect of compost and manure soil amendments on nematode and on yields of potato and barley: a 7-years study. *Nematology,* 35:289–293.

Kolb, S. 2007. Understanding the Mechanisms by which a Manure-Based Charcoal Product Affects Microbial Biomass Activity, PhD Thesis, University of Winsconsin, GreenBay, USA.

Lambert, K. and S. Bekal, 2002. Introduction to Plant-Parasitic Nematodes. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2002-1218-01 Revised 2009 by the Education Center Editorial Board Kris Lambert and Sadia Bekal University of Illinois, Department of Crop Sciences, Urbana, IL.

Lazcano, C., Goméz-Brandón, M., Domínguez, J.2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere*, 72, 1013-1019.

Lehmann, Gauntand, J. and Rondon, M. 2006. Bio-char Sequestration in Terrestrial Ecosystems –A Review. Mitigation and Adaptation Strategies for Global Change. 11:395-419.

Lehmann, J., Rillig, M. C. and Thies, J. 2011. Biochar effects on soil biota—A review. *Soil Biology and Biochemistry*, 43: 1812–1836.

Liu, K. S., 1997. Chemistry and Nutritional Value of Soybean Components. In: Soybean: Chemistry, Technology, and Utilization. *New York: Chapman & Hall*, pp 25-11

Liu, S., Kandoth, P., Warren, S., Yeckel, G., Heinz, R., Alden, J., Yang, C., Jamai, A., El-Mellouki, T., Juvale, P., Hill, J., Baum, T., Cianzio, S., Whitham, S., Korkin, D., Mitchum, M., & Meksem, K.2012. A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. *Nature*, 492:256-260.

Luc, M., Sikora, R.A. and Bridge, J. 2005. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, 2nd ed. CABI Publishing, Wallingford, UK.

Mahasi, J. M., Vanlauwe, B. Mursoy, R. C. Mbehero, P. and Mukalama, J. 2010. Increasing Productivity of Soybean in Western Kenya through Evaluation and Farmer Participatory Variety Selection.In:LA Wasilwa,J Ouda,IW Kariuki, NS Mangale and MN Lukuyu (eds).Transforming Agriculture for Improved Livelihoods through Agricultural Product Value Chain. Proceedings of the 12th KARI Biennial Scientific Conference. November 8-12, 2010. Nairobi -Kenya.

Mahmood, S., Finlay, R. D., Fransson, A. M. and Wallander, H. 2003. Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings. *Microbiol Ecology*, **43**: 121–131.

Mai, W.F. and P.G. Mullin (with H.H. Lyon and K. Loeffler) 1996. Plant-Parasitic Nematodes: A pictorial key to genera. Cornell University Press, Ithaca, NY 277 pp.

Manivannan, S.,Balamurugun, M.,Parsarathi, K.,Gunasekaran,G and Ranganathan, L.S.2009.Effect of Vermicompost on Soil Fertility and crop Productivity-beans(*Phaseolus vulgaris*).*Journal of Environmental Biology*, 30:275-281.

McSorley, R. and R. N. Gallaher. 1995b. Effect of yard waste compost on plant-parasitic nematode densities in vegetable crops. *Journal of Nematlogy*, 27:545-549.

Mehlich, A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. *Communication in Soil Science Plant Analysis*.15:1409-141.

Mehrdad, E. 2004. Problems facing the flower industry. In: M. N. Wabule, P. N. Ngaruiya, F. M. Kimmins, P. J. Silverside (Eds.),Registration for bio control agents in Kenya: Proceedings of the PCPB/KARI/DFID CPP workshop (pp. 7-8). Nakuru, Kenya, 14-16 May 2003.

Mian, I. H. and Rodriguez-Kabana, R. 1982c. Survey of the nematicidal properties of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria*. *Nematropica* 12:235–246.

Myaka, F. A., Kirenga, G., Malema, B. (Eds) (2005). Proceedings of the First National Soybean Stakeholders Workshop, 10–11 November 2005, Morogoro, Tanzania.

Neher, D. A. 2001. Role of nematodes in soil health and their use as bioindicators. *Journal of Nematology* 33:161 – 168.

Ngundo, B.W.and Taylor, D.P.1974. Effect of *Meloidogyne* spp on bean yields in Kenya. *Plant Disease Reporter* 58:1020-1023.

Nisho, M.a.O., S., 1991. Stimulation of the growth of alfalfa and infection of mycorrhizal fungi by the application of charcoal. Bulletin of the National Grassland Research Institute, 45: 61-71.

Norton, D.C. 1979. Relationship of physical and chemical factors to population of plant parasitic nematodes. Annual Review of *Phytopathology*, **17**: 279-299.

Oka, Y., Tkachi, N., Shuker, S., and Yermiyahu, U. 2007. Enhanced nematicidal activity of organic and inorganic ammonia-releasing amendments by Azadirachta indica extracts. *Journal of Nematiology*, 39:9–16.

Olabiyi T.I., Olayiwola A.O. and Oyediran G.O. 2009 Influence of Soil Textures on Distribution of Phytonematodes in the South Western Nigeria. *World Journal of Agricultural Sciences*, 5: 557-560.

Orisajo S.B, Afolami S.O, Fademi O, Atungwu J.J. 2008. Effects of poultry litter and carbofuran soil amendments on Meloidogyne incognita attacks on cacao. *Journal of Applied Biosciences*, 7: 214 – 221.

P .Castillo , A. I. Nico, C. Azcón-Aguilar, C. Del Río Rincón, C. Calvet and R. M. Jiménez-Díaz.2006. Protection of olive planting stocks against parasitism of root-knot nematodes by arbuscular mycorrhizal fungi, *Plant Pathology* 55:705-713.

PCPB (Pest Control Products Board). 2005. Annual report. Nairobi, Kenya.

Perry, R. N. and Evans, A. A. F. 2009. Survival mechanisms *in*: Perry, R.N., Moens, M., and Starr, J. L. (eds). Root-knot nematodes. CAB international. London. 201-220 pp.

Peterson, R.L., H.B. Massicotte and L.H. Melville, 2004. Mycorrhizas: Anatomy and Cell Biology. NCR Research Press, Ottawa, Canada.

Raul, A., Ricardo, G. and Marcia, T. 2013. Interactions between a Root Knot nematode (*Meloidogyne exigua*) and Arbuscular Mycorrhizae in Coffee Plant Development (Coffee Arabica).*American journal of plant sciences*, 4:19-23.

Renco, M. and Kovasik, P. 2012. Response of Plant Parasitic and Free Living Soil Nematodes to Composted Animal Manure Soil Amendments. *Journal of Nematology*, 44:329-336.

Ribeiro, C. F., Mizobutsi, E. H., Silva, D. G.; Pereira, J .C. R., Zambolim, L. 1998. Control of *Meloidognye javanica* on lettuce with organic amendments. *Fitopatologia Brasileira*, 23:42-44.

Rodriguez-Kabana, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *Journal of Nematology*, 18:129–135.

Rodriguez-Kabana, R., G. Morgan-Jones, G. Godoy, and H. O. Gintis. 1984. Effectiveness of species of *Gliocadium, Paecilomyces* and *Verticillium* for control of *Meloidogyne arenaria* in field soil. *Nematropica,* 14:155-158

Romina, R., Lidia, G. ,and Rosario, R.A. 2011. Soil Quality Index to Evaluate the Vermicompost Amendments Effects on Soil Properties. *Journal of Environmental Protection,* 2011,2,502-510.

Sanginga, N., 2003. Role of biological nitrogen fixation in legume based cropping systems; a case study of West Africa farming systems. *Plant and Soil*, 252:25-29.

Schreiner, P. and Pinkerton, J.**2002.** "Ring Nematodes (*Meso- criconema xenoplax*) Alter Root Colonization and Function of Arbuscular Mycorrhizal Fungi in Grape Roots in a Low P Soil." *Soil and Biochemistry*, 3:10-22.

Sikora, R.A. 1992. Managenent of Antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes.*Annual review of phytopathology,* 30 245-270.

Singh, A., Sharma, J., Rexer, K.H. and Varma, A. 2000. Plant productivity determinants beyond minerals, water and light. *Piriformospora indica*: a revolutionary plant growth promoting fungus. *Current Science*, 79:101-106.

Smith, S.E, and Read, D.J. 1997. Mycorrhizal symbiosis. 2nd ed. San Diego, California: Academic Press. 605 pp.

Smith, S.E. and D.J. Read, 2008. Mineral Nutrition,Toxic Element Accumulation and Water Relations of Arbuscular Mycorrhizal Plants. In Mycorrhizal Symbiosis. 3rd Edition, Academic Press, London, ISBN-10: 0123705266, pp: 145-148.

Stirling, G. R. 1991. Biological control of plant-parasitic nematodes. Wallingford, UK: CAB International.

Sultan, S.A and Ferris, H. 1991. The effect of soil moisture and soil particle size on the survival and population increase of *Xiphinerna index. Revue Nématol,* 14:345-351.

Tahat, M. M, Kamaruzaman, Sijam and R. Othman, 2010.Mycorrhizal fungi as a biocontrol agent. *Plant pathology*, 9:198-207.

Thacker J.R.M. 2002. An Introduction to Arthropod Pest Control. Cambridge University Press, Cambridge: 343p.

Thoden, T. C., Korthals, G. W., and Termorshuizen, A. J. 2011.Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? *Nematology,* 13:133–153.

Thomason, I.J and Caswell, E.P. 1987. Principles of nematode control. Pages 87-130 in H.R. Brown and B.R. Kerry eds. Principles and practice of nematode control in crops. Academic press London.

Thomason, I.J. 1987. Challenges facing nematology: environmental risks with nematicides and the need for new approaches. *In* JA. Veech & D.W. Dickson, eds. *Vistas on nematology,* pp. 469-476. Hyattsville, USA, Society of Nematologists.Tylenchidae Örley, 1980. *Revue de Nematologie,* 10:143-161.

Villenave, C., Leye J. and Duponnois, R. 2003. "Nematofauna Associated with Exotic and Native Leguminouspant Spe- cies in West Africa: Effect of Glomus Intradices Arbus-cular Mycorhizal Symbiosis." *Biology and Fertility of Soils*, 38:161-169.

Wachira, P.M., J.W. Kimenju, S.A. Okoth and R.K. Mibey, 2009. Stimulation of Nematode-Destroying Fungi by Organic Amendments Applied in Management of Plant ParasiticNematode. *Asian Journal of Plant Sciences*, 8: 153-159.

Walkley, A. and Black, I. A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science,* **37**: 29-38.

World Health Organization. Sound management of hazardous wastes from health care and from agriculture. New Delhi; WHO South- East Asia Regional Office, 2006.

Zasada, I. 2005. Factors affecting the suppression of Heterodera glycines by N-Viro Soil. *Journal of Nematology,* 37:220–225.

Zhang Xiao-Ke, Li Qi1, Liang Wen-Ju., Zhang Min, Bao Xue-Lian, and Xie Zu-Bin 2012. Soil Nematode Response to Biochar Addition in a Chinese Wheat Field. *Pedosphere,* 23: 98–103.

APPENDICES

Appendix 1: LSD Multiple comparisons of treatment effects on Herbivores at flowering.

Treatment		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I)	(J)				Lower Bound	Upper Bound
B	V	866.67*	253.266	**0.003**	329.77	1403.57
	M	650.00*	253.266	**0.021**	113.1	1186.9
	BV	683.33*	253.266	**0.016**	146.43	1220.23
	BM	766.67*	253.266	**0.008**	229.77	1303.57
	MV	983.33*	253.266	**0.001**	446.43	1520.23
	BVM	616.67*	253.266	**0.027**	79.77	1153.57
	MY	966.67*	253.266	**0.002**	429.77	1503.57

Appendix 2: Tests of Between-Subjects Effects on galling index.

Dependent Variable: galling index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	15.493[a]	17	0.911	5.361	0	0.717
Intercept	274.727	1	274.727	1616.04	0	0.978
Growth stage	2.081	1	2.081	12.24	0.001	0.254
treatment	8.76	8	1.095	6.441	0	0.589
Growth stage * treatment	4.653	8	0.582	3.421	0.005	0.432
Error	6.12	36	0.17			
Total	296.34	54				
Corrected Total	21.613	53				

a. R Squared = .717 (Adjusted R Squared = .583)

Appendix 3: LSD Multiple comparison galling index at flowering.

(I) treatment		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
B	MY	-.9333*	0.4037	**0.033**	-1.781	-0.085
V	BM	1.1000*	0.4037	**0.014**	0.252	1.948
	BVM	.8667*	0.4037	**0.046**	0.019	1.715
M	BM	.9333*	0.4037	**0.033**	0.085	1.781
BV	BM	1.0667*	0.4037	**0.017**	0.219	1.915
	NT	1.1667*	0.4037	**0.01**	0.319	2.015
BM	MY	-1.6000*	0.4037	**0.001**	-2.448	-0.752
MV	MY	-.8667*	0.4037	**0.046**	-1.715	-0.019
MY	BVM	1.3667*	0.4037	**0.003**	0.519	2.215
	NT	1.7000*	0.4037	**0.001**	0.852	2.548
NT	V	-1.2000*	0.4037	**0.008**	-2.048	-0.352
	M	-1.0333*	0.4037	**0.02**	-1.881	-0.185

*. The mean difference is significant at the 0.05 level.

Appendix 4: Multiple comparisons galling index at harvest

(I) treatment		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
B	V	-.767*	0.2524	**0.007**	-1.297	-0.236
	BM	.733*	0.2524	**0.009**	0.203	1.264
	MV	.567*	0.2524	**0.038**	0.036	1.097
	BVM	.733*	0.2524	**0.009**	0.203	1.264
	MY	.767*	0.2524	**0.007**	0.236	1.297
V	M	1.067*	0.2524	**0.001**	0.536	1.597
	BV	.833*	0.2524	**0.004**	0.303	1.364
	BM	1.500*	0.2524	**0**	0.97	2.03
	MV	1.333*	0.2524	**0**	0.803	1.864
	BVM	1.500*	0.2524	**0**	0.97	2.03
	MY	1.533*	0.2524	**0**	1.003	2.064
	NT	1.200*	0.2524	**0**	0.67	1.73
BV	BM	.667*	0.2524	**0.017**	0.136	1.197
	BVM	.667*	0.2524	**0.017**	0.136	1.197
	MY	.700*	0.2524	**0.013**	0.17	1.23

Appendix 5:Tests of Between-Subjects Effects (Predators)

Dependent Variable: Predators

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	213854.167[a]	17	12579.657	.769	.714	.266
Intercept	440104.167	1	440104.167	26.892	.000	.428
Treatment	57291.667	8	7161.458	.438	.890	.089
Growth stage	55104.167	1	55104.167	3.367	.075	.086
treatment * growth stage	101458.333	8	12682.292	.775	.627	.147
Error	589166.667	36	16365.741			
Total	1243125.000	54				
Corrected Total	803020.833	53				

a. R Squared = .266 (Adjusted R Squared = -.080)

Appendix 6: Tests of Between-Subjects Effects treatments and growth stage on bacteriovores

Dependent Variable: Bacteriovores

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	9079259.259[a]	17	534074.074	1.163	.340	.355
Intercept	89449074.074	1	89449074.074	194.861	.000	.844
Treatment	1980925.926	8	247615.741	.539	.819	.107
Growth stage	560185.185	1	560185.185	1.220	.277	.033
Treatment * Growth stage	6538148.148	8	817268.519	1.780	.114	.283
Error	16525416.667	36	459039.352			
Total	115053750.000	54				
Corrected Total	25604675.926	53				

Appendix 7:Tests of Between-Subjects Effects

Dependent Variable: Fungivores

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	423425.926[a]	17	24907.407	.805	.677	.275
Intercept	1742407.407	1	1742407.407	56.299	.000	.610
treatment	143842.593	8	17980.324	.581	.787	.114
Growth stage	2962.963	1	2962.963	.096	.759	.003
treatment * growth stage	276620.370	8	34577.546	1.117	.375	.199
Error	1114166.667	36	30949.074			
Total	3280000.000	54				
Corrected Total	1537592.593	53				

a. R Squared = .275 (Adjusted R Squared = -.067)

Appendix 8: Multiple comparison dry seed weight at harvest

(I) treatment		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
B	M	113.1000*	27.72506	**0.001**	54.8518	171.3482
	BV	79.6867*	27.72506	**0.01**	21.4385	137.9348
	BM	81.6533*	27.72506	**0.009**	23.4052	139.9015
	MV	106.9367*	27.72506	**0.001**	48.6885	165.1848
	NT	112.8967*	27.72506	**0.001**	54.6485	171.1448
V	M	83.8133*	27.72506	**0.007**	25.5652	142.0615
	MV	77.6500*	27.72506	**0.012**	19.4018	135.8982
	NT	83.6100*	27.72506	**0.007**	25.3618	141.8582
MY	M	90.0900*	27.72506	**0.004**	31.8418	148.3382
	BM	58.6433*	27.72506	**0.049**	0.3952	116.8915
	MV	83.9267*	27.72506	**0.007**	25.6785	142.1748
	NT	89.8867*	27.72506	**0.005**	31.6385	148.1348

Appendix 9: Key to Genera of Plant-feeding Nematodes

Key to Genera of Plant-feeding Nematodes

1	a	Stylet absent	**Not a plant feeder** **Go to alternate key**
	b	Stylet/spear present	**Go to 2**
2	a	2-part oesophagus, anterior slender, posterior glandular and muscular, spear, no metacorpus with valve	**Order Dorylaimida** **Go to 3**
	b	3-part oesophagus, metacorpus with valve, slender isthmus, glandular post corpus, Stylet usually with knobs	**Sub-Order Tylenchina** **Go to 7**
3	a	Spear short and curved, body thick, anus almost terminal	**Trichodoridae** **Go to 4**
	b	Spear long with long extension, body long and slender	**Longidoridae** **Go to 5**
	c	Spear short and straight	**Not a plant feeder** **Go to alternate key**
4	a	Diovarial, males without caudal alae	***Trichodorus***
	b	Diovarial, males with caudal alae	***Paratrichodorus***
	c	Monovarial, males without caudal alae	***Monotrichodorus***
	d	Monovarial, males without caudal alae	***Allotrichodorus***
5	a	Spear extension with basal flanges, guiding ring near base of spear	***Xiphinema***
	b	Spear extension without basal flanges, guiding ring near tip of spear	**Go to 6**

6	a	Amphid openings small and slit-like	***Longidorus***
	b	Amphid openings wide, posterior to lips	***Paralongidorus***
7	a	DEGO in metacorpus and difficult to see; metacorpus usually nearly as large as body diameter	**Superfamily Aphelenchoidea Go to 8**
	b	DEGO in procorpus; metacorpus usually less that 75% of body width	**Superfamily Criconematoidea or Tylenchoidea Go to 10**
8	a	Vulva with overlapping flap, vagina curved, male with small bursa at tail tip	***Bursaphelenchus***
	b	Vulva without flap, vagina not curved	**Go to 9**
9	a	Female tail bluntly rounded, lateral field with 6-15 lines, male with bursa and gubernaculum	***Aphelenchus***
	b	Female tail usually conoid and mucronate, lateral field with 3-5 lines, male without bursa or gubernaculum	***Aphelenchoides***
10	a	Head with setae, no plant feeders	**Go to 74**
	b	Head without setae, mainly plant feeders	**Go to 11**
11	a	Metacorpus absent or reduced, no valve.	***Nothanguina*, *Nothotylenchus***
	b	Metacorpus with valve	**Go to 12**
12	a	Mature females greatly enlarged (pear-shaped, kidney-shaped), embedded or attached to plant roots, or free in soil as cysts	**Go to 13**

	b	Mature females vermiform, slender to slightly swollen	**Go to 24**
13	a	Mature female body elongate-saccate or kidney shape and not hardened into a cyst	**Go to 14**
	b	Mature female body pear-, lemon-shaped or spherical, may be hardened into a cyst	**Go to 19**
14	a	Female diovarial	***Rotylenchulus***
	b	Female monovarial	**Go to 15**
15	a	Excretory pore in esophageal region, near nerve ring	**Go to 16**
	b	Excretory pore posterior to nerve ring	**Go to 17**
16	a	Mature female subspherical, may have a protruding vulva, cuticle marked with coarse reticulate pattern	***Sphaeronema***
	b	Mature female a thick spiral, without protruding vulva	***Trophonema***
17	a	Lip region elevated in females and juveniles	***Trophotylenchulus***
	b	Lip region not elevated, continuous with body contour	**Go to 18**
18	a	Excretory pore in posterior third of body, near vulva	***Tylenchulus***
	b	Excretory pore near basal region of esophagus	***Nacobbus***
19	a	Females with perineal pattern, excretory pore close to level of stylet, lip region with 2 lateral lips and 4 smaller sublateral lips, weak head frame, J2 stylet ≤20µm, host roots usually galled	***Meloidogyne***
	b	Females without perineal pattern, excretory pore posterior to metacorpus, lip region with 2 lateral lips smaller than 4 sublateral lips, J2 stylet ≥20µm, host roots not galled	**Go to 20**
20	a	V > 50% but well anterior to anus, cuticle striated	***Meloidodera***

	b	Vulva terminal or subterminal, cuticle striated or lace-patterned	**Go to 21**
21	a	Cuticle striated	***Cryphodera***
	b	Cuticle lace-patterned	**Go to 22**
22	a	Female body hardens to cyst, vulva terminal or on a terminal vulval cone, anus dorsal to vulva	**Go to 23**
	b	Female body does not harden to cyst, vulva and anus on terminal prominence	***Atalodera***
	c	Female body does not harden to cyst, vulva sunken into terminal vulval cone with anus on upper side of dorsal vulva lip, J2 stylet >38µm	***Sarisodera***
23	a	Cysts generally lemon-shaped, vulva on terminal vulval cone with fenestration, bullae present or absent, J2 stylet <30µm	***Heterodera***
	b	Cyst spherical or subspherical, bullae absent, J2 with 5 lines in lateral field	***Globodera***
24	a	c' ≥ 6, filiform with pointed or clavate terminus	**Go to 25**
	b	c' generally < 6, but if longer it is cylindroid rather than filiform	**Go to 29**
25	a	Female diovarial	**Go to 26**
	b	Female monovarial	**Go to 27**
26	a	Stylet without knobs, no cephalic sclerotization, tail filiform, usually with clavate terminus	**Go to 70**
	b	Stylet with knobs, heavy cephalic sclerotization, tail filiform with pointed terminus	***Brachydorus***

27	a	Esophagus criconematoid with swollen procorpus continuous with metacorpus, cuticle thick and coarsely striated	***Caloosia***
	b	Esophagus tylenchoid, procorpus not swollen,, cuticle thin, not coarsely striated	**Go to 71**
28	a	Blank couplet	
	b	Blank couplet	
29	a	Monovarial, vulva in posterior third of body	**Go to 30**
	b	Monovarial, V near 50%, lip region conical and not striated, female tail tip rounded, cuticle of tail swollen	***Trophurus***
	c	Diovarial, V near 50%	**Go to 45**
30	a	Procorpus not swollen and combined into metacorpus or, if swollen, offset from metacorpus by a constriction	**Go to 31**
	b	Procorpus swollen and continuous with metacorpus	**Superfamily Criconematoidea** **Go to 38**
31	a	Stylet delicate, ≤15µm, tail acute or subacute	**Go to 32**
	b	Stylet strong, >15µm, tail tapering or bluntly rounded	**Go to 34**
32	a	Ovary with oocytes in one or two rows, not arranged around central rachis, mature female slender to stout	**Go to 33**
	b	Ovary with multiple rows of oocytes arranged around central rachis, mature female usually obese, in seed, leaf or flower galls	***Anguina***
33	a	Ovary with one or two flexures, female moderately stout, in root galls of Graminae	***Subanguina***

	b	Ovary outstretched, female slender, in bulbs, stems, leaves, tubers	***Ditylenchus***
34	a	s≥1.5, c' generally ≤1.5	***Rotylenchoides***
	b	s<1.5, c' >1.5	**Go to 35**
35	a	Esophagus overlaps intestine ventrally	***Pratylenchus***
	b	Esophagus overlaps intestine dorsally	**Go to 36**
36	a	Lip region low, generally rounded, stylet knobs flattened anteriorly, sexual dimorphism	***Radopholoides***
	b	Lip region high, conoid, stylet knobs sloping anteriorly or indented, males present or absent	**Go to 37**
37	a	Female body swollen, stylet knobs sloping anteriorly, sexual dimorphism	***Acontylus***
	b	Female body slender, stylet knobs tapering anteriorly to a dentate tip, males absent	***Hoplotylus***
38	a	Mature female without extra cuticle or sheath	**Go to 39**
	b	Mature female with extra cuticle or sheath	**Go to 41**
39	a	Cuticle with prominent retrorse striations	**Go to 40**
	b	Cuticle without prominent retrorse striations	**Go to 42**
40	a	Cuticular striations of female with spines, scales, plates or stalks on posterior margins	***Criconema***
	b	Cuticular striations of female with smooth or crenate posterior margins	***Criconemoides***
41	a	Stylet knobs rounded, sloping anteriorly, usually <200 striations on cuticle	***Hemicriconemoides***

	b	Stylet knobs anchor-shaped, usually >200 striations on cuticle	***Hemicycliophora***
42	a	Cuticular striations of female with membranous structures on posterior margins	***Bakernema***
	b	Cuticular striations of female without membranous structures on posterior margins	**Go to 43**
43	a	Cuticle of female with minute tubercles	***Cacopaurus***
	b	Cuticle of female without minute tubercles	**Go to 44**
44	a	Female stylet ≤36µm	***Paratylenchus***
	b	Female stylet 45-120µm	***Gracilacus***
45	a	s≥2.5	**Go to 46**
	b	s generally <2.5	**Go to 50**
46	a	Esophageal glands not enclosed in a bulb, usually unequal in length, overlapping intestine	**Go to 47**
	b	Esophageal glands enclosed in a bulb, usually butting intestine	**Go to 48**
47	a	Average adult body length ≥1.75mm	***Belonolaimus***
	b	Average adult body length <1.75mm	**Go to 49**
48	a	Lip region continuous with body contour	***Macrotrophurus***
	b	Lip region offset from body contour by a constriction	***Dolichodorus***
49	a	Lateral field with 5 lines	***Morulaimus***
	b	Lateral field with 3 lines	***Carphodorus***
50	a	Phasmids absent	***Aphasmatylenchus***
	b	Phasmids present	**Go to 51**
51	a	c'<1.5	**Go to 62**

	b	c'≥1.5	**Go to 52**
52	a	Esophageal glands usually unequal in length, overlapping intestine dorsally or lateroventrally	**Go to 53**
	b	Esophageal glands enclosed in a bulb or equal in length, usually butting intestine	**Go to 60**
53	a	Weak to moderate cephalic framework, female head not low or flattened	**Go to 54**
	b	Well-developed cephalic framework, female head low , rounded or flattened	**Go to 57**
54	a	Well-developed stylet, lateral field with 5 lines	**Go to 55**
	b	Stylet slender with diverging knobs, lateral field with 4 lines	***Trichotylenchus***
55	a	Female tail cylindroid with rounded terminus	**Go to 56**
	b	Female tail elongate-conoid with blunt terminus	***Telotylenchus***
56	a	Stylet cone asymmetrical, c' around 2, tail with broadly rounded terminus	***Histotylenchus***
	b	Stylet cone symmetrical, female tail with broadly rounded to bulbous terminus and strongly thickened cuticle	***Telotylenchoides***
57	a	Esophagus overlapping intestine dorsally	**Go to 58**
	b	Esophagus overlapping intestine ventrally	**Go to 59**
58	a	Short overlap, no obvious sexual dimorphism	***Pratylenchoides***
	b	Long overlap, distinct sexual dimorphism	***Radopholus***
59	a	Tail tip mucronate	***Hirschmanniella***
	b	Tail tip not mucronate	***Zygotylenchus***
60	a	Lateral field with 4 lines, female tail not acute	**Go to 61**

	b	Lateral field with 6 lines, female tail acute or subacute	***Merlinius***
61	a	Female tail conoid with terminus bluntly rounded	***Tylenchorhynchus***
	b	Female tail cylindroid, tail with broadly rounded terminus and thick cuticle	***Paratrophurus***
62	a	Phasmids small, pore-like	**Go to 63**
	b	Phasmids enlarged	**Go to 64**
63	a	Esophagus overlapping intestine dorsally and laterally, lip region with or without striation, DEGO <0.25 of stylet length behind knobs	***Rotylenchus***
	b	Esophagus overlapping intestine ventrally, lip region without longitudinal striation, DEGO ≥0.25 of stylet length behind knobs	***Helicotylenchus***
64	a	Both phasmids posterior to vulva	**Go to 65**
	b	One phasmid anterior and one posterior to vulva	**Go to 66**
65	a	Phasmids nearly opposite each other in region of anus, lip region with transverse striations	***Scutellonema***
	b	Phasmids anterior to anus, not opposite each other, lip region without striations	***Peltamigratus***
66	a	Stylet knobs with anterior projections, ≤5 lines in lateral field, lateral field areolated throughout length	***Hoplolaimus***
	b	Stylet knobs rounded and without anterior projections, 5 lines in lateral field, lateral field areolated at phasmids and in anterior	***Aorolaimus***
67	a	Females diovarial	**Go to 68**

	b	Females monovarial	**Go to 71**
68	a	Tail short, subcylindrical, rounded; stylet very long (90-110µm)	**Macrotrophurus**
	b	Tail elongate, attenuated; stylet < 20µm	**Go to 69**
69	a	Cephalic framework sclerotized; vulva with lateral membranes; male cloaca with hypoptygma	**Antarctenchus**
	b	Cephalic framework not sclerotized; vulva withithout lateral membranes; male cloaca without hypoptygma	**Go to 70**
70	a	Head high, amphidial slit obvious; metacorpus posterior to middle of esophagus	**Psilenchus**
	b	Head low, amphidial slit indistinct; metacorpus anterior to middle of esophagus	**Atetylenchus**
71	a	Stylet very long (76-104µm)	**Tylodorus**
	b	Stylet long (38-52µm)	**Epicharinema**
	c	Stylet moderate (22-34µm)	**Go to 72**
	d	Stylet short (<22µm)	**Go to 73**
72	a	Cuticle with longitudinal ridges	**Campbellenchus**
	b	Cuticle without longitudinal ridges	**Gracilancea**
73	a	Head with setae	**Go to 74**
	b	Head without setae	**Go to 75**
74	a	Vulva covered by longitudinal flap; male without caudal alae; male cloaca with hypoptygma	**Atylenchus**
	b	Vulva with lateral flaps; male with caudal alae; male cloaca raised	**Eutylenchus**

75	a	Cuticle with longitudinal ridges	**Go to 76**
	b	Cuticle without longitudinal ridges	**Go to 80**
76	a	Cone about 1/3 of stylet length	**Go to 77**
	b	Cone almost 1/2 of stylet length	**Go to 79**
77	a	Transverse striations not visible through longitudinal ridges	**Basirienchus**
	b	Transverse striations and longitudinal ridges form block (tessellate) pattern; lateral field with 4 lines	**Go to 78**
78	a	Lip region with 2-3 striations, stylet without knobs	**Neothada**
	b	Lip region with 6-7 striations, stylet without knobs	**Basirienchus**
79	a	Vulva covered by longitudinal flap; stylet 17-19µm	**Pleurotylenchus**
	b	Vulva with lateral flaps; stylet <81µm	**Coslenchus**
80	a	Cone about 1/3 of stylet length	**Go to 81**
	b	Cone almost 1/2 of stylet length	**Go to 94**
81	a	Head high, with distinct lateral amphid slits	**Go to 82**
	b	Head variously shaped, amphid slit longitudinal	**Go to 85**
82	a	Female body ventrally curved or spiral; female with offset spermatheca and oocytes in multiple rows	**Boleodorus**
	b	Female body straight; oocytes not in multiple rows	**Go to 83**
83	a	Tail bent or hook-shaped near tip	**Basirienchus**
	b	Tail more or less straight	**Go to 84**
84	a	Stylet without knobs, anterior part with wide lumen	**Neopsilenchus**
	b	Stylet with or without knobs, anterior conical with narrow lumen	**Basiria**
85	a	Head with disc-like structure	**Go to 86**

	b	Head with smooth contour	**Go to 88**
86	a	Head with small disc	**Go to 87**
	b	Head with large dome-shaped structure	**Cucullitylenchus**
87	a	Very slender (a=62-76); caudal alae concave posteriorly	**Mitranema**
	b	Less slender; caudal alae rounded	**Filenchus**
88	a	Very slender (a=60-180); caudal alae lobed	**Go to 89**
	b	Body width variable; caudal alae rounded if present	**Go to 90**
89	a	Head quadrangular; pore-like amphid apertures; body constricted after vulva	**Ecphyadophora**
	b	Head flattened; long amphid apertures; body not constricted after vulva	**Ecphyadophoroides**
90	a	Cuticle deeply incised	**Go to 91**
	b	Cuticle not deeply incised	**Go to 92**
91	a	Head quadrangular; body striations with zigzag pattern; male without caudal alae	**Miculenchus**
	b	Head flattened; male with caudal alae	**Malenchus**
92	a	Very slender; indistinct striation; head very flat; long, sinuous amphid aperture	**Lelenchus**
	b	Larger body diameter; distinct striation; head quadrangular; aperture not sinuous	**Go to 93**
93	a	Head high with longitudinal amphid apertures lateral; clavate stylet knobs; DEGO > 1/2 stylet length behind knobs	**Irantylenchus**
	b	Head quadrangular; distinct striation; round stylet knobs; DEGO < 1/2 stylet length behind knobs	**Filenchus**

94	a	Vulva with lateral flaps	**Go to 95**
	b	Vulva without flaps	**Go to 96**
95	a	Lateral field with 2 lines; vagina thin; post-vulval sac short	**Allotylenchus**
	b	Lateral field with 3 lines; vagina thickened; post-vulval sac short	**Aglenchus**
	c	Lateral field with 4-6 lines; vagina not thickened; post-vulval sac long	**Cephalenchus**
96	a	Lateral field and striations inconspicuous; caudal alae very small	**Polenchus**
	b	Lateral field and striations distinct; caudal alae distinct	**Tylenchus**

Sources:

Adopted and compiled with slight modification from:

Mai, W.F. and P.G. Mullin (with H.H. Lyon and K. Loeffler) 1996. Plant-Parasitic Nematodes: A pictorial key to genera. Cornell University Press, Ithaca, NY 277p.

Geraert, E. and Raski, D.J. 1987. A reappraisal of Tylenchina (Nemata) 3. The family Tylenchidae Örley, 1980. Revue de Nematologie 10:143-16