DYNAMICS OF NEMATODE COMMUNITIES AS INFLUENCED BY SOIL FERTILITY MANAGEMENT PRACTICES AND BIOCONTROL AGENTS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my late mother. family members and the young Brian.

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ANOVA-Analysis of Variance

C-P –colonizer-persister rating

CN – Carbon: Nitrogen ratio

c.v- coefficient of variation

LSD-Least Significant Difference

MIRCEN-Microbiological Research Centre

PDA-Potato Dextrose Agar

pH- Hydrogen ion concetration

PPN –Plant parasitic nematodes

RKN- root knot nematodes

t ha^{-1}-ton per hectare

'N /E– minutes north/east

USDA- United States Department of Agriculture

ABSTRACT

Two field experiments were conducted to investigate the effect of soil fertility management practices and bio-control agents on the dynamics of nematode communities in Kakamega forest and the neighboring farmlands. Efficacy of a bio-control agent, *Bacillus subtilis* was tested alongside *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674 for nodule formation in common bean (*Phaseolus vulgaris* L.var Rosecoco). Three *Bacillus subtilis* strains (K158, K194 and K263), singularly or in combination with *Rhizobium leguminosarum biovar phaseoli* were tested in beans and soil fertility management practices which included inorganic N: P: K (75: 26: 46) kgha^{-1}, farm yard manure (5 tons ha^{-1}), PRE-PAC (800 kg P ha^{-1}) and (80 kg N ha^{-1}) were investigated in maize. The experiments were established on four farms representing age sequences since conversion from forest of 1-10, 10-20, 20-40 and >40 years.

Bacillus subtilis strain K194 in combination with *Rhizobium* treatment on beans and PRE-PAC on maize reduced the populations of root-knot nematodes leading to an increase in bean yields from 150 to 560 kg ha^{-1}. Diversity index analysis showed that application of PRE-PAC reduced nematode numbers compared to farmyard manure and inorganic fertilizers. Inoculating beans with *Rhizobium Leguminosarum biovar phaseoli* strains USDA 2674 enhanced nodulation and biomass production.

Continued land conversion leads to loss of soil fertility and land degradation that ultimately results in loss of nematode biodiversity. Use of PRE-PAC resulted in modification of soil fauna environment that led to reduction in nematode numbers. For instance, the population of *Pratylenchus* sp. reduced by over 30 %. On the other hand, use of inorganic fertilizers released N that promoted multiplication of nematodes as observed in this study where *Pratylenchus* sp. population increased by over 40 %.

This study further demonstrated that *Bacillus spp.* is a viable component of integrated nematode management packages. Potential *Bacillus subtilis* strains as biocontrol agent for root-knot nematode, *Meloidogyne* spp. as well as growth promoting agent in beans was demonstrated in the field. Strain K194 gave very consistent trends over three seasons where the population of *Meloidogyne* sp. was reduced by over 60 %. Success of growing *Bacillus* sp. and *Rhizobia spp.* in one medium for production of a bio-inoculant was demonstrated and has been packaged.

Suitability of the nematode diversity as bioindicators of land use change/ intensification gradient was demonstrated. As nematode populations change during the growth of a crop, it is desirable, to standardize sampling on a stage of crop growth, this is often the seedbed or immediately after harvest. Nematode populations increased from planting time, peaking at bean flowering then reduced at harvesting time.

High nematode populations were observed in the long rains than the short rains in plots planted with maize. This information indicates that priority ought to be given to plant parasitic nematodes in the long rains when designing pest management programmes in cereals. The significant interactions among soil fertility management practices, time of nematodes sampling and farm age cluster suggest that the populations of soil nematodes is influenced by fertility level, time of sampling and land conversion periods. It is recommended that, soil P and N levels be addressed as direct influence on plant parasitic nematodes. Use of organics, where available, should be recommended to the farmers.

CHAPTER ONE

1.0 INTRODUCTION

Nematodes are multicellular, bilaterally symmetrical, non-segmented worm-like organisms with well developed reproductive and digestive systems but primitive excretory and nervous systems (Coleman and Crossley, 1996). The respiratory and circulatory systems are absent. They are bisexual and undergo four molting stages from egg to adult. Nematodes are present in almost all agro-ecosystems where they interact directly and indirectly with plants and other microfauna, regulating decomposition and release of nutrients to the plants (Coleman *et al.*, 1984; Yeates *et al.*, 1993).

Soil inhabiting nematodes are very small (0.3-0.5mm long as adults) wormlike animals which are very abundant in million M^{-2} and diverse greater than 30 taxa in all soils (Yeates, 1979). Nematodes are a group of organisms of diverse biology. Their lifespans vary from several days to several years. A number of nematode species withstand anaerobic conditions. In polluted soils, nematodes are present after the macrofauna has disappeared and they have a relatively rapid turnover compared to the macrofauna. The short generation time of some nematode species means that the composition of the nematode fauna can react rapidly to disturbances (Lambshead, 1986). Plant-parasitic nematodes are slender, elongated, fusiform, tapering towards both ends and circular in cross section. *Meloidogyne arenaria, M. incognita* and *M. javanica* are the most encountered species in the tropical regions (Netscher and Sikora, 1990). In almost every soil sample, nematodes from five trophic levels namely bacteriovores, fungivores, herbivores, predators and omnivores are represented (Yeates, 1999). Due to their biological diversity and particularly feeding habits, nematodes are an integral part of the food webs in soil ecosystems (Yeates *et al.*, 1993). According to Yeates (1999), nematode diversity is greatest in ecosystems

experiencing long-term human interference. The changes in nematode community may be a reflection of changes in soil and ecological processes. Nutrient enriched soils show a reduced biodiversity and under such conditions the populations of short-lived r-strategists increase relative to other nematode groups. Soil disturbances, such as tillage or use of chemicals alter the structure of the soil ecosystem and discriminate against predatory or omnivorous nematodes, K-strategists, (Ferris and Matute, 2003). The diversity of nematodes in agro-ecosystems and the total abundance of members of different trophic levels are largely controlled by the biophysical, chemical and hydrological conditions of the soil (Yeates and Bongers, 1999). The soil as a habitat for nematodes can therefore be changed through management practices such as monoculture, tillage, drainage, application of agrochemicals, irrigation and organic mulch (Yeates, 1999).Organic mulch tends to increase soil water holding capacity besides improving the soil structure whereas agrochemicals increases soil acidity. Crop yield losses due to root-knot nematodes have been on the increase in the tropics and sub-tropics (Netscher and Sikora, 1990). This has been attributed to replacement of shifting cultivation with continuous cropping systems in almost all subsistence farming, monocropping and narrow rotations in large scale vegetable production especially where irrigation is practiced and poor nematode management due to perception that nematodes are not important crop pests (Bridge, 1996).

Common bean (*Phaseolus vulgaris* L.) is the most important legume, second only to maize as a food crop in Kenya (Gethi *et al.*, 1997). The crop is grown under a wide range of environmental conditions mainly at altitude of between 900-2700 metres above sea level (Acland, 1971). Beans are primarily grown by smallhold growers, mainly intercropped with other crops such as maize, coffee, bananas, sorghum, millet potatoes and cassava (Wortmann, 1998). Beans are the main

source of proteins in Africa and Latin America where diets lack adequate amounts of animal proteins. Beans not only improve soil fertility through biological nitrogen fixation but they also have very high nutritional value due to the low water content. Beans require minimum processing for human consumption and are relatively easy to store (Skerman, 1976). Common bean is attacked by a wide range of plant parasitic nematodes with *Meloidogyne spp.* being the most important (Kimenju *et al.*, 1999). Apart from causing diseases in plants, plant-parasitic nematodes also act as wounding agents and break host resistance to other plant pathogens particularly soil-borne (France and Abawi, 1994). Nodulation potential of leguminous plants is adversely affected by root-knot nematode infection, thus interfering with biological nitrogen fixation (Karanja, 1988). Yield losses of up to 60% have been recorded in beans in fields heavily infested with root-knot nematodes (Bridge, 1996).

The last decade has witnessed increased sensitivity to loss of nematode diversity resulting from; pollution, agricultural intensification, greenhouse effect, modification of global carbon and nitrogen cycles (Asner *et al.*, 1997). The status of belowground biodiversity is however, not conclusively documented and little is known of the effects of land use on nematode diversity especially in the tropical ecosystems. Various strategies including nematicides, cultural practices, organic amendments and resistant varieties have been developed for the management of pathogenic nematodes (Sikora, 1992; Bridge, 1996). Their potential has, however, not been fully exploited due to limited knowledge (Sikora, 1992). Nematicides for instance are too expensive and hence not affordable by small-scale bean producers (Oka *et al.*, 1993). Cultural practices such as fallowing and crop rotation are not practical due to scarcity of arable land but their effectiveness is also interfered with due to the broad host range nature of plant parasitic

nematodes. Organic amendments have successfully been used in the control of nematodes (Sikora, 1992). Despite the efficacy of organic amendments in nematode control, widespread use of this strategy is limited by the large quantities needed for effective control (Luc *et al.*, 1990; Oka *et al.*, 1993). Inorganic fertilizers containing ammoniacal nitrogen or formulations releasing this form of N in the soil are most effective for suppressing nematode populations. Anhydrous ammonia has been shown to reduce soil populations of *Tylenchorhynchus claytoni, Helicotylenchus dihystera,* and *Heterodera glycines* (Rodriguez-Kabana, 1986). The rates required to obtain significant suppression of nematode populations are generally in excess of 150 kg N/ha. Urea also suppresses several nematode species, including *Meloidogyne* spp., when applied at rates above 300 kg N/ha. Additional available carbon must be provided with urea to permit soil microorganisms to metabolize excess N and avoid phytotoxic effects. There is a direct relation between the amount of "protein" N in organic amendments and their effectiveness as nematode population suppressants. Organic soil amendments containing mucopolysaccharides (e.g., mycelial wastes, chitinous matter) are also effective nematode suppressants (Rodriguez-Kabana, 1986). It is therefore, important to continuously seek alternative control strategies that can be used either singly or in combination with nematicides. Biological control is a viable alternative strategy in nematode management (Bridge, 1996; Sikora, 1997). Studies on the effect of *Bacillus spp.* on both nodulation and plant parasitic nematodes control have received little attention, hence the need to address the possibility of using locally isolated indigenous *Bacillus spp.* which is an economically viable method that would enhance nodulation and to improve plant health through nematode control.

1.1 Study objectives

The broad objective was to determine abundance and diversity of nematodes and identify appropriate and sustainable integrated management strategies for plant parasitic nematodes.

Specific objectives:

1. To characterize soil subjected to different periods of cultivation.
2. To assess the effect of soil disturbance on nematode diversity.
3. To evaluate the effect of selected soil fertility management practices on dynamics of nematodes.
4. To assess the efficacy of *Bacillus subtilis* strains in controlling root-knot nematodes on beans (*Phaseoulus vulgaris* L.).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nematode ecology

Nematodes are generally free-living in marine, freshwater and soil environments, but a large number of species are parasitic on different kinds of plants and animals. The factors that influence nematode distributions are; soil, temperatuire, pH, salinity, moisture and vegetation (Boag & Yeates 1998). The parasitic species are of considerable agricultural, clinical and veterinary importance as pests of plants and parasites of man and livestock, respectively (Mai and Lyon, 1975). Nematodes are found at the bottom of lakes, rivers and at enormous depths in the oceans. Some species can withstand temperatures constantly below freezing point while others live in hot springs (Moss and Webster, 1970). The soil nematode fauna has affinities to the freshwater fauna but differs much from the marine fauna (Sohlenius, 1990). The number of coexisting species may vary from 23 in polar areas to about 62 in deciduous forests. Boag & Yeates (1998) reported 6-228 species in grassland and Lawton *et al.* (1996) found 432 morphospecies in 25 sites in a Cameron rainforest.

2.2 General nematode morphology

Nematode morphology is only visible after special procedures for extraction and fixation, and by means of complex instruments of observation. These procedures and instruments completely determine the knowledge of nematode morphology, because they impose a strict limit on the level of details with which we can study nematodes (Hooper, 1969). These procedures may lead to substantial errors through the incorrect interpretation of the artifacts which they can generate (Mayr, 1969). Describing the morphology of nematodes requires a great degree of jargon, because of their diversity and uniqueness, as well as a great degree of caution and training in microscopical observation (Mai and Lyon, 1975). All soil nematodes have a vermiform body tapering more or less strongly towards the anterior and posterior ends, and lacking any

appendages capable of independent movement (Mai and Lyon, 1975). The posterior end is called the tail. The anterior end is usually not referred to as the head, as it is often not clearly offset from the body and because it does not contain the "brain" of the nematode (Hooper, 1969). The anterior end instead, is variously referred to as lip region, cephalic region, or simply anterior end (Hooper, 1969; Mai and Lyon, 1975). An elastic, relatively tough and more or less impermeable cuticle covers the entire body (Hooper, 1969). The only openings on the cuticle are those of the digestive, reproductive and "excretory" systems, as well as those of various sensory and/or secretory organs (Mai and Lyon, 1975).

2.2.1 Mode of Reproduction

There are three main methods of reproduction in nematodes; amphimixis, parthenogenesis and hermaphroditism (Lee, 2002; Gaugler and Anwar, 2004). Amphimixis is also referred to as bisexual reproduction, cross-fertilization or gonochorism (Perry and Wright, 1998). The sexes are separate and the females produce oocytes which are fertilized by the sperms from the males. Well known examples include, *Anguina tritici*, some *Meloidogyne* species and the mycophagous species, *Aphelenchus avenae* (Luc *et al.*, 2005). In amphimixis, the males and females are present in equal numbers and copulation is required and females do not produce sperms (Gaugler and Anwar, 2004).

Parthenogenesis also referred as autotoky is common where males are very rare, absent or non-functional and are not involved in reproduction (Perry and Wright, 1998). Copulation is optional and females reproduce without sperms. There are two main kinds of parthenogenesis; meiotic and mitotic. The two types differ in whether or not a first meiotic division takes place in the oocytes. Meiotic parthenogenesis is in *Rhabditis*, *Meloidogyne*, *Heterodera*, some longidoridae

as well as *Aphelenchus avenae*. Mitotic parthenogenesis occurs in *Helicotylenchus* and *Pratylenchus* (Gaugler and Anwar, 2004).

Hermaphroditism (automixis or self-fertilization) is as in *Caenorhabditis elegans* (Perry and Wright, 1998; Luc *et al.*, 2005), in which a single gonad produces both the oocytes and sperms. This together with amphimixis is common in rhabditids and is reported in other free-living nematodes and plant parasites, including criconematids and in several predatory species. It is indicated by the occurrence of hermaphrodite females whose spermathecae contain sperms in the absence of males in the population. The hermaphrodite is morphologically female but has a syngonic (an ovotestis) usually acting protandrically -sperm produced first (Perry and Wright, 1998).

2.2.2 Life cycle

The life cycles of most plant parasitic nematodes are in general quite similar in that all have four larval stages (Lee, 2002). Eggs may be laid singly or stuck together in masses in a gelatinous matrix secreted by the females (Gaugler and Anwar, 2004). In root-knot nematodes, all the eggs are laid in an egg sac which may be buried partially within the host-derived root gall which *Meloidogyne* spp. induce during feeding. Egg sacs and cysts serve to protect the eggs from desiccation and natural enemies (Luc *et al.*, 2005). The juvenile within the egg develops to adult through four moults. The first moult normally occurs within the egg (Gaugler and Anwar, 2004). The egg develops into a first stage juvenile (J1). The juvenile coils several times within the eggshell and lies still (Perry and Wright, 1998). The J1 grows in size and undergoes the first moult within the egg and then hatches as a J2. The J2 is fully developed except that it lacks reproductive organs and is small in size. The J2 undergoes a second moult and becomes a J3 and

then undergoes a third moult to become a J4. The J4 undergoes a fourth moult and differentiates into adult females and males and then matures (Lee, 2002). A life cycle from egg to adult can be completed within 3-4 weeks under optimum environmental conditions such as temperature of 27^0C and pH of 4.0-8.0. In *Longidorus spp.* the life cycle takes 2 years while in *Ditylenchus dipsaci* it takes 19-23 days (Gaugler and Anwar, 2004). In certain plant nematode species, the parasitic life cycle is synchronized closely with that of the host with the aid of environmental and host derived stimuli, to maximize the reproductive success of the nematode (Lee, 2002; Gaugler and Anwar, 2004). Each egg contains a single juvenile, which hatches by cutting the egg-shell with its stylet by striking it with intermittent rhythmic blows or by rupturing the egg-shell with its tail tip as in *Heterodera iri*, or through normal rupture of the egg-shell due to juvenile enzymatic secretions and movement (Lee, 2002; Luc *et al.*, 2005). The eggs of the cyst nematodes survive in the soil in round (*Globodera*) or lemon-shaped (*Heterodera*) cysts each containing several hundred eggs. There are small openings at the neck and the vulval ends of the cyst through which the hatched juveniles escape (Gaugler and Anwar, 2004). Once hatched the J2s of *Globodera rostochiensis* and *G. pallida* can survive for < 2 weeks without feeding. *Meloidogyne javanica* and *Tylenchulus semipenetrans* can persist in the field for months (Lee, 2002; Gaugler and Anwar, 2004).

2.3 Plant-parasitic nematodes affecting beans

2.3.1 Biology and life cycle

All plant-parasitic nematodes have similar life cycles (Agrios, 1988). When the host, temperature and surroundinsg are unfavorable, females of *Meloidoyne spp.* produce a few eggs or may not produce at all (Agrios, 1988). Under less favorable conditions, such as extreme temperatures, a

single female produces 300- 500 eggs while under optimum temperatures of 27^0C it can produce more than 2800 eggs (Agrios, 1988). Females lay eggs in sac-like gelatinous matrices. A new generation can arise within 25 days but under less favorable conditions, the life cycle may be prolonged to 30 or 40 days or development may cease entirely (Agrios, 1988). During this dormant stage, each egg takes on a thick outer covering to protect it during the inactive period. The first larval stage develops inside the egg and undergoes the first moult within the egg to become second-stage larva. The latter emerges from the egg into the soil where it moves until it finds a susceptible root. Only the second-stage juveniles are active (Agrios, 1988). The J3 lacks a stylet whereas J4 can be distinguished either as male or female while the final moult becomes a free-living male nematode or a parasitic adult female. The importance of temperatures in the life cycle of root-knot nematodes was demonstrated by Agrios (1988). Plant penetration by second-stage juveniles occurs between 10°C and 35°C, with 27°C, being the optimum depending on the species.

Many plant-parasitic nematodes have been associated with leguminous crops (Mani *et al.*, 1982). Root-knot nematodes (*Meloidogyne spp.*), lesion nematodes (*Pratylenchus spp., Tylenchus spp., Criconemella spp., Aphelenchus spp.*, sheath nematodes (*Hemicycliophora spp.*), stubby root nematodes (*Trichodorus spp.*) and others are associated with beans (Kimenju *et al.*, 1999). *Meloidogyne* spp. are of considerable importance due to their wide distribution, especially in the warm regions of the world coupled with their polyphagus nature (Luc *et al.*, 1990).

2.3.2 Nematode pathology

Plant parasitic nematodes (PPNs) are biotrophic parasites which obtain nutrients from the cytoplasm of living root, stem and leaf cells for development, growth and survival (Gaugler and Anwar, 2004; Luc *et al.*, 2005). Nematodes have evolved diverse parasitic strategies and feeding relationships with their host plants (Perry and Wright, 1998). They possess a hollow and a protrusible feeding structure, the stylet and a pharynx, which has undergone morphological and physiological adaptations to suit the feeding relationships (Lee, 2002; Gaugler and Anwar, 2004). Depending on the species, they feed from the cytoplasm of unmodified living plant cells or have evolved to modify root cells into elaborate feeding cells as in root knot nematodes (Lee, 2002; Luc *et al.*, 2005). The nematodes use their stylet to pierce and penetrate the cell wall of a plant cell, inject gland secretions through the stylet orifice into the cell and withdraw and ingest nutrients from the cytoplasm (Perry and Wright, 1998). Nematodes that enter root tissue also use their stylet to cut openings and/or inject secretions to dissolve (intracellular migration) or weaken (intercellular migration) the cell wall or middle lamella (Lee, 2002; Gaugler and Anwar, 2004).

Generally, all PPNs damage plants by direct mechanical injury using the stylet during penetration and/or by secretion of enzymes into the plant cells while the nematode is feeding (Gaugler and Anwar, 2004). The physical presence of endoparasitic nematodes inside the host also affects the functioning of the host. As a result of nematode feeding, the architecture and extent of the root system is altered, so that it is less efficient at taking up nutrients and water from soil (Lee, 2002). The extent of nematode damage depends to a large extent on the inoculum density (level of infestation). Low or moderate numbers of nematodes may not cause much injury but large numbers severely damage or kill their hosts (Luc *et al.*, 2005).

2.4 Root-knot nematodes *(Meloidogyne spp.)*

2.4.1 Classification of root-knot nematodes

Root-knot nematodes belong to the kingdom; *Animalia,* phylum; *Nematoda,* class; *Nemata,* subclass; *Sercenentea,* order; *Tylnchida,* suborder; *Tylenchina,* family; *Meloidogynidae,* and genus; *Meloidogyne* (Chitwood, 1956). There are 51 species of *Meloidogyne* (Jepson, 1987), of which *M.incognita* (Chitwood, 1956), *M. javanica* (Chitwood, 1956), *M. arenaria* and *M. hapla* (Chitwood, 1956) are of economic importance in bean production across the world (Luc *et al.,* 1990). Root-knot nematode populations consist of male and female, which are easily distinguished morphologically. The males are wormlike and are about 1.20 - 1.50 mm long and 30 - 60µm in diameter (body width). Mature females are pear shaped and about are 0.40 - 1.30 mm long by 0.27 - 0.75 mm in diameter. Second-stage juveniles are vermiform in shape while third and fourth stage juveniles are sausage shaped and microscopic in size (Agrios, 1988).

2.4.2 Effect of root-knot nematode infection on nodulation of bean roots

Root knot nematodes establish a feeding site in the vascular bundle inside the nodule and induce formation of giant cells that lead to premature senescence of the nodules (Vones *et al.,* 1998). Root-knot nematodes also affect nodulation through competition for ecological niches and nutrients and suppression of lateral root formation thus reducing sites for nodule formation. This leads to early degradation of nodules due to their infection (Taha, 1993). Besides beans, the main leguminous crop cultivated in association with maize in Western Kenya, being affected by these nematodes, *S. sesban* and *T. vogelii* are also good hosts for *Meloidogyne spp.* (Faridah and Van der Maesen, 1997) and heavy root infestations have been observed in the area (Desaeger and Rao 1999).

2.5 Effect of agricultural intensification on nematodes

Agricultural intensification is frequently associated with increased disturbance of the soil through tillage, indiscriminate use of mineral fertilizers and pesticides, manipulation of organic residues and planting of a narrow range of plant genotypes or complete monotypes (Yeates *et al.*, 1999). These attributes inevitably interfere, in the long run, with the functions of any ecosystem (Giller *et al.*, 1997). Among other fundamental ecosystems' functions, biological control of pests and diseases such as plant-parasitic nematodes is disrupted leading to population build-up. The decrease in diversity with increasing intensity of management is attributed to physical disturbance, change in quantity and quality of organic matter returned to the soil and increase in numbers of specific plant feeding nematodes that are favored by the crops selected (Yeates and Bongers, 1999).

2.6 Effect of soil on nematodes

Soil texture and structure, which are directly related to water holding capacity and aeration, influence nematode survival, egg hatching and disease severity (Netscher and Sikora, 1990). Soil type and soil pH have been found to influence nematodes distribution (Taylor *et al.*, 1982). Most *Meloidogyne species* survive and reproduce at pH levels ranging from 4.0 to 8.0. Soil type influences the type of crops grown; this in turn affects nematode distribution, population build-up and crop damage intensity (Prot and Van Gundy, 1981).

2.7 Influence of organic and inorganic fertilizers on nematodes

Free-living nematodes may accelerate the decomposition of soil organic matter (Abrams and Mitchell, 1980). Numbers of free-living nematodes increase rapidly in the soil following the addition of the inorganic fertilizers (Marshall, 1977). Increase in plant-pathogenic nematodes

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with increased levels of potassium application has been reported (Badra and Yousif, 1979). It appears that pest and disease control in amended soil is the product of several mechanisms operating through their effects on soil, host plant and the pathogen (Akhtar and Alam, 1993). The mechanisms may include increased activity of nematode antagonists, accumulation of deleterious decomposition end products and microbial metabolites and increased disease resistance and tolerance (Akhtar and Alam, 1993). Man has added organic and inorganic amendments to soil for centuries to improve soil fertility and increase crop yield. The nematicidal effect of some of these amendments has been recognized for some time, and reviews on the subject have been published (Muller and Gooch, 1982).

Other researchers examined different forms of nitrogen to determine their relative effectiveness against nematodes. Eno *et al.*, (1955) demonstrated the effectiveness of anhydrous ammonia in field soil infested with species of *Hoplolaimus, Criconemoides, Trichodorus,* and *Belonolaimus.* More recently, Rodrlguez-Kabana *et al.,* (1982) reexamined the nematicidal properties of anhydrous ammonia. In greenhouse studies, ammonia reduced soil populations of *Tylenchorhynchus claytoni* and *Helicotylenchus dihystera* when applied at rates of 62 mg N/kg soil or higher; root populations of *H. dihystera* or of *Hoplolaimus galeatus* were reduced only with rates of 125 mg N/kg soil. In three field experiments with soybean *(Glycine max),* planting time applications of anhydrous ammonia at rates of 0-224 kg N/ha were relatively ineffective in reducing late-season juvenile population densities of *Meloidogyne arenaria* (Neal) Chitwood, although significant yield increases were obtained in one experiment in response to the treatments. In another field experiment, ammonia at 56 and 112 kg/ha reduced population densities of juveniles of *Heterodera glycines* in soil samples collected 14 days after planting.

These field experiments also demonstrated that planting time applications of ethylene dibromide (4.7-18.6 liters/ha) together with anhydrous ammonia (56 or 112 kg N/ha) resulted in a soybean yield increase and accompanying control of M. *arenaria* and *H. glycines* superior to that obtained when each chemical was applied singly. Similar results were also obtained with combinations of ammonia and 1,3-dichloropropene in other soybean field experiments for control of *M. arenaria* and M. *incognita* Rodrlguez-Kabana *et al.*, (1982). Rodrlguez-Kabana *et al.*, (1981) were in agreement with Vassalo (1968) in attributing the nematicidal properties of anhydrous ammonia principally to its plasmolysing effect in the immediate vicinity of its application point in the soil; however, their data on the effectiveness of ammonia against *H. glycines* also suggested other mechanisms were operating. They believed it possible that ammonia could exert a selective influence for microbial antagonists of *H. glycines*, particularly fungi (Ownley *et al.*, 1983). It was reasoned that since NH~+-N is the preferred source of N for many soil fungi (Cochrane, 1958), some fungal parasites of *H. glycines* could have increased in numbers following applications of NH3 to soil. Proliferation of such fungal parasites in turn could have resulted in the observed reductions in *H. glycines* juvenile populations. Walker (1971), studied organic--peptone, soybean meal, skim milk, urea--and inorganic-- KNO3, (NH4)~SO4, (NH4)2CO3, NH4OH--nitrogen sources and found that ammoniacal and organic nitrogen sources were more detrimental to nematodes than nitrate. Other common fertilizers also have been studied, and findings generally indicate that those containing ammoniacal nitrogen are more damaging to nematodes than those with nitrate nitrogen (Badra and Khattab, 1980). Since ammoniacal nitrogen is detrimental to nematodes, urea has been studied as a nematicide. The compound is readily converted to ammonia by urease present in the soil, a necessary conversion if urea is to be effective both as a fertilizer and as a nematicide.

2.8 Impact of land use change and farming systems on nematodes

Any crop husbandry can result in a rise or fall in nematode population levels in a site (McSorley, 2001). Cropping systems such as continuous monoculture, continuous cropping, various forms of fallow, crop rotation, polycultures and mixed cultivars are particularly important because they influence microbial communities in the soil. In multiple cropping systems, the individual crops may be arranged in time (crop sequences), in space (intercropping), or in various combinations of time and space. A good cover crop provides a good niche for nematode antagonistic fauna and flora (Caswell *et al.*, 1990). Fallow period with no susceptible plants for a period of about two years, can significantly reduce the population size of the plant parasitic nematodes. This is because nematodes are not able to grow and reproduce in the absence of host plant (Bridge, 1996). Plants play both direct and indirect roles in structuring of the nematode communities. This is because nematodes are heterotrophs and therefore ultimately depend on autotrophs such as higher plants (Yeates, 1999). Consequently, different land use types result in different types of plant community structures and ultimately in different decomposition and nutrient cycling pathways (Cadish and Giller, 1997). Rhizosphere processes link plants to the soil, and root-feeding nematodes are known to increase the supply of carbon from roots to the soil microbial biomass (Yeates, 1999).

2.9 Management strategies for plant-parasitic nematodes

Several methods, such as use of chemicals, resistant plants, physical methods, biological agents, use of antagonistic and trap crops and cultural methods are available for the control of plant parasitic nematodes (Dropkins, 1988). Their use is limited by; cost, crops, nematode species, arable land, market preferences for certain crops and environmental factors.

2.9.1 Chemical control

Use of chemicals is justifiable when other methods fail to sufficiently suppress plant-parasitic nematode populations (Oka *et al.*, 1987). Nematicides used in control of root-knot nematodes are either fumigants or non-fumigants (Ware, 1983). The fumigants are usually in liquid form and enter the soil solution in a gas phase while non-fumigants are water soluble granules or liquid compounds (Bridge, 1996). Environmental toxicity problem associated with nematicide use is relatively serious more so the chlorinated and bromated fumigants (Bridge, 1996). Their use is rapidly declining, primarily due to their costs, health and environmental considerations.

2.9.2 Plant resistance

Use of resistant varieties is perhaps the best method of controlling root-knot nematodes, (Bridge, 1996). Resistance to *Meloidogyne species* may be due to failure of larvae to penetrate plant roots or to reproduce after penetration and feeding have taken place (Madumadu, 1979). However, these plant varieties are usually resistant to only one or two species of *Meloidogyne*. This method therefore, is limited to situations in which one or perhaps two *Meloidogyne* species are present. Resistance may not provide protection against even one species, since numerous intraspecific races and biotypes are known to exist in nature (Omwega *et al.*, 1990). Use of resistant varieties is limited by high number of races attacking different plant varieties, unavailability of resistant materials to farmers and breakdown of resistance after a few years of use (Ngundo, 1977). Plant parasitic nematodes often interact with other soil pathogens, causing more plant damage than either pathogen would cause alone, thus rendering plant resistance ineffective (Bridge, 1996). In some cases, resistance is incomplete meaning that the nematode levels will build up if host plants

are grown but their numbers will increase more slowly than if a susceptible plant is grown (Omwega *et al.*, 1990).

2.9.3 Physical control

Root-knot nematodes have been controlled through flooding and also by soil solarisation (Gaur and Perry, 1991). *Meloidogyne* densities drop significantly when soils are flooded for prolonged periods of time such as three months (Stover, 1979). Soil solarisation has been used to raise temperatures to lethal levels to control root-knot nematodes and other diseases (Gaur and Perry, 1991). However, this technique is only viable in regions where sufficient solar energy is available for sufficiently long periods. Application of this form of control is limited by such factors as terrain, time and availability of water.

2.9.4 Cultural methods

Cultural methods attempt to adopt husbandry practices so as to minimize the effects of nematodes (Madumadu, 1979). These include crop rotations, quarantine, fallowing and use of organic amendments. Crop rotation systems have been developed to make full use of crops, maintain soil fertility and reduce build-up of pests and diseases (Bridge, 1996). The major constraints to the use of crop rotation is the broad host-range of many economically important nematodes, occurrences of several nematode species in a given field, lack of resistant or tolerant cultivars, lack of agronomically adopted cultivars and limitation of land (Bridge, 1996).

2.9.5 Use of antagonistic and trap crops

While marigolds (*Tagetes* species) are typically grown for ornamental purposes as bedding plants, studies have found that they can be highly toxic to plant-parasitic nematodes and are capable of suppressing a wide range (up to 14 genera) of nematode pests. The nematicidal potential varies with the marigold species and cultivar, (Koon-Hui *et al.*, 2007). The marigold species most often used for nematode control are *Tagetes patula*, *T. erecta*, and *T. minuta*. The key mode by which marigolds suppress plant-parasitic nematodes is through a biochemical interaction known as allelopathy. Allelopathy is a phenomenon where a plant releases compounds that are toxic to other plants, microorganisms, or other organisms, such as nematodes (Hooks *et al.*, 2006). Marigold plants produce a number of potentially bioactive compounds, among which α-therthienyl is recognized as one of the most toxic. This sulfur-containing compound is abundant in marigold tissues, including roots. It has nematicidal, insecticidal, fungicidal, antiviral, and cytotoxic activities, and it is believed to be the main compound responsible for the nematicidal activity of marigold. Thus nematodes may be killed either by entering the root system of a marigold plant or contacting soil containing marigold's bioactive compounds (Ploeg, 2002). Nematicidal compounds apparently permeate from marigolds' root tissues into nematodes attached to the root, but they are also believed to kill nematodes found in the rhizosphere, the soil near marigold roots. Thus, marigold is believed to be most effective in suppressing plant-parasitic nematodes when actively growing, but it is not as effective when incorporated as crop residues or root extracts (Ploeg, 2002). Several other plants with nematicidal properties, including sunn hemp (*Crotalaria juncea*), are believed to release nematicidal compounds when incorporated into the soil and thus do not require root penetration to effectively kill nematodes. Some researchers believe that marigold root exudates prevent the

nematodes from developing and their eggs from hatching. Another marigold species, *T. erecta* behaves as a trap crop: root knot nematodes are attracted to and enter its roots, but the development of their offspring is impeded. In other cases, marigolds may behave as a trap crop by allowing penetration of nematodes but inhibiting their subsequent development and reproduction (Koon-Hui *et al.*, 2007).

2.9.6 Biological control of nematodes

Biological control may be defined as the reduction of inoculum or disease producing capacity of a pathogen accomplished through one or more organisms other than man (Baker and Cook, 1974). Control of soil borne pathogens especially nematodes has been achieved by use of their natural enemies residing in the soil which act through such mechanisms as parasitism, predation, competition and antibiosis (Sikora, 1992). Several fungi, bacteria and nematophagus nematodes have been used in the control of plant-parasitic nematodes (Mankau, 1995). Plant health promoting rhizobacteria (PHPR) that reduce plant infection and stimulate plant growth have been widely investigated for practical use (Kloepper and Schroth, 1981). The ability of rhizobacteria, especially *Bacillus spp.* and fluorescent *Pseudomonas* spp. to improve plant growth and/or health has been demonstrated (Becker *et al.*, 1988; Weller, 1988; Oostendorp and Sikora, 1990). This leads to improved nutrient uptake, enhanced atmospheric nitrogen fixation, induced disease resistance, competition for nutrients and/or niches, parasitism or alteration of chemical components of root exudates (Sikora, 1992; Sikora and Hoffmann-Hergarten, 1992). *Bacillus spp.* was reported to improve nodulation in leguminous plants (Srinivasan *et al.*, 1996). Their use in nematode control would be an added advantage as this would lead to increased nodulation. Rhizobacteria are particularly desirable biocontrol agents because they are able to colonize plant roots and can be applied as seed treatment making them cost effective (Sikora, 1995). Many

natural enemies attack plant parasitic nematodes in the soil and reduce their populations. They include bacteria, fungi, protozoa, tardigrades, mites, and insects (Brown and Kerry, 1987; Nickle, 1991). It is important to determine the nature and extent of such attacks on nematode multiplication in order to establish whether these enemies can be exploited to reduce damage and increase crop yield (Nickle, 1991). Two types of biological control are (i) induced, where the biological control agents are applied by man, and (ii) natural, where indigenous agents suppress nematode multiplication without being specifically introduced (Sikora, 1992).

2.9.6.1 Success stories on use of biological controls

Biological controls do not have negative impacts on biodiversity (Corry and Myres, 2000). Biological control is now being considered for an increasing number of crops and managed ecosystems as the primary method of pest control. One reason for its growing popularity is its record of safety during the past 100 years considered as the era of modem biological control (Waage and Greathead, 1988). No microorganism or beneficial insect deliberately introduced or manipulated for biological control purposes has, itself, become a pest so far as can be determined. There is no evidence so far of measurable or even negligible negative effects of biocontrol agents on the environment (Cook and Chairman, 1987). Another reason for considering biological control over other methods is untapped potential. Biological control is underused, underexploited, underestimated and often untried and therefore unproven. There are two commercial bionematicidal agents based on *Bacillus* species. Through a PGPR research program of the ARS (Agriculture Research Service, USA), a commercial transplant mix (Bio YieldTM, Gustafson LLC) containing *Paenobacillus macerans* and *Bacillus amyloliquefaciens* has been developed to control plant-parasitic nematodes on tomato, bell pepper and strawberry

(Meyer, 2003). Another product, used in Israel, is BioNem, which contains 3% lyophilized *Bacillus firmus* spores and 97% nontoxic additives (plant and animal extracts) to control root-knot nematodes as well as other nematodes (Giannakou & Prophetou-Athanasiadou, 2004).

2.9.6.2 Positive aspects of *Bacillus subtilis*

Microbial growth and survival in an ecosystem are dependent on abiotic factors such as pH, water potential, nutrient availability (Stotzky and Burns, 1982) and biotic factors such as predation, bacteriostasis (Rissler, 1984). *Bacillus subtilis* is known to inhibit penetration of nematodes into plant roots thus reducing root galling (Rao, *et al.*, 2000). Reduction of infection and suppression of development by plant parasitic nematodes in the plants by several *Bacillus sp.* is due to production of toxic or inhibitory metabolites (Mankau, 1995). Oosterndorp and Sikora (1990) reported that presence of *Bacillus spp.* in the rhizosphere caused modification of root exudates thus affecting nematode attraction or recognition of the host. Several biocontrol strains are known to produce multiple antibiotics that can suppress one or more pathogens. For example, *Bacillus cereus* strain UW85 is known to produce both zwittermycin (Silo-Suh *et al.*, 1994) and kanosamine (Milner *et al.*, 1996). The ability to produce multiple classes of antibiotics, that differentially inhibit different pathogens, is likely to enhance biological control.

2.9.6.3 Mode of action of *Bacillus subtilis*

The major modes of action include alteration of root exudates, production of toxic metabolites and reducing the activity of egg hatching factors (Sikora and Hoffmann-Hergarten, 1993). Some bacteria (e.g. *Bacillus spp.*, *Fluorescent Pseudomonas* and *Telluria chitinolytica*) have been shown to inhibit penetration of nematodes into the roots thereby reducing root galling

(Oostendorp and Sikora, 1990). These bacteria may interfere with host identification through receptor blockage on the roots or by modifying root exudates of the host plant. This hinders the attraction, hatching or penetration behaviour of nematodes (Oostendorp and Sikora, 1990). Metabolites produced by *Bacillus thuringiensis Berliner* and *Bacillus subtilis Ernberg* are known to be toxic to *Meloidogyne* spp. (Oostendorp and Sikora, 1990). The metabolites produced by *Bacillus* spp. include bactracin, circulins, polymyxins, tyrocidins and surfactin (Brandbury, 1986). Presence of *Bacillus* spp in the rhizosphere is known to modify root exudates affecting nematode attraction to or recognition of the host (Oosterndorp and Sikora, 1990). Aerobic endospore-forming bacteria (AEFB) (mainly *Bacillus spp.*) and *Pseudomonas spp.* are among the dominant populations in the rhizosphere that are able to antagonize nematodes (Krebs *et al.*, 1998). Numerous *Bacillus* strains can suppress pests and pathogens of plants and promote plant growth. Some species are pathogens of nematodes (Li *et al.*, 2005). The most thoroughly studied is probably *Bacillus subtilis* (Siddiqui, 2002). In addition, a number of studies have reported direct antagonism by other *Bacillus spp.* towards plant-parasitic nematode species belonging to the genera; *Meloidogyne, Heterodera* and *Rotylenchulus* (Meyer, 2003).

The rhizobacteria usually comprise a complex assemblage of species with many different modes of action in the soil (Siddiqui, 2002). Rhizobacteria reduce nematode populations mainly by regulating nematode behaviour (Sikora & Hoffmann-Hergarten, 1993), and/or interfering with plant–nematode recognition (Oostendorp & Sikora, 1990). Similarly by competing for essential nutrients (Oostendorp and Sikora, 1990). Also by promoting plant growth (El-Nagdi & Youssef, 2004) or inducing systemic resistance (Hasky-G¨unther *et al.*, 1998) or directly antagonizing by means of the production of toxins, enzymes and other metabolic products (Siddiqui, 2002). Most rhizobacteria act against plant-parasitic nematodes by means of metabolic by-products, enzymes

and toxins. The effects of these toxins include the suppression of nematode reproduction, egg hatching and juvenile survival, as well as direct killing of nematodes (Siddiqui, 2002).

2.9.6.4 Adhesives and carriers used in *Bacillus* spp. formulations

Rhizobacteria (e.g. *Bacillus spp.*) have unique advantage of biocontrol agents since they can be pelleted onto seed, applied through drip irrigation systems or directly applied to transplants (Sikora and Hoffmann-Hergarten, 1992). Several materials such as sucrose, polyvinyl alcohol and methylcellulose have been used for bacteria adhesion onto seed (Racke and Sikora, 1992). Gum Arabica was found to be the best protector of cell against biotic stress hence increasing survival of bacterial cells on the seed (Rodriguez-Navorro *et al.*, 1991). Direct application of a biocontrol agent to seeds or other plant parts gives it competitive advantage over the pathogen (Oostendorp and Sikora, 1989), by reducing production cost and simplifying formulation and application (Sikora, 1997). Soil amendments such as peat, cotton seed cake, bacto peptone clandosan, polymer gel have been used but with varying effects as carriers for rhizobacteria on nematode control (Oka *et al.* 1993). Peat was found to considerably increase bacteria population on seed than those in granular formation (Xi k Stephens and Verma, 1996).

2.9.6.5 Limitations of biocontrols

The efficacy of biological control agents is influenced by the environment (Brown and Kerry, 1987). Each agent has its own optimum conditions particularly of pH, temperature and moisture. The biological control is influenced by the multiplication rate of the nematode and by the time nematode is exposed to the antagonist (Stirling, 1991). Endoparasitic nematodes are relatively harder to control by biological means as they spend a great part of their lives inside the roots

(Gaugler and Anwar, 2004). The major restriction on the development of effective biological control of nematodes is the large bulk of soil that must be treated to ensure contact between host and agent (Brown and Kerry, 1987). Efforts to acquire sustained biological control in the field have been limited by the fact that soil is a powerful buffer (Kerry, 1987).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The study was conducted in farmers' fields in Kakamega district within a radius of 0-5 km from the forest in Lurambi division. Soils were sampled from farms having varying history of cultivation. The farms were grouped into four clusters:

1 Farm in Lusero village had been cultivated for 5 years

2 Farm in Mutsami area had been cultivated for 15 years

3 Musisi area had a farm that had been cultivated for 30 years

4 The farms in Ivakale had been under cultivation for 55 years

The area lies between latitude: 0^{o} 10' N and 0^{o} 21' N longitude: 34^{o} 47' E and 34^{o} 58' E. The altitude ranges between 1500 m and 1600 m above sea level. Mean annual temperature ranges between 18-27^{o}C with an annual precipitation of 2080 mm (Kenya Soil survey 2004). The area has a bimodal rainfall pattern, with peaks in April (long rains) and November (short rains). The soils are predominantly Luvisols and Lixisols (FAO-UNESCO, 1992). Geologically, the soils are associated with Kavirondian sediments (mudstones) and granite. The soils are moderately acidic (pH 5.0-5.9) with predominantly clay texture (Siderius, 1976).

3.2 Experiment 1: Effects of selected soil fertility management practices on nematodes' dynamics

Selected sampling points in the croplands were used for soil sampling. Sampling was done on farms of different periods of cultivation ranging from 0 – 100 years which were clustered in four as follows: 0 – 10 years Lusero, 10 – 20 years Mutsami, 20 – 40years Musisi and over 40 years

Ivakale. The bestbet technologies were chosen after the macrofauna survey. These were picked based on what local farmers use.The four on farm fertility improvement treatments were; Farm Yard Manure at 5 t ha^{-1}, PRE-PAC at 800 Kg ha^{-1} rock phosphate plus 80 Kg ha^{-1} urea and N: P: K to supply 75 Kg N ha^{-1}, 26 Kg P ha^{-1} and 46 K Kg ha^{-1}. Untreated plots acted as controls. Plots were 5 m x 5 m in size with 1m guard rows. The test crop was Maize Western variety, which was spaced at 75 cm x 25 cm giving a population of 53,334 plants ha^{-1}. Cultivation and pest control were done according to the local practice.

3.2.1 Experimental design

A randomized complete block design was used replicated four-times. The soil fertility management practices treatments formed the main plots with the location (farms of different periods of cultivation) making the subplots.

3.2.2 Soil sampling

Soil samples for nematode extraction were taken at; prior to planting, six weeks after germination, milk stage and harvesting following the technique described by Hooper (1990). In each plot, a soil auger (5 cm diameter) was used to collect sample from five evenly distributed sites in each plot from a 5-30 cm depth. The five cores were mixed to form a composite sample. The samples were placed in paper bags, sealed and stored in an insulated box for transportation to the laboratory where they were stored at 4°C awaiting nematode extraction. Nematodes were extracted using the floatation and sieving methods described by Flegg and Hooper (1990), at the Nematology Laboratory, University of Nairobi. Nematodes were identified to the genus level using an identification key and descriptions by Mai and Lyon (1975). The abundance of

nematodes was expressed as number of individuals per $100cm^3$ of soil.

3.3 Experiment 2: Efficacy of *Bacillus subtilis* as a biocontrol of *Meloidogyne* spp. on bean roots and dynamics of nematodes in the treated soils

Parallel experiments were set up in the farms. Three *Bacillus subtilis* strains namely; K158, K194 and K263 were used singly or in combination with *Rhizobium Leguminosarum biovar phaseoli* strain USDA 2674. The Rose Coco beans were inoculated with the bacteria strains following the procedure for applying Rhizobia inoculants developed by the MIRCEN Programme, University of Nairobi. Each isolate was applied aseptically using 70% alcohol to avoid mixing. Serial dilutions of *Bacillus* isolates and *Bacillus* + *Rhizobium* were made and 30ml of $10^{-6}cfml^{-1}$ dilutions inoculated onto 15g of sterilized Ondiri peat that was then used to dress the bean seeds using gum arabica as a sticker before the seeds were planted in each plot (Oka *et al.*, 1993). Control plots were included.

3.3.1 Experimental design

Plots measuring 2 m x 2 m and separated by 1 m wide paths were established in a randomized complete block design, replicated four-times. The bean seeds were spaced at 20 cm x 20 cm giving a plant population of 100 plants per plot. The treatments were;

1. *Bacillus subtilis* strain K194 (K194)
2. *Bacillus subtilis* strain K158 (K158)
3. *Bacillus subtilis* strain K263 (K263)
4. *Rhizobium Leguminosarum biovar phaseoli* strain USDA 2674 (USDA 2674)

5. *Bacillus subtilis* strain K194 + *Rhizobium Leguminosarum biovar phaseoli* strain USDA 2674 (K194 + USDA 2674)
6. *Bacillus subtilis* strain K158 + *Rhizobium Leguminosarum biovar phaseoli* strain USDA 2674 (K158 + USDA 2674)
7. *Bacillus subtilis* strain K263 + *Rhizobium Leguminosarum biovar phaseoli* strain USDA 2674 (K263 +USDA 2674)
8. Control (no *Bacillus subtilis* isolate)

3.3.2 Soil sampling for nematodes

Soil sampling for nematode determination was done prior to planting, six weeks after germination, at 60-75% flowering and during harvesting of beans using the technique described by Hooper (1990). Nematode counts and identification were done as described in section 3.2.2.

3.3.3 Nodule number and dry matter determination

Three plants were randomly selected from each plot and carefully dug out at 70% flowering stage, 45 days after emergence. The plants were separated into shoots and roots. The roots were dipped in bucket of water to remove the soil. The roots with undisturbed nodules were placed in labeled plastic bags and then taken to the laboratory. Nodules were manually removed from the roots and their numbers recorded for each plant. The shoots and roots, which were placed separate paper bags, were oven-dried at 70^{O}C for 48 hours for dry weight determination.

3.3.4 Yield and yield components assessment

At pod maturity, ten plants were randomly selected from each plot and tagged. Pods were harvested and placed in paper bags. The harvested pods from the sampled plants were shelled and seeds counted for each plant. The average numbers of seeds per plant/plot were obtained. The final grain yield was determined by weighing all the seeds from the sampled plants and converting the yield into kg ha^{-1}.

3.3.5 Enumeration of *Bacillus subtilis* and *Rhizobia* populations from soil

Bacillus subtilis were enumerated using Most Probable Number (MPN) and Plate counts following the procedure described by Zuberer (1994). Enumeration of *Bacillus subtilis* at levels ranging from 10^4 to 10^6 cells g^{-1} of soil was done. Microbial cultures from each dilution were spread onto YEM broth. Plates were incubated at 30°C for 4 days before enumeration.

3.4 Diversity determination

The Shannon-Wiener index (Yeates, 2003) was used to assess nematode diversity. The indices were calculated as follows:

diversity $H' = -\sum_{i=1}^{s} P_i \log P_i$ ---------- (Equation 1)

dominance $\lambda = \Sigma P_i^2$ ----------(Equation 2)

diversity $H_2 = -\log_e \lambda$ ----------(Equation 3)

evenness $J' = \frac{H_2}{H' \max}$ where $H' \max = \log_e s$ ----------(Equation 4)

richness $SR = \frac{S-1}{\log_e N}$ ----------(Equation 5)

Where; N, the number of individuals identified, (abundance),

S, the number of taxa identified, i.e. genera,

P_i the proportion of individuals in the ith taxon,

H estimates the probability of correctly predicting the species of an individual randomly drawn from the population. H confounds the number of species and the evenness.

3.5 Data analysis

The nematode counts, *Bacillus subtilis* counts, bean nodules and crop yield data were entered into a Microsoft excel spread sheet and then subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat (Lawes Agricultural Trust Rothamsted Experimental Station, 1998, version 8). The differences among the treatment means were compared using Fisher's Protected LSD test at 5% probability level. Data collected for soil characteristics were entered into an excel spreadsheet and subjected to multivariate analysis of an unbalanced design using GenStat regression and GLM SAS version 8.0. (2005).

CHAPTER FOUR

4.0 RESULTS

4.1 Soil characteristics as influenced by length of disturbance

The soil pH, exchangeable calcium, magnesium, potassium, carbon, nitrogen and carbon: nitrogen ratio of the soils are shown in table 1 and were significantly ($P<0.05$) different among the land use conversion periods. The soil pH tended to acidity with land use conversion period as P content declined before increasing in the 20-40 year farm (Table 1). All parameters except P showed significant differences with the length of disturbance. Exchangeable magnesium was lowest in the >40 years farm as exchangeable Ca and pH showed no clear trend, while there were consistent decreases in total C and total N along the chronosequence. The C: N ratio increased from forest to farms 20 years where a decrease is experienced before an increment in farms cultivated for 20 years and over. Two groups are definite in all elements, forest to 20 years old and >20 years old farms where soil fertility decline eminently.

Table 1. Properties of soil under varying land coversion periods.

LUS/Age cluster	pH	ExCa	ExMg	ExK	ExP	C	N	C:N
Nf	6.8^{a}	13.07^{a}	2.11^{b}	0.34^{abc}	3.76^{a}	3.77^{a}	0.39^{a}	9.68^{c}
Sf	6.3^{ab}	8.39^{b}	3.29^{a}	0.31^{bc}	3.12^{a}	3.39^{a}	0.32^{a}	10.40^{c}
1-10 yrs	5.9^{bc}	10.53^{ab}	2.22^{b}	0.39^{ab}	2.02^{a}	3.77^{a}	0.32^{a}	12.31^{b}
10-20 yrs	5.3^{c}	11.00^{ab}	1.52^{bc}	0.48^{a}	3.55^{a}	3.58^{a}	0.34^{a}	10.56^{c}
20-40 yrs	5.7^{bc}	5.30^{c}	0.80^{c}	0.19^{c}	6.66^{a}	1.73^{b}	0.13^{b}	13.66^{a}
>40 yrs	5.7^{bc}	4.50^{c}	0.74^{c}	0.23^{bc}	3.33^{a}	1.51^{b}	0.11^{b}	13.79^{a}
P	0.0353	<. 0001	<. 0001	0.0005	0.4878	<. 0001	<. 0001	<. 0001
$LSD_{(p=0.05)}$	0.837	2.674	1.014	0.164	4.216	0.939	0.804	1.243
CV%	14.53	30.12	56.80	47.37	103.06	26.79	27.90	10.50

Means with the same letter in column are not significantly different

LUS – Land use system, Sf – Secondary forest, Nf – Natural forest

4.2 Effect of selected soil fertility management practices on dynamics of nematodes

4.2.1. Moisture content in soils treated with various soil fertility management practices

Soil moisture content was significantly ($P<0.05$) influenced by the treatments (Table 2). Addition of manure increase moisture content more than other interventions while PRE-PAC influenced the amount of moisture in soil during the long rains. In both seasons, the trend for moisture content was in the order: farm yard manure>nitrogen fertilizer >control>PRE-PAC.

Table 2. Percent moisture content during the short rains of 2005 and long rains of 2006 prior to planting.

Soil Fertility Management Practices	Short rains	Long rains
Control	8.19[b]	13.43[b]
FYM	8.60[a]	13.87[a]
Nitrogen	8.23[b]	13.76[ab]
PRE-PAC	7.88[b]	12.91[c]
$LSD_{(p=0.05)}$	0.36	0.42
C.V (%)	12.41	9.01

Means with the same letter in column are not significantly different

4.2.2 Diversity of nematodes

Addition of nitrogenous fertilizer resulted in higher number of nematode abundance whereas PRE-PAC had least numbers of nematode genera and abundance in both seasons (Table 3). The Shannon-Wiener diversity index- H_2 was highest in PRE-PAC (2.55) and in the nitrogen fertilizer (5.56) while being lowest in the control (2.42) and farm yard manure (2.40) treatments in two seasons. Species richness (SR) was highest in PRE-PAC and lowest in the nitrogenous fertilizer treatments in both seasons.

Table 3. Comparison of nematode abundance (number/100cm³) and diversity under various soil fertility management practices.

Soil Fertility Management Practices	N		S		SR		H_2		J	
	SR	LR	SR	LR	SR	LR	SR	LR	SR	LR
Control	307	300	24	24	4.02	4.03	2.49	2.42	0.78	0.76
Nitrogen	407	500	24	24	3.83	3.70	2.48	2.56	0.78	0.81
FYM	351	388	23	24	3.75	3.86	2.40	2.46	0.77	0.77
PRE-PAC	251	252	24	24	4.16	4.16	2.55	2.46	0.80	0.77

H_2 the Shannon–Weiner index (Yeates 2003)
Where N=Nematode abundance S = number of genera SR= Species richness
H_2=Diversity J=Species evenness
FYM = Farmyard manure
PRE-PACK= rock phosphate and Urea applied at a rate of 800 kg ha^{-1} and 80 kg ha^{-1} respectively.

4.2.3. Nematode communities and their distribution

Nematode communities and their distribution shown in table 4 had more free-living than plant-parasitic nematodes during the two seasons. Higher nematode abundance and diversity was recorded during the long rains than in the short rains. Majority of the nematodes belonged to the plant feeding trophic group and family hoplolaimidae was predominant. The most common C-P rating was 3, sourced from Bongers (1990). The most dominant genera were; *Meloidogyne, Tylenchus, Pratylenchus, Achromadora, Teratocephalus, Discolaiminae* and *Acrobeles.* The N and P released led to higher populations of these nematode genera.

Table 4. Nematode diversity and abundance (number/100cm³) under different soil fertility management practices.

Family	Genus	C-P rating	Trophic group	Soil Fertility Management Practices							
				Control		FYM		Nitrogen		PRE-PAC	
				SR	LR	SR	LR	SR	LR	SR	LR
Pratylenchidae	*Pratylenchus*	3	PF	51	55	68	74	73	76	34	41
Tylenchidae	*Tylenchus*	2	PF	21	22	31	26	28	41	18	19
Meloidogynidae	*Meloidogyne*	3	PF	13	11	18	14	18	21	8	9
Hoplolaimidae	*Helicotylenchus*	3	PF	17	10	10	15	13	18	7	7
Tylenchidae	*Ditylenchus*	2	PF/FF	7	5	6	7	8	13	8	5
Dolichodoridae	*Dolichodorus*	3	PF	1	3	6	4	5	7	3	3
Hoplolaimidae	*Scutellonema*	3	PF	3	2	2	4	3	4	3	3
Hoplolaimidae	*Rotylenchulus*	3	PF	5	2	5	5	7	7	2	2
Paratylenchidae	*Paratylenchus*	3	PF	13	8	9	14	12	19	7	7
Hemicycliophoridae	*Hemicycliophora*	3	PF	1	2	1	2	2	2	2	1
Creconematidae	*Hemicriconemoides*	3	PF	1	2	2	2	4	3	2	1
Heteroderidae	*Heterodera*	3	PF	1	1	0	2	1	2	1	2
Aphelenchoididae	*Aphelenchoides*	2	FF	4	2	3	3	3	4	2	2
Tylenchulidae	*Tylenchulus*	2	PF	2	2	1	7	5	10	3	2
Tylenchidae	*Anguina*	2	PF	1	2	6	2	2	2	2	3
Hoplolaimidae	*Aorolaimus*	3	PF	1	1	1	4	2	2	1	2
Pratylenchidae	*Hirschmanniella*	3	PF	1	1	1	2	2	2	2	1
Cephalobidae	*Acrobeles*	2	BF	26	26	0	34	31	43	22	28
Dorylaimidae	*Discolaiminae*	4	Pre	23	22	26	29	29	38	17	20
Creconematidae	*Criconemella*	3	FF	22	21	24	28	30	37	20	18
Dorylaimidae	*Dorylaiminae*	4	OM	23	26	25	29	32	35	20	19
Cyatholaimidae	*Achromadora*	3	BF	25	26	27	27	32	40	23	20
Ironidae	*Cryptonchus*	4	FF	25	25	27	26	32	38	24	21
Teratocephalidae	*Teratocephalus*	3	BF	26	25	26	29	34	39	23	23

C-P Values taken from Bongers (1990)

Where; C-P = consister-persister index BF = Bacterial Feeding PF = Plant Feeding FF = Fungal (Hyphal) Feeding
Pre = Predation on protozoan and soil animals OM = Omnivores SR=Short rains *LR=Long rains*

4.2.4. Plant-parasitic nematode populations (number/100cm^3) in soils treated with various soil fertility management practices

The populations of plant parasitic nematodes increased with maize growth period up to the milk stage and then declined at harvesting time. There were significant plant parasitic nematode population differences ($P<0.05$) in the four nematode sampling times in both seasons as indicated in tables 5 and 6. The interactions among soil fertility management practices, time of nematode sampling and period of cultivation were significant ($P<0.05$) for plant parasitic nematodes populations in both seasons.

There was an increase in population of plant parasitic nematodes where nitrogenous fertilizer and farm yard manure were applied but a decline was observed in the PRE-PAC in the long rains (Tables 5 and 6). PRE-PAC significantly reduced PPN populations. Increment in plant parasitic nematode populations was observed with farm conversion period in both seasons. There were significant plant parasitic nematode population differences ($P<0.05$) in the farm age clusters in both the short and long rains seasons.

Table 5. Plant parasitic nematode populations (number/100cm^3) in soil treated with different fertility management practices in the short rains of 2005.

Farm conversion Period (years)	Sampling Time	Treatments			
		Control	FYM	Nitrogen	PRE-PAC
0-10	P_0	54	93	46	58
	P_1	70	204	66	98
	P_2	110	234	164	123
	P_3	91	155	88	109
10-20	P_0	143	79	113	48
	P_1	168	139	144	79
	P_2	186	260	260	151
	P_3	184	180	185	89
20-40	P_0	86	109	120	64
	P_1	118	144	174	108
	P_2	271	284	368	153
	P_3	126	173	239	86
>40	P_0	66	70	173	69
	P_1	89	114	228	100
	P_2	290	294	378	179
	P_3	146	144	261	135

	$LSD_{(p=0.05)}$
Farm conversion period	5
Sampling time	5
Treatment	5
Farm conversion period x sampling time	10
Farm conversion period x treatment	10
Sampling time x treatment	10
Farm conversion period x sampling x treatment	20
C.V (%)	9.7

Where
P_0 = initial population
P_1 = population at 6 weeks after planting
P_2 = population at the milky stage
P_3 = population at maize harvesting

Table 6. Plant parasitic nematode populations (number/100cm^3) in soil treated with different fertility management practices in the long rains of 2006.

Farm conversion Period (years)	Sampling Time	Treatments			
		Control	FYM	Nitrogen	PRE-PAC
0-10	P_0	54	141	85	39
	P_1	113	166	129	95
	P_2	156	196	240	134
	P_3	81	191	226	108
10-20	P_0	75	114	170	35
	P_1	155	170	221	69
	P_2	160	258	284	156
	P_3	120	184	209	64
20-40	P_0	78	158	164	84
	P_1	115	218	209	110
	P_2	229	259	354	160
	P_3	86	173	179	128
>40	P_0	96	98	244	76
	P_1	163	201	288	148
	P_2	244	274	414	175
	P_3	153	179	300	91

	$LSD_{(p=0.05)}$
Farm conversion period	4
Sampling time	4
Treatment	4
Farm conversion period x sampling time	8
Farm conversion period x treatment	8
Sampling time x treatment	8
Farm conversion period x sampling x treatment	17
C.V (%)	7.4

Where
P_0 = initial population
P_1 = population at 6 weeks after planting
P_2 = population at the milky stage
P_3 = population at maize harvesting

4.2.5. Effect of soil fertility management on root lesion nematodes

There was an increase in *Pratylencus spp.* populations with farm conversion periods in both seasons (Tables 7 and 8). There were significant differences of *Pratylencus spp.* population ($P<0.05$) among all the farm conversion periods in both seasons. There was an increase in population of *Pratylencus spp.* in both seasons where nitrogenous fertilizer and farm yard manure were applied but a decline was observed in PRE-PAC treatment. Significant *Pratylencus spp.* population differences ($P<0.05$) among all the treatments were reported in both seasons.

An increment was observed in *Pratylencus spp.* populations from maize planting to the milky stage but a decline was noticed at harvesting time in both seasons. Significant *Pratylencus spp.* population differences ($P<0.05$) in the four nematode sampling times were observed in both rains seasons.

The interactions among soil fertility management practices, time of nematode sampling and period of cultivation were highly significant ($P<0.05$) for *Pratylenchus sp.* population in the short and long seasons (Tables 7 and 8).

Table 7. Population of *Pratylenchus* spp. (number/100cm^3) in soil treated with different fertility management practices in the short rains season of 2005.

Farm conversion period (years)	Sampling Time	Treatments			
		Control	FYM	Nitrogen	PRE-PAC
0-10	P_0	20	55	13	21
	P_1	26	70	25	30
	P_2	44	85	54	41
	P_3	36	78	29	34
10-20	P_0	40	45	45	14
	P_1	58	61	63	26
	P_2	81	106	90	48
	P_3	60	65	70	28
20-40	P_0	30	30	60	28
	P_1	43	43	83	38
	P_2	85	121	111	59
	P_3	49	41	95	43
>40	P_0	40	44	83	16
	P_1	55	61	98	29
	P_2	96	128	144	59
	P_3	51	61	109	30

	$LSD_{(p=0.05)}$
Farm conversion period	2
Sampling time	2
Treatment	2
Farm conversion period x sampling time	5
Farm conversion period x treatment	5
Sampling time x treatment	5
Farm conversion period x sampling x treatment	10
C.V (%)	12.3

Where
P_0 = initial population
P_1 = population at 6 weeks after planting
P_2 = population at the milky stage
P_3 = population at maize harvesting

Table 8. *Pratylenchus* spp. populations (number/100cm^3) in soil treated with different fertility management practices in the long rains season of 2006.

Farm conversion period (years)	Sampling Time	Treatments			
		Control	FYM	Nitrogen	PRE-PAC
0-10	P_0	21	51	24	18
	P_1	38	64	31	29
	P_2	79	96	63	63
	P_3	35	81	35	35
10-20	P_0	28	39	48	14
	P_1	75	61	74	25
	P_2	88	111	121	63
	P_3	40	68	69	26
20-40	P_0	20	53	46	36
	P_1	34	88	88	50
	P_2	91	113	131	73
	P_3	34	78	63	64
>40	P_0	44	39	74	16
	P_1	66	58	111	40
	P_2	120	121	148	80
	P_3	71	64	88	24

	$LSD_{(p=0.05)}$
Farm conversion period	2
Sampling time	2
Treatment	2
Farm conversion period x sampling time	5
Farm conversion period x treatment	5
Sampling time x treatment	5
Farm conversion period x sampling x treatment	9
C.V (%)	10.9

Where
P_0 = initial population
P_1 = population at 6 weeks after planting
P_2 = population at the milky stage
P_3 = population at maize harvesting

4.2.6. Diversity of free-living nematodes in soils treated with various fertility management practices

A decline in the population of free-living nematodes in all the soil fertility management practices were observed in both seasons (Tables 9 and 10). Differences in number of free-living nematodes were significant ($P<0.05$) among the soil fertility treatments tested in the two seasons. A decline in free-living nematode populations was observed with conversion period of the farms in both seasons. The >40 years farm conversion period had significantly higher populations ($P<0.05$) than farm conversion periods in both rains seasons.

Significant differences ($P<0.05$) in free-living populations were recorded during the growth stages with milk stage giving significantly higher numbers than the rest in both seasons. There were significant ($P<0.05$) differences in the interactions between soil fertility management practices, stage of growth and farm conversion period for free-living nematodes in the short and long rains seasons (Tables 9 and 10).

Table 9. Free-living nematode populations (number/100cm³) in soils subjected to different soil fertility management practices in the short rains season of 2005.

Farm conversion period (years)	Sampling Time	Treatments			
		Control	FYM	Nitrogen	PRE-PAC
0-10	P_0	171	115	149	85
	P_1	251	170	195	108
	P_2	300	325	403	300
	P_3	215	195	249	108
10-20	P_0	106	140	158	101
	P_1	144	188	193	138
	P_2	295	251	344	214
	P_3	131	188	269	179
20-40	P_0	95	90	198	94
	P_1	118	124	238	115
	P_2	184	240	313	196
	P_3	181	159	293	149
>40	P_0	114	126	93	108
	P_1	121	211	100	130
	P_2	150	236	205	146
	P_3	141	179	118	195

	$LSD_{(p=0.05)}$
Farm conversion period	3
Sampling time	3
Treatment	3
Farm conversion period x sampling time	7
Farm conversion period x treatment	7
Sampling time x treatment	7
Farm conversion period x sampling x treatment	14
C.V (%)	5.5

Where
P_0 = initial population
P_1 = population at 6 weeks after planting
P_2 = population at the milky stage
P_3 = population at maize harvesting

Table 10. Free-living nematode populations (number/100cm³) in soils subjected to different soil fertility management practices in the long rains season of 2006.

Farm conversion period (years)	Sampling Time	Treatments			
		Control	FYM	Nitrogen	PRE-PAC
0-10	P_0	100	118	174	93
	P_1	193	269	234	158
	P_2	254	293	403	223
	P_3	195	249	344	149
10-20	P_0	139	136	256	114
	P_1	188	169	298	148
	P_2	251	271	348	221
	P_3	175	171	296	136
20-40	P_0	118	151	150	95
	P_1	150	185	213	138
	P_2	228	270	325	205
	P_3	159	215	295	143
>40	P_0	115	149	196	94
	P_1	140	185	214	133
	P_2	174	204	300	195
	P_3	145	190	241	128

	$LSD_{(p=0.05)}$
Farm conversion period	2
Sampling time	2
Treatment	2
Farm conversion period x sampling time	5
Farm conversion period x treatment	5
Sampling time x treatment	5
Farm conversion period x sampling x treatment	9
C.V (%)	3.4

Where
P_0 = initial population
P_1 = population at 6 weeks after planting
P_2 = population at the milky stage
P_3 = population at maize harvesting

4.3. Effect of selected *Bacillus subtilis* strains on nematodes

4.3.1. Nematode abundance (number/100cm³) as influenced by *Bacillus subtilis*

Generally control plots had higher nematode abundance compared to where *Bacillus subtilis* strain K194 and *Rhizobium. Leguminosarum biovar phaseoli* strain USDA 2674 were applied in both seasons (Table 11). Plots treated bi-inoculants had high nematode species richness (SR) and diversity index -H_2. Species evenness was highest in soils treated with *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674 and lowest in the control plots.

Table 11. Comparison of nematode abundance (number/100cm³) and diversity as influenced by biocontrol agents in the short rains of 2005 and long rains of 2006.

Bio-inoculants	N		S		SR		H_2		J	
	Sr	Lr	Sr	Lr	Sr	Lr	Sr	Lr	Sr	Lr
Control	355	379	34	34	5.62	5.56	2.26	2.34	0.64	0.66
K158	305	339	34	34	5.77	5.66	2.13	2.41	0.60	0.68
K158 + USDA	314	347	34	34	5.74	5.64	2.42	2.61	0.65	0.74
K194	291	348	34	34	5.82	5.64	2.71	2.61	0.69	0.74
K194 + USDA	267	307	34	34	5.91	5.76	2.71	2.59	0.69	0.73
K263	318	343	34	34	5.73	5.65	2.63	2.49	0.64	0.71
K263 + USDA	315	334	34	34	5.74	5.68	2.49	2.50	0.75	0.71
USDA	276	326	34	34	5.89	5.73	2.71	2.65	0.77	0.75

H_2 the Shannon–Weiner index (Yeates 2003)

Where N=Nematode abundance S = number of genera SR= Species richness
H_2=Diversity J=Species evenness Sr = Short rains
Lr = Long rains

4.3.2. Nematode communities and their distribution in soil treated with different bio-inoculants

There were higher populations of free-living nematodes compared to the parasitic ones in both seasons (Table 12). There was an increment in nematode populations from the short to the long rains seasons. Marked reduction of nematodes (*Meloidogyne sp.*) population was observed in plots treated with *Bacillus subtilis* strain K194 and *Rhizobium* strain USDA2674 whereas control and *Bacillus subtilis* strains K158 and K263 had almost constant nematode populations in both seasons. The population of plant parasitic nematodes reduced in the long rains season but the converse was true for the free-living nematodes in the short rains season.

The most common C-P rating was 3 while the most prominent nematode family was Hoploimidae. There were less predacious nematodes than the bacteriavores. A few genera exhibited omnivorous feeding habits. A few others were fungivores. The dominant genera were; *Meloidogyne, Tylenchus, Acrobeles, Discolaiminae, Thelastoma, Achromadora,* in both seasons.

Table 12. Nematode communities and their distribution in soil treated with different bio-controls.

Family	Genus	C-P Rating	Trophic group	Bio-inoculants															
				Control		K158		K158 + USDA		K194		K194 + USDA		K263		K263 + USDA		USDA	
				SR	LR	SR	LR	SR	LR	SR	LR	SR	LR	SR	LR	SR	LR	SR	
Meloidogynidae	*Meloidogyne*	3	PF	62	99	43	82	63	69	24	51	28	55	49	77	56	72	27	
Tylenchidae	*Tylenchus*	2	PF	81	29	52	16	44	18	24	10	29	8	40	14	44	15	28	
Pratylenchidae	*Pratylenchus*	3	PF	13	10	15	7	10	9	12	5	12	4	16	7	15	7	11	
Hoplolaimidae	*Helicotylenchus*	3	PF	8	7	8	9	10	9	10	3	8	5	12	6	10	6	9	
Tylenchidae	*Ditylenchus*	2	PF/FF	7	6	10	4	7	5	7	3	8	3	11	5	9	5	7	
Dolichodoridae	*Dolichodorus*	3	Pf	4	5	3	3	5	4	4	2	3	3	7	3	5	4	3	
Hoplolaimidae	*Scutellonema*	3	PF	3	10	2	6	3	7	4	4	3	5	2	6	1	7	3	
Nacobbidae	*Rotylenchulus*	2	PF	6	3	5	4	5	2	3	1	4	1	7	2	2	2	3	
Paratylenchidae	*Paratylenchus*	3	PF	14	5	11	4	10	5	8	2	5	3	11	5	11	5	7	
Hemicyclophoridae	*Hemicycliphora*	3	PF	1	2	1	2	2	1	0	1	2	2	2	2	1	3	1	
Creconematidae	*Hemicreconemoides*	3	PF	5	4	1	2	2	3	1	2	2	3	2	4	1	4	2	
Heteroderidae	*Heterodera*	3	PF	2	2	1	3	1	4	1	2	1	2	2	2	4	2	1	
Aphelenchoididae	*Aphelenchoides*	2	FF	1	3	2	2	1	2	0	2	1	2	2	2	1	1	1	
Tylenchulidae	*Tylenchulus*	2	PF	5	4	4	3	3	3	2	2	1	5	1	3	2	4	2	
Pratylenchidae	*Radopholus*	3	PF	1	2	1	3	1	1	1	1	0	2	0	1	0	1	1	
Dorylaimidae	*Dorylaiminae*	4	OM	17	5	18	5	17	3	24	4	18	4	17	6	19	5	18	
Tylenchidae	*Anguina*	2	PF	2	3	1	1	1	2	1	2	0	1	1	2	1	1	1	
Hoplolaimidae	*Rotylenchoides*	3	PF	2	3	2	2	1	2	1	1	0	1	0	2	1	1	1	
Pratylenchidae	*Hirschmanniella*	3	PF	2	1	1	1	1	2	1	1	1	1	2	2	1	2	0	
Aphelenchidae	*Aphelenchus*	2	FF	0	6	0	5	0	5	0	4	0	5	0	6	0	6	0	
Tylenchulidae	*Sphaeronema*	3	PF	0	3	0	2	0	2	0	2	0	1	0	1	0	2	0	
Trichodoridae	*Trichodorus*	3	OM	0	5	0	2	0	2	0	1	0	1	0	4	0	2	0	
Heteroderidae	*Cryphodera*	3	PF	0	3	0	2	0	3	0	1	0	1	0	2	0	2	0	
Longidoridae	*Xiphinema*	5	PF	0	3	0	2	0	2	0	2	0	1	0	3	0	2	0	
Cephalobidae	*Acrobeles*	2	BF	26	22	24	24	26	20	30	32	27	27	26	27	24	26	28	
Dorylaimidae	*Discolaiminae*	4	Pre	19	15	21	18	20	18	25	26	22	23	19	19	22	19	21	
Creconematidae	*Criconemella*	3	PF	11	17	11	18	13	19	16	28	12	22	14	19	13	18	18	
Thelastomatidae	*Thelastoma*	3	BF	17	15	16	16	13	18	18	27	17	20	13	19	15	17	17	
Ironidae	*Cryptonchus*	4	Bf	0	14	0	14	0	17	0	25	0	20	0	15	0	13	0	
Cyatholaimidae	*Achromadora*	3	OM	14	14	14	14	17	18	17	24	16	19	15	14	14	14	16	
Tylenchidae	*Neotylenchinae*	2	PF	10	17	14	17	12	20	18	24	15	18	16	17	16	18	16	
Belondiridae	*Belondira*	1	PF	12	15	12	17	12	19	20	21	14	16	15	17	14	17	15	
Teratocephalidae	*Teratocephalus*	3	BF	0	16	0	16	0	20	0	20	0	16	0	18	0	19	0	
Tobrilidae	*Tobrilus*	3	BF	0	17	0	17	0	20	0	20	0	12	0	18	0	20	0	
Mononchidae	*Mylonchus*	4	Pre	12	0	12	0	15	0	19	0	14	0	15	0	14	0	16	
Hoplolaimidae	*Rotylenchus*	3	PF	6	0	5	0	5	0	3	0	4	0	7	0	2	0	3	
Hoplolaimidae	*Aorolaimus*	3	PF	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1	

C-P Values taken from Bongers (1990) Where; C-P = consister-persister index, BF = Bacterial Feeding, PF = Plant Feeding, FF = Fungal (Hyphal) Feeding, Pre = Predation on protozoan and soil animals, OM = Omnivores

4.3.3. Plant-parasitic nematodes (number/100cm^3) under selected bio-inoculants

An increment in the population of plant-parasitic nematodes in all the biocontrol treatments including the control was observed in both rain seasons (Tables 13 and 14). Significant PPNs population differences ($P<0.05$) were recorded in the selected biocontrol agents in both seasons with *Bacillus subtilis* strain K158 being the least effective in controlling of nematodes and gave counts as high as those by the control. Strain K194 and *Rhizobium* strain USDA2674 showed significantly lower counts, thus, effectively controlling nematodes.

An increase in plant-parasitic nematode populations was observed with period of cultivation in both seasons. There were significant plant-parasitic nematodes population differences ($P<0.05$) in the farm age clusters in both seasons. The >40 years giving significantly higher populations while the 0-10 years showed significantly lower populations.

An increase in the population of plant-parasitic nematodes was recorded from bean planting up to the flowering stage with a decline at harvesting time in both seasons. There were significant plant-parasitic nematodes population differences ($P<0.05$) among the four nematode sampling times in both seasons. The interactions among biocontrols, time of nematode sampling and period of bean cultivation were highly significant ($P<0.05$) for plant-parasitic nematodes populations in both seasons. The percentage changes in nematode populations were higher in the control and *Bacillus* isolates K158 and K263 but lower in K194 and *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674 in both seasons, implying the effectiveness of strain K194 plus *Rhizobium*.

Table 13. Plant parasitic nematode populations (number/100cm^3) in soil treated with different bio-inoculants in the short rains season of 2005.

Farm conversion period (Years)	Sampling Time	Bio-noculants*							
		Control	K158	K158 + USDA	K194	K194 + USDA	K263	K263 + USDA	USDA 2674
0-10	P_0	103(40)	85(38)	71(44)	54(40)	39(48)	73(54)	60(55)	60(25)
	P_1	161	100	93	79	53	145	79	74
	P_2	171	138	128	90	75	159	133	80
	P_3	143	113	81	75	49	131	108	79
10-20	P_0	109(47)	75(47)	81(41)	58(47)	61(48)	66(60)	79(45)	54(39)
	P_1	149	93	106	75	88	94	99	69
	P_2	206	143	138	109	118	166	143	89
	P_3	159	90	105	74	71	88	106	65
20-40	P_0	110(52)	78(58)	74(50)	59(46)	74(40)	84(50)	85(44)	84(25)
	P_1	131	114	103	83	109	110	120	101
	P_2	228	183	146	109	123	168	153	111
	P_3	141	120	104	80	89	123	98	91
>40	P_0	131(44)	125(39)	96(42)	65(42)	81(45)	88(54)	93(52)	79(35)
	P_1	168	166	118	73	101	126	113	81
	P_2	235	205	165	113	148	193	193	121
	P_3	163	159	110	91	96	110	110	89

* *Bacillus subtilis* isolates; K158, K194, K263 and *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	3
Sampling time	3
Treatment	4
Farm conversion period x sampling time	5
Farm conversion period x treatment	7
Sampling time x treatment	7
Farm conversion period x sampling x treatment	15
C.V (%)	9.9

Where

P_0 = initial population

P_1 = population at 6 weeks after planting

P_2 = population at 75% flowering

p3 = population at bean harvesting

N/B: Values in brackets are the percentage changes from P_0 to P_2

Table 14. Plant parasitic nematode populations (number/100cm^3) in soil treated with different bio-inoculants in the long rains season of 2006.

Farm conversion period (Years)	Sampling Time	Bio-inoculants*							
		Control	K158	K158 + USDA	K194	K194 + USDA	K263	K263 + USDA	USDA 2674
0-10	P_0	130(50)	121(46)	85(56)	56(51)	63(60)	99(51)	76(61)	96(38)
	P_1	203	180	163	98	113	153	148	149
	P_2	263	223	193	115	158	200	198	155
	P_3	180	165	131	83	96	125	114	115
10-20	P_0	154(43)	108(52)	104(51)	63(52)	70(57)	96(56)	100(51)	94(46)
	P_1	230	159	164	128	105	145	153	110
	P_2	273	226	214	131	161	218	204	176
	P_3	231	143	128	103	90	123	116	83
20-40	P_0	160(44)	104(55)	111(50)	76(57)	74(57)	116(50)	89(59)	100(44)
	P_1	215	179	176	115	91	156	155	141
	P_2	288	233	223	178	171	233	219	180
	P_3	210	141	140	114	108	156	159	128
>40	P_0	184(40)	114(56)	141(43)	64(660	89(53)	138(46)	123(51)	103(46)
	P_1	228	168	186	63	141	199	184	130
	P_2	308	256	246	186	189	254	249	191
	P_3	213	153	165	84	126	170	160	125

**Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	3
Sampling time	3
Treatment	4
Farm conversion period x sampling time	6
Farm conversion period x treatment	8
Sampling time x treatment	8
Farm conversion period x sampling x treatment	16
C.V (%)	7.6

Where
P_0 = initial population
P_1 = population at 6 weeks after planting
P_2 = population at 75% flowering
p3 = population at bean harvesting
N/B: Values in brackets are the percentage changes from P_0 to P_2

4.3.4 *Meloidogyne spp.* populations (number/100cm³) under selected bio-inoculants

There was an increase in the population of *Meloidogyne spp* in all the treatments in both the long rain seasons (Tables 15 and 16). There were significant *Meloidogyne spp.* population differences ($P<0.05$) in the selected biocontrol agents in both seasons with control showing significantly higher population whereas *Bacillus subtilis* strain K194 and *Rhizobium* strain USDA2674 singularly and in combination giving significantly lower populations.

Similarly an increment in *Meloidogyne spp.* populations was observed with period of cultivation in both seasons. There were significant *Meloidogyne sp.* population differences ($P<0.05$) in the farm age clusters in the two rain seasons with the >40 years age clusters giving significantly higher populations while the 0-10 recording the lowest populations in both rain seasons.

There was an increment in the population of *Meloidogyne spp.* from bean planting to flowering stage followed by a drop at harvesting time. There were significant *Meloidogyne spp* population differences ($P<0.05$) at the four nematode sampling times in both rain seasons. The interactions among biocontrols, time of nematode sampling and period of bean cultivation were significant ($P<0.05$) for *Meloidogyne spp* population in the short and long rain seasons. Generally, the percentage change in *Meloidogyne* spp. populations was greater in the control and *Bacillus* isolates K158 and K263 but less in the isolate K194 and *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674.

Table 15. *Meloidogyne* spp. populations (number/100cm^3) in soil treated with different bio-inoculants in the short rains season of 2005.

Farm conversion period (Years)	Sampling Time	Bio-inoculants*							
		Control	K158	K158 + USDA	K194	K194 + USDA	K263	K263 + USDA	USDA 2674
0-10	P_0	36(44)	25(53)	21(68)	13(57)	13(62)	15(63)	18(67)	11(57)
	P_1	60	29	53	18	23	31	44	20
	P_2	65	54	66	29	33	40	54	36
	P_3	56	28	35	21	23	25	39	18
10-20	P_0	38(49)	24(61)	28(67)	13(60)	13(62)	30(58)	33(56)	19(52)
	P_1	43	29	35	23	18	65	69	28
	P_2	74	61	84	31	33	65	74	39
	P_3	36	29	50	19	21	63	71	26
20-40	P_0	34(56)	34(51)	46(51)	20(54)	23(52)	34(52)	31(58)	19(52)
	P_1	55	61	70	30	29	50	43	26
	P_2	76	69	95	44	48	70	75	39
	P_3	56	66	65	30	30	53	53	25
>40	P_0	59(46)	38(53)	58(51)	25(51)	25(53)	34(62)	48(51)	25(44)
	P_1	89	45	76	31	36	38	66	35
	P_2	110	81	118	51	54	89	98	45
	P_3	89	50	93	28	39	43	64	31

* *Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	2
Sampling time	2
Treatment	3
Farm conversion period x sampling time	4
Farm conversion period x treatment	6
Sampling time x treatment	6
Farm conversion period x sampling x treatment	11
C.V (%)	18.1

Where
P_0 = initial population
P_1 = population at 6 weeks after planting
P_2 = population at 75% flowering
P_3 = population at bean harvesting
N/B: Values in brackets are percentage change from P_0 to P_2

Table 16. ***Meloidogyne*** **spp. populations (number/100cm^3) in soil treated with different bio-inoculants in the long rains season of 2006.**

Farm conversion period (Years)	Sampling Time	Bio-inoculants*							
		Control	K158	K158 + USDA	K194	K194 + USDA	K263	K263 + USDA	USDA 2674
0-10	P_0	55(58)	43(63)	20(78)	11(70)	11(84)	25(75)	26(71)	21(75)
	P_1	106	84	51	16	34	66	45	50
	P_2	133	116	93	38	70	99	91	84
	P_3	78	73	36	23	28	48	48	46
10-20	P_0	48(66)	56(55)	20(81)	33(55)	10(87)	33(73)	43(63)	26(70)
	P_1	128	108	38	68	38	80	74	50
	P_2	139	126	106	73	78	119	115	88
	P_3	83	88	40	56	43	76	65	29
20-40	P_0	58(63)	51(64)	34(74)	43(53)	25(76)	43(69)	38(70)	25(61)
	P_1	110	63	68	90	58	81	81	40
	P_2	156	141	128	90	103	138	126	90
	P_3	88	54	63	78	61	73	68	48
>40	P_0	61(64)	65(57)	53(75)	51(57)	28(77)	48(65)	48(62	38(63)
	P_1	121	83	105	76	91	79	88	83
	P_2	171	153	168	119	120	138	128	100
	P_3	69	68	76	43	75	81	78	73

* *Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	2
Sampling time	2
Treatment	3
Farm conversion period x sampling time	4
Farm conversion period x treatment	5
Sampling time x treatment	5
Farm conversion period x sampling x treatment	10
C.V (%)	10.3

Where

P_0 = initial population

P_1 = population at 6 weeks after planting

P_2 = population at 75% flowering

P_3 = population at bean harvesting

N/B: Values in brackets are percentage change from P_0 to P_2

4.3.5. Free-living nematodes (number/100cm^3) under selected bio-inoculants

There was an increase in the population of free-living nematodes in all the biocontrol treatments including the control in both seasons (Tables 17 and 18). Both seasons showed significant free-living nematodes population differences ($P<0.05$) in the selected biocontrol agents with *Bacillus subtilis* strain K194 and *Rhizobium* strain USDA2674 giving significantly higher populations while *Bacillus subtilis* strain K158 and control recorded the lowest populations.

Free-living nematode populations declined with period of cultivation in both seasons. There were significant free-living nematodes population differences ($P<0.05$) in the farm age clusters with the 0-10 years age cluster showing significantly higher populations whereas the >40 years recorded the lowest counts in the both rain seasons.

There was an increase in the population of free-living nematodes from bean planting to flowering stage with a decrease at harvesting time in both seasons. There were significant free-living nematodes population differences ($P<0.05$) at the four nematode sampling times in both seasons with bean flowering stage yielding significantly higher populations while the prior to planting time recorded the lowest populations. The interactions among biocontrols, sampling time and period of bean cultivation were highly significant ($P<0.05$) for free-living nematodes populations in (Tables 19 and 20). *Bacilli isolate* K194 and *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674 showed greater percentage change in populations of free-living nematodes than the control and isolates K158 and K263 in the two seasons.

4.3.5. Free-living nematodes (number/100cm^3) under selected bio-inoculants

There was an increase in the population of free-living nematodes in all the biocontrol treatments including the control in both seasons (Tables 17 and 18). Both seasons showed significant free-living nematodes population differences ($P<0.05$) in the selected biocontrol agents with *Bacillus subtilis* strain K194 and *Rhizobium* strain USDA2674 giving significantly higher populations while *Bacillus subtilis* strain K158 and control recorded the lowest populations.

Free-living nematode populations declined with period of cultivation in both seasons. There were significant free-living nematodes population differences ($P<0.05$) in the farm age clusters with the 0-10 years age cluster showing significantly higher populations whereas the >40 years recorded the lowest counts in the both rain seasons.

There was an increase in the population of free-living nematodes from bean planting to flowering stage with a decrease at harvesting time in both seasons. There were significant free-living nematodes population differences ($P<0.05$) at the four nematode sampling times in both seasons with bean flowering stage yielding significantly higher populations while the prior to planting time recorded the lowest populations. The interactions among biocontrols, sampling time and period of bean cultivation were highly significant ($P<0.05$) for free-living nematodes populations in (Tables 19 and 20). *Bacilli isolate* K194 and *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674 showed greater percentage change in populations of free-living nematodes than the control and isolates K158 and K263 in the two seasons.

Table 17. Free-living nematode populations (number/100cm^3) in soil treated with different bio-inoculants in the short rains season of 2005.

Farm conversion period (Years)	Sampling Time	Bio-inoculants*							
		Control	K158	K158 + USDA	K194	K194 + USDA	K263	K263 + USDA	USDA 2674
0-10	P_0	98(49)	90(56)	103(50)	159(45)	116(42)	129(46)	114(53)	130(47)
	P_1	136	145	139	203	144	164	151	205
	P_2	191	203	204	291	200	239	239	245
	P_3	115	114	124	183	135	136	133	200
10-20	P_0	113(40)	116(41)	108(47)	128(53)	109(46)	110(47)	103(50)	98(53)
	P_1	139	145	139	163	168	194	146	145
	P_2	189	198	201	269	201	209	204	206
	P_3	123	125	116	169	141	159	131	148
20-40	P_0	90(52)	90(54)	106(46)	124(50)	96(54)	105(44)	100(51)	100(51)
	P_1	146	148	154	180	146	148	143	139
	P_2	186	195	198	248	210	189	204	204
	P_3	143	121	153	203	128	134	133	115
>40	P_0	94(46)	91(53)	109(39)	114(43)	93(59)	73(51)	94(53)	118(42)
	P_1	140	138	135	160	194	119	151	159
	P_2	175	194	179	201	245	149	199	203
	P_3	119	118	125	189	118	110	134	129

* *Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	2
Sampling time	2
Treatment	3
Farm conversion period x sampling time	4
Farm conversion period x treatment	6
Sampling time x treatment	6
Farm conversion period x sampling x treatment	11
C.V (%)	5.2

Where

P_0 = initial population

P_1 = population at 6 weeks after planting

P_2 = population at 75% flowering

P_3 = population at bean harvesting

N/B: Values in brackets are percentage change from P_0 to P_2

Table 18. Free living nematode populations (number/100cm^3) in soil treated with different bio-inoculants in the long rains season of 2006.

Farm conversion period (Years)	Sampling Time	Bio-inoculants*							
		Control	K158	K158 + USDA	K194	K194 + USDA	K263	K263 + USDA	USD 267
0-10	P_0	128(51)	139(45)	154(41)	221(29)	158(38)	155(42)	149(42)	161(3
	P_1	166	184	203	266	180	191	196	199
	P_2	259	250	263	314	254	269	258	255
	P_3	158	171	180	246	186	179	190	185
10-20	P_0	119(49)	143(42)	141(46)	190(36)	141(41)	118(52)	120(53)	145(4
	P_1	189	194	204	255	210	183	188	198
	P_2	235	248	261	299	239	248	254	248
	P_3	150	166	155	233	168	145	148	185
20-40	P_0	109(52)	138(40)	130(47)	211(28)	133(43)	129(47)	123(52)	130(4
	P_1	141	198	195	251	180	190	185	168
	P_2	225	228	244	293	234	244	254	240
	P_3	133	140	148	256	184	153	161	171
>40	P_0	103(48)	93(48)	119(44)	165(38)	146(37)	118(51)	96(59)	138(4
	P_1	154	144	194	224	205	195	179	201
	P_2	196	176	229	266	231	241	234	238
	P_3	130	121	169	206	191	150	145	166

* *Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	3
Sampling time	3
Treatment	4
Farm conversion period x sampling time	5
Farm conversion period x treatment	7
Sampling time x treatment	7
Farm conversion period x sampling x treatment	15
C.V (%)	5.7

Where

P_0 = initial population

P_1 = population at 6 weeks after planting

P_2 = population at 75% flowering

P_3 = population at bean harvesting

N/B: Values in brackets are percentage change from P_0 to P_2

4.3.6. Change in population of *Bacillus subtilis* in soil

There was no increase in the total *Bacillus subtilis* counts in all the treatments in both seasons (Tables 19 and 20). Significant *Bacillus subtilis* count differences ($P<0.05$) in the selected biocontrol agents in both seasons were recorded with *Bacillus subtilis* strain K194 and *Rhizobium* strain USDA2674 giving significantly higher counts while *Bacillus subtilis* strains K158 and K263 showing significantly lower counts. A decrease in *Bacillus subtilis* count was observed with period of cultivation in both seasons. There were significant *Bacillus subtilis* counts differences ($P<0.05$) in the farm age clusters in both seasons with the 1-10 years cluster showing significantly higher counts and the >40 years cluster recording significantly lower counts.

There was an increase in the *Bacillus subtilis* counts from bean planting to harvesting time. In both seasons, significant *Bacillus subtilis* counts differences ($P<0.05$) were observed at the two *Bacillus subtilis* sampling times with the prior to planting time showing significantly lower counts than at the harvesting time. The interactions among biocontrols, time of *Bacillus subtilis* sampling and period of bean cultivation were highly significant ($P<0.05$) for *Bacillus subtilis* counts in both seasons.

Table 19. Mean *Bacillus subtilis* counts under selected bio-inoculants in soil under bean in the short rains season.

arm onversion eriod ears)	Sampling Time	Bio-inoculants*							
		Control	K158	K158+ USDA	K194	K194+ USDA	K263	K263+ USDA	USDA 2674
-10	P_0	$3.73x10^6$	$3.68x10^5$	$7.20x10^5$	$9.15x10^6$	$6.25x10^6$	$8.20x10^5$	$4.18x10^6$	$8.23x10^6$
	P_1	$3.80x10^6$	$4.68x10^5$	$7.73x10^5$	$9.75x10^6$	$6.65x10^6$	$8.73x10^5$	$4.75x10^6$	$8.75x10^6$
0-20	P_0	$2.73x10^6$	$2.13x10^5$	$5.25x10^5$	$8.68x10^6$	$4.65x10^6$	$6.25x10^5$	$3.18x10^6$	$4.23x10^6$
	P_1	$3.05x10^6$	$2.48x10^5$	$5.73x10^5$	$9.25x10^6$	$5.18x10^6$	$6.73x10^5$	$3.60x10^6$	$4.75x10^6$
0-40	P_0	$1.25x10^6$	$6.15x10^4$	$4.65x10^5$	$2.68x10^6$	$3.15x10^6$	$9.15x10^4$	$3.18x10^6$	$2.23x10^6$
	P_1	$1.40x10^6$	$6.65x10^4$	$4.78x10^5$	$5.38x10^6$	$3.65x10^6$	$9.58x10^4$	$2.40x10^6$	$2.73x10^6$
-40	P_0	$1.23x10^6$	$3.73x10^4$	$1.18x10^5$	$2.70x10^6$	$2.64x10^6$	$7.20x10^4$	$1.23x10^6$	$2.20x10^6$
	P_1	$1.65x10^6$	$4.15x10^4$	$1.65x10^5$	$3.38x10^6$	$3.08x10^6$	$7.65x10^4$	$1.75x10^6$	$2.73x10^6$

* *Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	47359.1
Sampling time	33488.0
Treatment	66975.9
Farm conversion period x sampling time	66975.9
Farm conversion period x treatment	133951.9
Sampling time x treatment	94718.3
Farm conversion period x sampling x treatment	189436.5
C.V (%)	5.0

Where
P_0 = initial population
P_1 = population at bean harvesting planting

Table 20. Mean *Bacillus subtilis* counts under selected bio-inoculants in soil under bean in the long rains season.

m nversion iod ars)	Sampling Time	Bio-inoculants*							
		Control	K158	K158+ USDA	K194	K194+ USDA	K263	K263+ USDA	USDA 2674
10	P_0	$3.88x10^6$	$9.25x10^5$	$8.15x10^5$	$9.65x10^6$	$7.65x10^6$	$6.65x10^5$	$4.73x10^6$	$7.75x10^6$
	P_1	$3.93x10^6$	$9.6x10^5$	$8.65x10^5$	$9.75x10^6$	$8.05x10^6$	$7.20x10^5$	$5.73x10^6$	$8.75x10^6$
-20	P_0	$3.58x10^6$	$5.23x10^5$	$5.15x10^5$	$9.43x10^6$	$7.55x10^6$	$4.23x10^5$	$4.40x10^6$	$6.20x10^6$
	P_1	$3.65x10^6$	$5.75x10^5$	$5.68x10^5$	$9.73x10^6$	$8.55x10^6$	$4.65x10^5$	$4.68x10^6$	$6.75x10^6$
-40	P_0	$1.75x10^6$	$2.68x10^5$	$4.75x10^5$	$7.70x10^6$	$5.68x10^6$	$7.73x10^4$	$3.75x10^6$	$5.73x10^6$
	P_1	$2.23x10^6$	$3.68x10^5$	$6.15x10^5$	$8.95x10^6$	$6.68x10^6$	$9.23x10^4$	$4.75x10^6$	$6.73x10^6$
40	P_0	$1.60x10^6$	$6.73x10^4$	$2.73x10^5$	$4.18x10^6$	$3.20x10^6$	$7.68x10^4$	$2.25x10^6$	$2.73x10^6$
	P_1	$1.70x10^6$	$6.80x10^4$	$3.73x10^5$	$5.18x10^6$	$3.65x10^6$	$8.68x10^4$	$4.25x10^6$	$3.60x10^6$

* *Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	61711.0
Sampling time	43636.2
Treatment	87272.5
Farm conversion period x sampling time	87272.5
Farm conversion period x treatment	174545.0
Sampling time x treatment	123421.9
Farm conversion period x sampling x treatment	246843.8
C.V (%)	4.9

P_0 = initial population

P_1 = population at bean harvesting planting

4.4 The efficacy of *Bacillus subtilis* strains on root-knot damage to *P. vulgaris*

4.4 .1 Bean nodule counts under selected bio-controls

There was an increment in the bean nodule counts in all the treatments in the two seasons (Tables 21 and 22). Both seasons recorded significant bean nodule count differences ($P<0.05$) in the selected

biocontrol agents with *Bacillus subtilis* strain K194 and *Rhizobium* strain USDA2674 giving significant higher counts, being most effective. Hence reduced root damage by nematodes. Strain K158 showed significantly lower counts almost equal to control.

A decline in bean nodule counts was observed with period of cultivation in both seasons. There were significant bean nodule count differences ($P<0.05$) in the farm age clusters in both seasons with the 0-10 years cluster recording significantly higher nodule counts and the >40 years cluster giving lower nodule counts. The interactions between biocontrols and period of bean cultivation were highly significant ($P<0.05$) for bean nodule counts in both seasons.

Table 21. Mean bean nodule numbers under different bio-inoculants in the short rains. Season.

Farm Conversion period (years)	Bio-inoculants*							
	Control	K158	K158+ USDA	K194	K194+ USDA	K263	K263+ USDA	USDA 2674
0-10	14	33	37	115	122	26	36	129
10-20	13	23	33	74	99	24	33	88
20-40	11	18	21	62	92	18	24	87
>40	11	17	18	50	34	16	20	75

**Bacillus subtilis* isolates; K158, K194, K263 and *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

$LSD_{(p=0.05)}$

Farm conversion period	3
Treatment	4
Farm conversion period x treatment	9
CV (%)	13.7

Table 22. Mean bean nodule numbers under different bio-inoculants in the long rains. Season.

Farm conversion period (years)	Bio-inoculants*							
	Control	K158	K158+ USDA	K194	K194+ USDA	K263	K263+ USDA	USDA 2674
0-10	16	39	50	128	123	34	50	148
10-20	15	27	40	84	116	32	42	131
20-40	13	24	28	81	110	24	29	126
>40	11	21	25	59	38	20	24	92

**Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	3
Treatment	4
Farm conversion period x treatment	9
CV (%)	13

4.4.2. Bean haulm dry weights under selected bio-inoculants

Bean haulm dry weights increased in all the treatments in both seasons (Tables 23 and 24). There were significant differences ($P<0.05$) between treatments with *Bacillus subtilis* strain K194 and *Rhizobium* strain USDA2674 giving higher weights compared to the control.

A reduction in bean haulm dry weights was observed with age of farms in both seasons. Whereas farms that were 1-10 years had significantly higher weights, those that were >40 years cluster showed significantly lower weights. The interactions between biocontrols and period of bean cultivation were highly significant ($P<0.05$) bean haulm dry weights in the short rains season but not significant in the long rains season (Tables 23 and 24).

Table 23. Mean bean haulm dry weights (kg ha^{-1}) under different bio-inoculants in the short rains season.

Farm conversion period (years)	Bio-inoculants*							
	Control	K158	K158+ USDA	K194	K194+ USDA	K263	K263+ USDA	USDA 2674
0-10	83.3	265.3	211.0	292.5	269.3	202.8	238.8	249.8
10-20	67.0	206.3	127.0	244.8	226.3	163.3	168.0	220.5
20-40	46.5	57.3	74.5	226.8	202.3	128.3	142.8	189.5
>40	43.0	33.3	41,0	178.3	187.3	92.5	97.0	131.0

**Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	6
Treatment	9
Farm conversion period x treatment	17
CV (%)	19.4

Table 24. Mean bean haulm dry weights (kg ha^{-1}) under different bio-inoculants in the long rains season.

Farm conversion period (years)	Bio-inoculants*							
	Control	K158	K158+ USDA	K194	K194+ USDA	K263	K263+ USDA	USDA 2674
0-10	148.3	261.8	241.0	310.0	352.0	225.3	272.8	282.8
10-20	122.5	257.0	191.0	301.5	257.5	182.0	247.3	220.3
20-40	96.5	191.3	185.3	206.8	175.3	159.3	162.8	193.8
>40	63.8	156.5	162.0	172.0	161.5	114.0	126.8	180.8

**Bacillus subtilis* isolates; K158, K194, K263 & *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	11
Treatment	16
Farm conversion period x treatment	31
CV (%)	28.0

4.4.3. Effect of selected bio-inoculants on bean grain yield

There was an increment in the bean grain dry weights in all the treatments in both seasons (Tables 25 and 26). Significant bean grain dry weight differences ($P<0.05$) in the selected biocontrol agents were observed in both seasons with *Bacillus subtilis* strain K194 and *Rhizobium* strain USDA2674 giving significantly higher weights while the control and *Bacillus subtilis* strain K158 showed significantly lower weights.

Bean grain dry weights declined with period of cultivation in both seasons. There were significant bean grain dry weight differences ($P<0.05$) in the four farm age clusters with 0-10 years recording higher weights and >40 years cluster showing lower weights in the short and long rain seasons. The interactions between biocontrols and period of bean cultivation were not significant for bean grain dry weights in both seasons (Tables 25 and 26).

Table 25. Mean bean grain yield (kg ha^{-1}) in the short rains season.

Farm conversion period (years)	Bio-inoculants*							
	Control	K158	K158+ USDA	K194	K194+ USDA	K263	K263+ USDA	USDA 2674
0-10	400.0	660.0	587.6	1493.6	1231.6	776.6	905.0	1059.0
10-20	337.6	588.6	252.0	1119.0	921.2	575.6	670.6	878.6
20-40	274.6	495.0	207.0	983.0	709.6	507.6	575.6	708.0
>40	198.0	401.0	158.8	800.6	676.6	438.0	407.0	519.6

* *Bacillus subtilis* isolates; K158, K194, K263 and *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	16
Treatment	22
Farm conversion period x treatment	44
CV (%)	23.8

Table 26. Mean bean grain yield (kg ha^{-1}) in the long rains season.

Farm Conversion period (years)	Bio-inoculants*							
	Control	K158	K158+ USDA	K194	K194+ USDA	K263	K263+ USDA	USDA 2674
0-10	461.0	913.6	765.6	1579.6	1105.6	632.6	1087.6	1235.6
10-20	390.0	726.6	649.0	1171.0	989.0	520.0	852.0	840.0
20-40	311.6	628.0	514.6	906.6	972.6	499.0	655.0	701.0
>40	225.6	526.0	474.0	838.6	738.0	216.0	609.6	609.6

* *Bacillus subtilis* isolates; K158, K194, K263 and *Rhizobium leguminosarum biovar phaseoli* strain USDA 2674

	$LSD_{(p=0.05)}$
Farm conversion period	20
Treatment	28
Farm conversion period x treatment	57
CV (%)	27.8

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Soil quality as affected by cultivation period

Results of this study generally show that continued land cultivation may lead to decline in soil pH, exchangeable; calcium, magnesium, potassium and phosphorus as well as total carbon and nitrogen. However the C: N increases with land conversion time. Phosphorus uptake by plants is greatly influenced by soil moisture, being largely controlled by diffusion rates, and P depletion in the rhizosphere (Gahoonia, Raza and Nielsen, 1994). Soil moisture influence N availability from organic N materials differently depending on source of N. Soil moisture and temperature are the major environmental factors affecting N availability from organic N sources. Because urea is readily soluble in water, urea hydrolysis is largely dependent on diffusion of dissolved urea in soil (Agehara and Warncke, 2005). The initial decline in total soil C and N can probably be attributed to the C and N being distributed more evenly through the plough layer after the initial farm cultivation after forest (Saggar *et al.*, 2001). As N is taken up by plants, C increases leading to higher C:N ratio. After cultivation, bases are used up by plants as well as leached resulting in lower pH.

5.2 Effect of soil properties on nematodes

The host plant plays a major role in the structure of nematode communities. This role can be direct through quantity and quality of the substrate or indirect through changes in soil properties (Kandji *et al.*, 2001). In cropped systems; soil texture, soil moisture and availability of substrate are critical in determining the diversity of nematode. In this study, higher nematode populations were recorded in the long rains than in the short rains. The nematode diversity was composed of

both the native species that had survived agricultural management systems and species that could have been introduced through different dispersion agents. Long-term cycles of land use influenced the proportion of various nematode taxa at particular times (Yeates, 1999) and agroecosystem may contain in excess of 50 nematode taxa in varying proportions, which is the case in this study where over 30 genera of nematodes were found in the bean plots and this agrees with (Yeates and Bongers, 1999).

Nematode populations increased with maize and bean physiological changes as the highest numbers were recorded at milk stage which agrees with the findings of Bloemers and Hodda (1995). This study, assessment of the effect of soil disturbance on nematode diversity, supports the findings that the populations of free-living nematodes reduce from younger conversions (age of the farm) to older ones and vice verse for plant parasitic ones (Geraert, 1965). The decline may be due to a combination of the use of pesticides, loss of organic inputs because of the use of herbicides and lower organic matter inputs generally by the conversion from forest to maize cropping (Saggar *et al.*, 2001; Yeates *et al.*, 1998). The increase in the population of plant parasitic nematodes could be attributed to predation on other soil organisms, continued planting of host crops and creation of good niches through cultivation (Saggar *et al.*, 2001). An intensive New Zealand study showed the greater importance of the soil rather than month and/or year of management practices in determining the composition of the nematode fauna, i.e. diversity and richness were all greatest in the young, mostly textually heterogeneous soil. Soil texture is important in affecting the movement, feeding and reproduction of all nematodes; however it is uncertain whether the soil mineralogy has a direct effect on the nematode fauna (Yeates and Bongers, 1999).

5.3 Effect of selected soil fertility management practices on dynamics of nematodes

The variability in nematode abundance and diversity observed in the cropping systems of Kakamega in Western Kenya is an indication that land management has a significant impact on nematode communities. The treatments in this study represented a range of soil fertility management improvement practices and crops that are likely to influence the he heterogeneity of the below-ground soil food webs including the nematode community. The more than 30 nematode taxa observed in this study; (Table 3) was less than in a Swedish arable cropping system of 50 (Sohlenius *et al.*, 1987).

Nematodes in the genera *Meloidogyne, Pratylenchus, Scutellonema,* and *Helicotylenchus* were widely distributed in maize/bean fields in Kenya which agrees with those reported by (Karanja *et al.*, 2006). Warm and wet conditions prevailing in the district (Jaetzold and Schmidt, 1983), coupled with long-term continuous growing of *Phaseolus vulgaris* (bean) and maize have influenced the build-up of the plant parasitic nematode population.

Increased nematode built-up following inorganic fertilizer application could be related to the important production of root biomass that triggers increased biological activity in the soil (Wasilewska, 1989; Lavelle, 1994). Similarly, Arancon *et al.*, (2004) reported that soils from all of the vermicompost-treated plots contained smaller populations of plant parasitic nematodes than soil from inorganic fertilizer-treated plots. Application of organic farm yard manure recorded lower nematode populations confirming findings by Miano (1999) who observed remarkable reduction in activity and/or mobility when second stage *Meloidogyne* juveniles were treated with extracts from organic amendments suggesting that substances released by decomposing amendments had nematostatic effects. According to Sikora (1992), organic

amendments have been used in the control of nematodes and specifically on root knot nematode densities (Mojumder *et al.*, 2000; Jonathan *et al.*, 2000; Leon *et al.*, 2000). According to Rodriguez-Kabana (1986) and Sayre and Starr (1988) presence of high organic matter stimulates the activity of indigenous soil microorganisms some of which are antagonistic to nematodes and their decomposition results in accumulation of compounds with nematicidal effects. Soils rich in organic matter have high quantities of available carbon and nitrogen which are key elements for the growth and multiplication of the actinomycete isolates (Porter, 1971). Actinomycetes produce antibiotics that are inhibitory to the growth of plant pathogen tested *invitro* (Muiru, 2000). Antibiotics production could be a survival mechanism that protects the actinomycetes from faster growing bacteria and fungi and nematodes in the soil ecosystem (Muiru, 2000; Porter, 1971).

Nematode populations in PRE-PAC treatment which is primarily a phosphate fertilizer were the lowest compared to the control. This is consistent with findings by Sinha and Neog (2003) who observed maximum nematode reduction in the soil (69.8%) and root (38.2%) in treatment with 240 g P and 200 g K/plant. According to Yeates and Bongers (1999), there are positive correlations between total nematodes and pH and phosphorus. Ammonia phosphate has been widely reported to adversely affect survival or germination of certain soil-borne fungi and nematodes (Rodriguez-Kabana, 1986; Muiru, 2000).

Increasing the intensity of cropland management, which is use of agrochemicals, is usually intended to increase plant production. This implies not only a greater plant resource for plant feeding nematodes but also larger populations of bacteria feeding nematodes, which contribute to

nutrient cycling and larger populations of predacious nematodes to feed on. In croplands there are clear relationships between the diversity of nematodes and soil texture, plant species, fertilizers and management practices (Yeates and Bongers, 1999). This relates well with the results of this study which show higher nematode populations in soils treated with N than the other treatments at maize harvesting stage.

5.4 Efficacy of *Bacillus subtilis* in controlling plant-parasitic nematodes

Bacillus subtilis strain K194 and *Rhizobium, Leguminosarum biovar phaseoli* strain $USDA$ 2674 suppressed nematode populations and this was in agreement with findings by others (Macharia, 2002; Kimenju *et al.*, 1998; Karanja *et al.*, 2006). The ability of these strains to suppress nematodes as observed in this study could be due to *Bacillus subtilis* production of toxic metabolites which kill nematodes (Mankau, 1995; Sikora and Hoffman-Hergarten, 1992). According to Oostenderp and Sikora (1989), penetration of *Heterodera schachtii* in sugar beet was inhibited through modification of root exudates after inoculation with *Bacillus subtilis*. Other mechanisms include induced systemic resistance and improved plant nutrition (Hallman *et al.*, 1998; Nagana-Parmar and Dadarwal, 1997). The presence of symbiotic microorganisms and their interaction with plants could also have led to nematode suppression through competition and/or antibiosis (Sikora, 1992). Likewise, Karanja *et al.*, (2006) reported lowest juvenile numbers in non-sterile soil treated with *Bacillus* isolate K194. Results of this study show that *Bacillus subtilis* counts increased with soil moisture content (long rains) and period of bean growth (harvesting time) but reduced with farm cultivation period (farm age clusters)

Bean plants treated with K194 yielded more biomass and grain than those treated with other *Bacillus subtilis* isolates (Tables 38 & 40). Growth promotion in plants treated with rhizobacteria especially

Bacillus and *Pseudomonas* spp. has also been reported by Pal *et al.*, (1999) and. Growth promotion has been associated with improved nutrient uptake, enhanced atmospheric nitrogen supply or induced disease resistance (Tuzun and Ku, 1991; Sikora, 1992). According to Sikora (1992), increased root hair formation provided increased surfaces for nutrient uptake. With respect to yield, the results show that the strategic use of biocontrols in *Meloidogyne spp* is inevitable.

The increased nodulation when some *Bacillus* isolates were applied together with rhizobium strains is consistent with earlier reports by Araujo *et al.*, (1999), El-Sayed (1999) and Pal *et al.*, (1999) in different crops. *Bacillus* isolates K194 and K273 consistently promoted bean nodulation and growth (Macharia, 2000). Other studies have also demonstrated that *Bacillus* and other organisms in the rhizosphere enhance nodule formation in leguminous plants (Srinivasan and Holl, 1996; Grimes and Mount, 1980). Increased nodulation could be attributed to increased root hair formation, production of phytohormones especially auxin and increased nitrogenase activity, (Holl *et al.*, 1988; Srinivasan *et al.*, 1996). The increased plant growth was observed in plants that had higher numbers of nodules and is consistent with findings by Srinivasan *et al.*, (1997), Araujo *et al.*, (1999) and El-Sayed, (1999). Nodulation is affected by microorganisms which alter the composition and activity of microflora in the rhizosphere (Schroth and Ole Becker, 1990). Several of the microbes referred to as nodulation promoting rhizobacteria (NPR) have been identified (Meyer, 2003; Li *et al.*, 2005). They belong to the genera; *Azospillum* (Schmidt *et al.*, 1988), *Pseudomonas* (Bolton *et al.*, 1990), *Streptomyces* (Li and Alexander, 1990) and *Bacillus* (Halverson and Handelsman, 1990).

5.4.1 Effect of combining *Bacillus* strains with *Rhizobium* bean strains on root-knot nematodes

The reduced plant damage by root-knot nematodes associated with dual inoculations of bean plants with *Bacillus subtilis* K194 and *Rhizobia, Leguminosarum biovar phaseoli* (strain USDA 2674) is consistent with earlier reports by Siddiqui (2002) which showed that use of *Bacillus subtilis* and *Bradyrhizobia japonica*, reduced nematode multiplication and wilting index. In this study, *Bacillus subtilis* strain K194 and *Rhizobium*, *Leguminosarum biovar phaseoli* strain USDA 2674 inoculation improved nodulation of the beans (Table 36). Srinivasan *et al.*, (1996) demonstrated that co-inoculation of *Rhizobium etli* 182 with *Bacillus* spp. induced root hair proliferation on *Phaseolus vulgaris* and enhanced nodulation due to production of indole-acetic acid (IAA) from *Bacillus* spp. In another study Srinivasan *et al.*, (1997), showed that co-inoculation of *Rhizobium etli* TAL 182 with *Bacillus megaterium* 549 resulted in early nodulation of *Phaseolus vulgaris* compared to single inoculation with *Rhizobium etli.* Formation provided increased surfaces for nutrient uptake.

The habitat surrounding crops play an important role in supporting and sustaining important natural enemies of plant parasitic nematodes (Maredia *et al.*, 1992; Landis *et al.*, 2000). Leguminous plants are known to form nodules on their roots resulting from symbiotic association with Bradyrhizobium. This process involves a sequential exchange of chemical signals between bacteria and the host plants (Fisher and Long, 1992; Relic *et al.*, 1994; Fellay, *et al.*, 1995). The insignificant interactions among biocotrols, time of nematode sampling and farm age cluster indicate that the efficacy of biocontrol is not inhibited by other factors in as so far as management of plant parasitic nematodes in beans is concerned.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Use of inorganic fertilizers release N and P that promotes multiplication of nematodes as observed in this study. Addition of soil organic amendments like farm yard manure reduced populations of nematodes due to production of toxic substances with nematocidal tendencies.

Potential *Bacillus subtilis* strains as biocontrol agent of root-knot nematode, *Meloidogyne* spp. as well as growth promoting agent in beans was demonstrated in the field. The strain K194 gave very consistent trends over three seasons.

The success of growing *Bacillus* sp. and *Rhizobia spp.* in one medium for production of a bi-inoculant was demonstrated and has been packaged.

Suitability of the nematode diversity as bioindicators of land use change/ intensification gradient was demonstrated.

High nematode populations were observed in the long rains than the short rains in plots planted with maize. This information indicates that priority may be given to plant parasitic nematodes in the long rains when designing pest management programmes in cereals. The significant interactions among soil fertility management practices, time of nematodes sampling and farm age cluster suggest that the populations of soil nematodes is influenced by fertility level, time of sampling and land conversion periods

6.2 RECOMMENDATIONS

1. Soil P and N levels need to be addressed as direct influence on plant parasitic nematodes. More research to be conducted in different climatic conditions to evaluate their effect on nematodes.
2. Use of farm yard manure need to be encouraged to the farmers as a way of managing soils sustainably through reduction of effects of pathogenic nematodes.
3. *Bacillus subtilis* strains K194 may be used to control nematodes, though research is required on efficacy and interaction with field abiotic and biotic conditions-soil/climate/cropping.

REFFERENCES

Abrams, B. I., Mitchell, M. J. 1980. Role of nematology - bacterial interaction in heterotrophic systems with emphasis on sewage sludge decomposition. Oikos 35, 404-410.

Acea, M. J., Alexander, M. 1988. Growth and survival of bacteria introduced into carbon-amended soil. *Soil Biol. Biochem.* 20:703-709.

Acland, J. D. 1971. East African Crops. Longman Group Limited, London 22p.

Agehara, S., Warncke, D. D. 2005. Soil Moisture and Temperature Effects on Nitrogen Release from Organic Nitrogen Sources. *Soil Sci. Soc. Am. J.* 69:1844-1855.

Agrios, G. N. 1988. Plant Pathology. Academic Press Incl. San Diego, California P.803.

Akhtar, M., Alam, M. M. 1993. Utilization of waste materials in nematode control: *a review: Bioresource Technology* 45, 1-7.

Arancon, N. Q., Galvis, P., Edwards, C., Yardim, E. 2004. The trophic diversity of nematode communities in soils treated with vermicompost. *Pedobiologia*, Vol. 47, pp. 736-740(5).

Araujo, F. F. de., Hungaria, M. de., Araujo, F. F. 1999. Nodulation and yield of soybeans co-inoculated with *Bacillus subtilis* and *Bradyrhizobium japonicaum/Bradyrhizobium elkanii. Pesquisa Agropecuaria Briasileira* 34:1633-1643.

Asner, G. P., Seastedt, T. R., Townsend, A. R. 1997. The decoupling of terrestrial carbon and nitrogen cycle: Human influences on land cover and nitrogen supply are altering natural biogeochemical links in the biosphere. *Bioscience* 47:226-234.

Badra, T., Khattab, M. M. 1980. The effect of nitrogen fertilizers on the growth of olive and in relation to infestations of *Rotylenchulus reniformis. Nematologia. Medit.* 8:67-72.

Badra, T., Yousif, G. M. 1979. Comparative effects of potassium levels on growth and mineral composition of intact and nematised cowpea and sour orange seedlings. *Nematol. Medit.* 7, 21-27.

Baker, K. F., Cook, R. J. 1974. Biological control of plant pathogens. *Phytopathology* 49:648-650.

Becker, J. O., Zavaleta-mejia, E., Colbert, S. F., Schroth, M.N., Weinhord, A. R., Hancock, J. G., Van Gundy, S. D. 1988. The effect of rhizobacteria on root-knot nematodes and gall formation. *Phytopathology* 78:1466-1469.

Bloemers, G. F., Hodda, M. 1995. A method for extracting nematodes from Tropical forest soil. *Pedobiologia* 39: 331-343.

Bolton, H. Jr., Elliott, L. F., Turco, R. F., Kennedy, A. C. 1990. Rhizoplane colonization of pea seedling by *Rhizobium leguminosarum* and a deleterious root colonizing Pseudomonas spp. and effect on plant growth. *Plant and Soil*, 123:121-124.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Ecologia* 83:14-19.

Brandbury, J. F. 1986. Guide to plant pathology bacteria. CABI. Mycological Institute Kew, Surrey England.

Bridge, J. 1996. Nematode management In Subsistence Agriculture. *Annual Review Phytopathology* 34; 201-225.

Brown, R. H., Kerry B. R. 1987. Principles and practice of nematode control in crops. Academic Press New York.447 pp.

Cadish, G., Giller, K. E. 1997. Driven by Nature-Plant Litter Quality and Decomposition. CAB International, Wallingford. 409 pp.

Caswell, E.P., Sarah, J. L., Apt, W. J. 1990. Nematode parasites of pineapple. Pp. 519-537 in M. Luc R. A. Sikora and J. Bridge eds. Plant Parasitic nematodes in Subtropical and Tropical Agriculture. Wallingford, UK; CAB International.

Chitwood, B. G. 1956. Root-knot nematode. A revision of genus *Meloidogyne* Geoldi 1887. *Proc. Helminthol. Soc.* Was.16: 90-104.

Cochrane, V. W. 1958. Physiology of fungi. New York: John Wiley.

Coleman, D. C., Crossly, D. A. 1996. Fundamentals of Soil Ecology. Pp. 51-108. Academic Press, San Diego.

Coleman, D. C., Cole, C.V., Elliot, E. T. 1984. Decomposition, organic matter turnover and nutrient dynamics in agroecosystems. In: Lawrence, B.R. Stinner and G.J. House (Eds), *Agricultural Ecosystems*. Unifying Concepts. Wiley, New York, pp. 84-440.

Cook, R., Chairman, J. 1987. Research Briefing Panel on Biological Control in Managed Ecosystems. Committee on Science, Engineering, and Public Policy, National Academy of Sciences, National Academy of Engineering, and Institute of Medicine. National Academy Press, Washington, DC. 12 pp.

Corry, J., Myers, J. 2000. Direct and indirect ecological effects of biological control Trends in Ecology & Evolution. 15, 4, 137-139.

Desaeger, J., Rao, M. R. 1999. The root-knot nematode (*Meloidogyne spp*) problem in Sesbania fallows and scope for management in western Kenya. Agrof. Sys. 47:273-288.

Dropkins, V. H. 1988. Introduction to Nematology. A Wilsey-Interscience Publ, NY. 293 pp.

El-Nagdi, W. M. A., Youssef, M .M. A. 2004. Soaking faba bean seed in some bio-agent as prophylactic treatment for controlling *Meloidogyne incognita* root-knot nematode infection. *J. Pest Sci.* 77: 75–78.

El-Sayed, S. A. M. 1999. Influence of Rhizobium and phosphate solubility bacteria on nutrient uptake and yield of lentil in the New Valley (Egypt). *Egyptian J. Soil Sci.* 39:175-186.

Eno, C. F., Blue, W. G., Good, J. M. 1955. The effect of anhydrous ammonia on nematodes, fungi, bacteria, and nitrification in some Florida soils. Proceedings *Soil Science Society of America* 19:55-58.

FAO-Unesco, 1992. Soil Map of thee World, 1:5000000, ten vols.

Faridah, H. I., Van der Maesen, L. J. G. 1997. Plant resources of Southeast Asia, No. 11. Auxiliary Plants. Backhuys Publishers, Leiden. The Netherlands, 389 pp.

Fellay, R., Rochepeau, P., Relic, B., Broughton, W. J. 1995. Signals to and emanating from *Rhizobium* largely controlled symbiotic specificity. In Pathogenesis and host Specificity in Plant Disease: *Histopathological, Biochemical, Genetic and Molecular basis.* Vol. I. Pregamon/ Elsevier Science Ltd. Tarrytown, N. Y. pp. 199-220.

Ferris, H., Matute, M. M. 2003. Structural and functional succession in nematode fauna of a soil food web. *Applied Soil Ecology*, 23, 93-110.

Fisher, R. F, Long, S. R. 1992. Rhizobium-plant signal exchange. *Nat.* 358:655-660.

Flegg, J. J. M., Hooper, D. J. 1970. Extraction of Free-living stages from soil. In Laboratory Methods for work with plant and soil nematodes (Ed., J.F. Southey), pp. 5-22, Technical Bulletin 2, Commonwealth Agricultural Bureau, Rothanmsted.

France, R. A., Abawi, G. S. 1994. Interaction between *M. incognita* and *Fusarium oxysporum f. sp. phaseoli* on selected bean genotype. *Journal of Nematol.* 26:467-474.

Freckman, D.W., Ettema, C. H. 1993. Assessing nematode communities in agro-ecosystems of varying human intervention. *Agric, Ecosys. and Environ.* 45, 239-261.

Gahoonia, D. S., Raza, S., Nielsen, N. E. 1994. Phosphorus depletion in the rhizosphere as influenced by soil moisture. *Plant and Soil* 159: 213-218.

Gaugler, R., Anwar, B. L. 2004. Nematode behaviour. CABI Publishing.

Gaur, H. S., Perry, R. S. 1991. The use of soil solarization for control of plant parasitic nematodes. *Nematol. Abstract*. 60(40): 153-167.

Geraert, E. 1965. The genus *Paratylenchus. Nematologica* 11:301-334.

Gethi, M., Muriithi, F. M., Macharia, N., Njoroge, K. 1997. Maize bean intercropping system in medium potential area of Kenya. Farm practice and research challenges. *African Crop Science* Conference Proceeding Vol. 3:756-770.

Giannakou, I. O., Prophetou-Athanasiadou, D. 2004. A novel non-chemical nematicide for the control of root-knot nematodes. *Appl. Soil Ecol.* 26: 69–79.

Giller, K. E., Beare, M. H., Lavelle, P., Izac, A. M. N., Swift, M. J. 1997. Agricultural Intensification, soil biodiversity and ecosystem function. *Appl. Soil Eco.* 6, 3-16.

Grimes, H. D., Mount, M. S. 1984. Influence of *Pseudomonas putida* on nodulation of *Phaseolus vulgaris*. *Soil Biology and Biochemistry*. 16-27-30.

Hallmann, J., Hasky-Guther, K., Hoffmann-Hergarten, S., Reitz, M., Sikora, R.A., Duffy, B., Roesnberger, U., Defago, G. 1998. Similarities and differences in the mode of action of two rhizosphere bacteria antagonistic to *Globodera pallida* on potatoes. Molecular approaches in biological control. Delemont, Switzerland, 15-18 September 1997. Bulletin-OILB-SROP 21:41-43.

Halverson, L. J., Handlesman, J. 1991. Enhancement of soybean nodulation by *Bacillus Cereus* UW85 in the field and in a growth chamber. *Appl. Environ. Microb.* 57:2767-2770.

Hasky-G¨unther, K., Hoffmann-Hergarten, S., Sikora, R. A. 1998. Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43). *Fund Appl. Nematol.* 21: 511–517.

Holl, F. B., Chanway, C. P., Turkington, R., Radley, R. A. 1988. Response of crested wheat grass (*Agropyron cristatum* L.) perennial rye grass (*Lolium perenne)* and white clove *(Triflolium repens* L.) to inoculation with *Bacillus polymyxa. Soil Biology and Biochemistry* 20:19-24.

Hooks, C. R .R., Wang, K. H., Fallon, D. 2006. An ally in the war against nematode pests: Using sun hemp as a cover crop to suppress root-knot nematodes. University of Hawai'i at Mānoa, College of Tropical Agriculture and Human Resources, Plant Disease publication PD-32. www.ctahr.hawaii.edu/ oc/freepubs/pdf/PD-32.pdf.

Hooper, D. J. 1969. Identification of plant and soil nematodes. In: *Nematodes of Tropical Crops* (Ed. Peachey, J.E.). St Albans, UK: Comm. Bur. Helminth., Tech. Commun. No. 40, pp. 37-66.

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, Wallington.

Jaertzold, R., Schmidt, H. 1983. Farm management Handbook of Kenya, Vol. III. Ministry of Agriculture, Nairobi, Kenya.

Jepson, S. B. 1987. Identification of root-knot nematodes. Wallingford. UK. CABI.

Jonathan, E. I., Gajendran, G., Manuel, W. W. 2000. Management of *Meloidogyne incognita* and *Helicotylenchus multicinctus* in banana with organic amendments. *Nematologia Mediterranea* 28:103-105.

Kandji, S.T., Ogol, C. K. P. O., Albrecht, A. 2001. Diversity of plant parasitic nematodes and their relationships with some soil physico-chemical characteristics in improved fallows in Western Kenya. *Applied Soil Ecology* 18:143-157.

Karanja, N. K. 1988. Selecting *Rhizobium phaseoli* strain for use with beans *Phaseolus vulgaris* in Kenya, PhD. Thesis University of Reading UK 221p.

Karanja, N, K., Kimenju, W. J., Macharia, I., Muiru M. D. 2006. Plant parasitic nematodes associated with common bean (*Phaseolus vulgaris* L.) and integrated management approaches. International Technical Workshop on Biological Management of Soil Ecosystems for Sustainable Agriculture. FAO. Case Study.

Kenya Soil survey Annual Report. 2004. Western Kenya, Part 11 29-56.

Kerry, B. R. 1987. Biological control. In principles and practice of nematode control in crops (ed) H.R. Brown, B.R. Kerry, 12:233-263. New York: Academic.

Kerry, B. R., de Leij, F. A. A. M. 1992. Key factors in the development of fungal agents for the control of cyst and root-knot nematodes. In *Biol. control of plant diseases*, P.139- 144. London, Plenum.

Kerry, B. R. 1990. An assessment of progress towards microbial control of plant parasitic nematodes. Supplements to *Journal of Nematology* 22(4): 621-631.

Kimenju, J. W. 1998. Identification and biological control of lesion nematodes (*Pratylenchus spp.)* affecting maize production in Kenya. PhD Thesis, Uni. of Nairobi, Kenya. 162pp.

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

Kimenju, J. W., Waudo, S. W., Mwang'ombe, A. W., Sikora, R. A., Schuster, R. P. 1998. Distribution of lesion nematodes associated with maize in Kenya and susceptibility of maize cultivar to *Pratylenchus zeae*. *African Crop Science Journal* 6:367-375.

Kloepper, J. W., Schroth, M. N. 1981. Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. *Phytopathology* 71:590-592.

Koon-Hui Wang, Cerruti Hooks, R., Antoon Ploeg. 2007. Protecting Crops from Nematode Pests: Using Marigold as an Alternative to Chemical Nematicides. Department of Plant and Environmental Protection Sciences, University of Hawai'i at Mänoab Department of Nematology, University of California, Riverside.

Krebs, B., Hoeding, B., Kuebart, S., Workie, M. A., Junge, H., Schmiedeknecht, G., Grosch, R., Bochow, H., Hevesi, M. 1998. Use of *Bacillus subtilis* as biocontrol agent. In: Activities and characterization of *Bacillus subtilis* strains. *Zeitschrift Pflanzenkrankh Pflanzenschutz* 105: 181–197.

Lambshead, P. J. D. 1986. Subcatastrophic sewage and industrial waste contamination as revealed by marine nematode fauna analysis. *Marine Ecology*. Ser. 29:247-260.

Landis, D., Wratten, B. D., Gurr, C. 2000. Habitat manipulation to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45:173-199.

Lavelle, P. 1994. Faunal activities and soil processes: adaptive strategies that determine ecosystem function. IN: Transactions of the 15th World Congress of Soil Science; 1994 July 10-16; Acapulco, Mexico. Volume 1. Mexico City, Mexico: *Mexican Society of Soil Science*; 189-220.

Lawton, J. H., Bignell, D. E., Bloemers, G. F. Eggleton, P., Hodda, M. E. 1996. Carbon flux and diversity of nematodes and termites in Cameroon forest soils. *Biodiversity and Conservation* 5, 261.

Lee, D. L. 2002. The biology of nematodes. Taylor and Francis, London UK. 635 pp.

Leon, L., Bancherol, L., Lopex, P., Bellow, A. 2000. Control of *Meloidogyne incognita* in tomato crops in Uruguay. Bulletin-de-Sanidad vegetal. *Plagal* 26:401-407.

Li, B., Xie, G.L., Soad, A., Coosemans, J. 2005. Suppression of *Meloidogyne javanica* by antagonistic and plant growth promoting rhizobacteria. *J. Zhejiang Uni. Sci.* 6B:496–501.

Li, D. M., Alexander, M. 1990. Factors affecting co-inoculation with antibiotic- producing bacteria to enhance rhizobial colonization and nodulation. *Plant and Soil* 131:195-205.

Luc, M., Sikora, R. A., Bridge, J. 1990. Plant Parasitic Nematodes in Subtropical Tropical Agriculture, Institute of Parasitology, CAB International, Wallingford, UK, pp.629.

Luc, M., Sikora, R. A., Bridge, J. 2005. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture CABI Publishing, Oxfordshire, UK. 871 pp.

Macharia, I. 2002. Biological control of root knot nematodes (*Meloidogyne* spp.) in common bean using *Bacillus* spp. MSc. Thesis University of Nairobi.

Madumadu, G. G. 1979. Inheritance of resistance to root-knot nematodes, *Meloidogyne javanica* (treab) Chitwood, and some yield characters in tomatoes *L. esculenthum mill.* MSc. Thesis University of Nairobi.

Mai, W. F., Lyon, H. H. 1975. *Pictorial key to genera of plant-parasitic nematodes*. Comstock Publishing associates, London.

Mani, A., Koshy, P. K., Swamp, P. G. 1982. Nematodes associated with grain and forage legumes. FAO. Technical Document No.129 7p.

Mankau, R. 1980. Biological control of *Meloidogyne* spp. population by *Bacillus penetrans* in West Africa. *Journal of Nematology*.1:12-17

Mankau, R. 1995. Bacteria antagonist of nematodes. *Biocontrol* 1:3, 15-28, 38.

Maredia, K. M., Gage, S. H., Landis, D. A., Wirth, T. M. 1992. Ecological observation on predatory Cocropillidae in southwestern Michigan. *Great Lakes Entom.* 25:265-270.

Marshall, V. G. 1977. Effect of manure and fertilizers on soil fauna: a review. Special Publication No.3. Commonwealth Bureau of Soils. CABI, Wallingford, UK.

Mateille, T., Trudgill, D. L., Trivino, C., Bala, G., Abdoussalam, S.,Vouyoukalou, M. E., 1969. *Principles of Systematic Zoology*. New York, USA: McGraw-Hill.

McSorley, R. 2001. Multiple Cropping Systems for nematode Management. A review soil and crop science society of Florida proceedings 49: 111-118.

Meyer, S. L. F. 2003. United States Department of Agriculture –Agricultural Research Service research programs on microbes for management of plant-parasitic nematodes. *Pest Manag Sci* 59: 665–670.

Miano, D. W. 1999. Optimization of carbon :nitrogen ratios in organic soil amendments for the control of root-knot nematodes (*Meloidogyne spp.*) in tomato. MSc. Thesis. Uo N.

Milner, J. L., Stohl, E. A., Handelsman, J. 1996. Production of Kanosamine by *Bacillus cereus* strain UW85. *Appl. Environ.Microbiol.* 62:3061-3065.

Milner, J. L., Stohl, E. A., Handelsman, J. 1996. Zwittermicin A resistance gene from *Bacillus cereus. J. Bacteriol.*, 07 1996, 4266-4272, Vol. 178, No. 14.

Mojumder, V., Nawal, S. S., Hoagland, R. W., Dilday, R. H., Reigosa, M. J. 2000. Ecofriendly technologies for management of phytoparasitic nematodes in pulses and vegetable crops. Allelopathy in ecological agriculture and forestry. Proceedings of the 3rd International congress on Allelopathy in Ecological Agriculture and Forestry 59-69.

Moss, W. W., Webster, W. A. 1970. Phenetics and numerical taxonomy applied to systematic Nematology. *Journal of Nematology* 2, 16-25.

Muiru, W. M. 2000. Isolation of soil actinomycetes characterization and screening of their antibiotics against economically important plant pathogens. MSc. Thesis Uni. of Nairobi.

Muller, R., Gooch, P. S. 1982. Organic amendments in nematode control. An examination of the literature. *Nematropica* 12:319-326.

Mutuo, P. K., Smithson, P. C., Buresh, R. J., Okalebo, R. J. 1999. Comparison of phosphate rock and triple superphosphate on phosphorus deficient Kenyan soil. Commun. *Soil Sci Plant anal.* 30:1091-1103.

Nagana-Parmar, Dadarwal, K. R. 1997. Rhizobacteria from rhizosphere and rhizoplane of chickpea (*Circer arietinum* L.). *Indian Journal of Microbiology.* 37:205-210.

Netscher, C., Sikora, R. A. 1990. Nematode parasites of vegetables. In: Plant parasitic nematodes in Subtropical and tropical agriculture. M. Luc, R.A. Sikora and J.B. Bridge (Eds) CAB International.

Ngundo, B. W. 1977. Screening of bean cultivars for resistance to *meloidogyne* spp. In Kenya. Plant Disease Reporter 61:991-993.

Nickle, W. R. 1991. Manual of Agric. Nematology. Marcel Dekker, Inc. NewYork 1035 pp.

Oka, Y., Chet, I., Spiegel, Y. 1993. Control of root-knot nematodes *Meloidogyne javanica* by *Bacillus cereas*. *Biocontrol Science and technology* 3:115-126.

Omwega, C. O., Thomason, I. J., Roberts, P. A. 1990. A single dominant gene in common bean conferring resistance to three root-knot nematode species. *Phytopathol.* 80:745-748.

Oostendorp, M., Sikora, R. A. 1989. Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugarbeets. *Review of Nematolology*. 12:77-83.

Oostendorp, M., Sikora, R. A. 1990. In vitro interrelationship between rhizosphere bacteria and *Heterodera schachtii*. *Rev. Nematol.* 13:269-274.

Ownley, G. B., Morgan-Jones, G., Rodriguez-Kabana, R. 1983. Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soybean field soil. *Nematropica* 13:181 -200.

Pal, K. K., Rinku, D., Bhatto, D. M., Chauhan, S., Dey, R. 1999. Enhancement of groundnut growth and yield by plant growth promoting rhizobacteria. International *Arachis* Newsletter 19:51-52.

Perry, R. N., Wright, D. J. 1998. The Physiology and Biochemistry of Free – Persistence of *Steinernema spp*. *Journal of nematology* 22, 440-445.

Ploeg, A. T. 2002. Effect of selected marigold varieties on root-knot nematodes and tomato and melon yields. *Plant Disease* 86: 505-508.

Porter, N. J. 1971. Prevalence and distribution of antibiotic producing actinomycetes. *Advances in Applied Microbiology* 14:73-92.

Prot, J. C., Van Gundy, S. D. 1981. Effects of soil texture and clay component on migration of *Meloidogyne incognita* second stage juveniles. *Nematology* 14:213-219.

Saggar, S., Yeates, G. W., Shepherd, T. G. 2001. Cultivation effects on soil biological properties, microfauna and organic matter dynamics in Eutric Gleysol and Gleyic Luvisol soils in New Zealand. *Soil Tillage Res* 58:55–68.

Sayre, R. M., Starr, M. P. 1988. Bacterial disease and antagonism of nematodes. *Diseases of Nematodes* pp.69-101.

Schmidt, W., Martin, P., Omay, S. H., Bangerth, F. 1988. Influence of *Azospirillum* on nodulation of legumes. *In Azospirillum Genetic, Physiology Ecology.* Vol. Springnger-Verlag. Heidelberrg. German pp 92-100.

Schroth, M. N., Ole Becker, J. 1990. Concept of ecological and physiological activities of rhizobacteria related to biological control and plant growth promotion. In *Biological Control of Soil-Borne Plant Pathogens.* CAB. International. Wallingford. UK. p389-414.

Siddiqui, I. A. 2002. Suppression of *Meloidogyne javanica* by *Pseudomonas aeruginosa* and *Bacillus subtilis* in tomato. *Nematologia Mediterranea* 30: 125–130.

Siderius, W. 1976. Environment and Characterization of Nitosol at N.A.L Kenya soil survey. Miscellaneous Soil paper No. 10,1976.

Sikora, R. A. 1990. The concept of using plant-promoting rhizobacteria for the biological control of plant parasitic nematodes. WPRS Bulletin. Interlaken Switzerland (Abst).

Sikora, R. A. 1991. The concept of using plant health promoting rhizobacteria for the biological control of plant parasitic nematodes. 2nd International workshop. Plant Growth Promoting Rhizobacteria. Interlaken Switzerland WPRS Bulletin XIV 8:3-10.

Sikora, R. A. 1992. Management of the antagonistic potential in agricultural systems for the biological control of plant parasitic nematodes. *Ann. review of phytopathol* 30:245-270.

Sikora, R. A. 1995. Utilization of symbiotic and mutualistic rhizosphere microorganism for biological control of plant parasitic nematode pg 233-236. In XXVII Annual meeting of the organization of nematologists of tropical America Rio Quente Brazil.

Sikora, R. A. 1997. Biological system management in the rhizosphere and inside-outside-in perspective. In *review of plant pathology* CABI. Abstracts 1998 volume 77(6):638.

Sikora, R. A., Hoffmann-Hergarten, S. 1992. Importance of plant health promoting rhizobacteria for the control of soil borne fungal diseases and plant parasitic nematodes. *Arab J.P.I. Prot.* 10: 48-53.

Sikora, R. A., Hoffmann-Hergarten, S. 1993. Biological control of plant parasitic nematodes with plant-health promoting rhizobacteria. Biologically based technology (Lumsden PD & Vaugh JL, eds), pp. 166–172. ACS Symposium series, USA.

Silo-Suh, L. A., Lethbridge, B. J., Raffel, S. J., He, H., Clardy, J., Handelsman, J. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus cereus*. UW85. *Appl. Environ. Microbio.* 60: 2023-2030.

Sinha, A. K., Neog, P. P. 2003. Effect of different levels of NPK fertilizers against citrus nematode (*Tylenchulus semipenetrans*) on Khasi mandarin. *Indian Journal of Nematology*, (Vol. 33) (No. 1) 61-62.

Skerman, P. J. 1976. Tropical forage legumes. FAO (Food and Agriculture Organization of the United Nations), Rome, Italy. pp. 186-194.

Sohlenius, B. 1990. Influence of cropping system and nitrogen input on soil fauna and microorganisms in a Swedish arable soil. Biology and *Fertility of Soils* 9, 168-173.

Sohlenius, B., Sandor, A. 1987. Vertical distribution of nematodes in arable soil under grass (*Festuca pratensis*) and barley (*hordeum distichum*). *Bio. & Fert. of Soils* 3, 19-25.

Sikora, R. A. 1995. Utilization of symbiotic and mutualistic rhizosphere microorganism for biological control of plant parasitic nematode pg 233-236. In XXVII Annual meeting of the organization of nematologists of tropical America Rio Quente Brazil.

Sikora, R. A. 1997. Biological system management in the rhizosphere and inside-outside-in perspective. In *review of plant pathology* CABI. Abstracts 1998 volume 77(6):638.

Sikora, R. A., Hoffmann-Hergarten, S. 1992. Importance of plant health promoting rhizobacteria for the control of soil borne fungal diseases and plant parasitic nematodes. *Arab J.P.I. Prot.* 10: 48-53.

Sikora, R. A., Hoffmann-Hergarten, S. 1993. Biological control of plant parasitic nematodes with plant-health promoting rhizobacteria. Biologically based technology (Lumsden PD & Vaugh JL, eds), pp. 166–172. ACS Symposium series, USA.

Silo-Suh, L. A., Lethbridge, B. J., Raffel, S. J., He, H., Clardy, J., Handelsman, J. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus cereus*. UW85. *Appl. Environ. Microbio.* 60: 2023-2030.

Sinha, A. K., Neog, P. P. 2003. Effect of different levels of NPK fertilizers against citrus nematode (*Tylenchulus semipenetrans*) on Khasi mandarin. *Indian Journal of Nematology.* (Vol. 33) (No. 1) 61-62.

Skerman, P. J. 1976. Tropical forage legumes. FAO (Food and Agriculture Organization of the United Nations), Rome, Italy. pp. 186-194.

Sohlenius, B. 1990. Influence of cropping system and nitrogen input on soil fauna and microorganisms in a Swedish arable soil. Biology and *Fertility of Soils* 9, 168-173.

Sohlenius, B., Sandor, A. 1987. Vertical distribution of nematodes in arable soil under grass (*Festuca pratensis*) and barley (*hordeum distichum*). *Bio. & Fert. of Soils* 3, 19-25.

Sohlehius, B., Bostrom, S., Sandor, A. 1987. Long-term dynamics of nematode communities in arable soil under four cropping systems. *J. appl. Ecol.*, 24:131- 144.

Southey, J. F. 1986. Laboratory methods for work with plant and soil nematodes. *soybean crop Science Journal* 45(3): 854-858.

Srinivasan, M., Petersen, D. J., Holl, F. B. 1996. Influence of IAA producing *Bacillus* isolates on the nodulation *of Phaseolus vulgaris* by *Rhizobium etli. Can. Journal. mirobiol.*42:1006-1014.

Srinivasan, M., petresen, D. J., Holl, F. B. 1997. Nodulation of *Phaseoulus vulgaris* by *Rhizobium etli* is enhanced by the presence of *Bacillus spp. Canadian J. Microbio.*43:1-8.

Stirling, G. R. 1991. Bio control of plant parasitic nematodes. CABI. Wallingford Oxon, UK.

Stotzky, G., Burns, G. R. 1982. The soil environment: clay-humus-microbe systematic Nematology. *Journal of Nematology* 2:16-25.

Stover, R. H. 1979. Flooding of soil for disease control. In soil disinfestations (Development in Agricultural and managed forestry ecology). Ed. D. Muller 6:19-28. Amsterdam. The Netherlands.

Taha, A. H. Y. 1993. Nematode interaction with root-nodule bacteria. In: Nematode interaction. Khan M.W. (Ed) pp175-202 Chapman and hall, London, UK.

Taylor, J. D., Day, J. M, Podley, C. L. 1982. The effect of Rhizobium inoculation and nitrogen fertilizer on nitrogen fixation and seed yield of dry beans (*Phaseolus vulgaris). Animals of Applied Biology* (103(3): 419-429.

Tuzun, S., Ku, J. 1991. Plant immunization. An alternative to pesticides for control of plant diseases in greenhouse and field. Pg30-40. In the biological control of plant diseases, food and fertilizer technology center for Asia and Pacific Region of Taipei Taiwan.

Vassalo, M. 1968. The nematicidal power of ammonia. Compte Rendue 8th International Symposium of Nematology. P. 128.

Vones, N., Castillo, P., Troccoli, A. 1998. Histology of nodular tissue of three leguminous hosts infected by three root-knot nematode species. *International J. Nemato.* 8:105-110.

Waage, J., Greathead, D. J. 1988. Biological control: challenges and opportunities. In R. K. S. Wood and M. J. Way (eds.). Biological Control of Pests, Pathogens and Weeds: Developments and Prospects. The Royal Soc., London. pp 1-18.

Walker, J. T. 1971. Populations of *Pratylenchus penetrans* relative to decomposing nitrogenous soil amendments. *Journal of Nematology* 3:43-49.

Ware, G. W. 1983. Pesticide theory and application, W.H. Freeman and Company. New York pp.70-73.

Wasilewka, L. 1989. Impact of human activities on nematode communities in terrestrial ecosystems. In: M. Clarholm and L. Begstroem (Editors). *Ecology of Arable Land.* Kluwer, Dor drecht, pp 123-132.

Weller, D. M., Cook, R. J. 1988. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* 73:463-469.

Wortmann, C. S. 1998. Assessment of yield loss caused by biotic stress on beans in Africa. CIAT Occasional publications series, No. 4, Dar Es Salaam, Tanzania.

Xi, K., Stephens, J. H. G., Vernna, P. R. 1996. Application of formulated rhizobacteria against root-rot of field peas. *Plant pathology* 45(6) 1150-1158.

Yeates, G. W. 1979. Soil nematodes in terrestrial ecosystem. *J. Nematol.* 11:213-229.

Yeates, G. W. 1999. Effects of plants on nematode community structure. *Ann. Rev Phytopathology.* 37:127-49.

Yeates, G. W. 2003. Nematodes as soil indicators: functional and biodiversity aspects. *Biol. Fertil. Soils* 37:199–210.

Yeates, G. W., Shepherd, T. G., Francis, G. S. 1998. Contrasting response to cropping of populations of earthworms and predacious nematodes in four soils. *Soil Tillage Res* 48:255–264.

Yeates, G. W., Bongers, T. 1999. Nematode diversity in agroecosystems *Agriculture, Ecosystems and Environment*, 74, 113-135.

Yeates, G.W., Bongers, T., de Goedes, R. G. M., Freckman, D. W., Georgiave, S. S. 1993. Feeding habits in soil nematode families and genera- an outline for soil ecologists. *Journal of Nematology* 25:325-331.

Zuberer, D. A. 1994. Methods of soil analysis. SSSA Bk series Mad. USA. Pp 119-144.

APPENDICES

Appendix 1: ANOVA for the soil pH measured across different land use types in selected age-clusters

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Age Cluster	5	10.25228918	2.05045784	2.75	0.0353
Rep	10	4.23939888	0.42393989	0.57	0.8262

Appendix 2: ANOVA for the soil ExCa measured across different land use types in selected age-clusters

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Age Cluster	5	522.1512656	104.4302531	17.00	<.0001
Rep	10	89.7218211	8.9721821	1.46	0.1997

Appendix 3: ANOVA for the soil ExMg measured across different land use types in selected age-clusters

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Age Cluster	5	44.41148874	8.88229775	9.37	<.0001
Rep	10	10.82843319	1.08284332	1.14	0.3638

Appendix 4: ANOVA for the soil ExK measured across different land use types in selected age-clusters

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Age Cluster	5	0.62249484	0.12449897	5.97	0.0005
Rep	10	0.40999130	0.04099913	1.97	0.0718

Appendix 5: ANOVA for soil ExP measured across different land use types

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Age Cluster	5	76.3968887	15.2793777	0.91	0.4878
Rep	10	173.4522170	17.3452217	1.03	0.4404

Appendix 6: ANOVA for soil C measured across different land use types

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Age Cluster	5	55.30714291	11.06142858	19.91	<.0001
Rep	10	17.55154518	1.75515452	3.16	0.0064

Appendix 7: ANOVA for the soil N measured across different land use types in selected age-clusters

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Age Cluster	5	0.63247388	0.12649478	26.09	<.0001
Rep	10	0.10356387	0.01035639	2.14	0.0506

Appendix 8: ANOVA for the soil CN measured across different land use types in selected age-clusters

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Age Cluster	5	125.8982638	25.1796528	16.02	<.0001
Rep	10	11.5355703	1.1535570	0.73	0.6877

Appendix 9: ANOVA for the percent soil moisture content in soil under maize treated with various soil fertility management practices in the short rains of 2005

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	3	16.6333578	5.5444526	5.32	0.0014
Rep	3	0.0707141	0.0235714	0.02	0.9954
Location	3	42.6593516	14.2197839	13.65	<.0001
Time	3	422.0979641	140.6993214	135.09	<.0001

Appendix 10: ANOVA for the percent soil moisture content in soil under maize treated with various soil fertility management practices in the long rains of 2006

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	3	35.55454	11.85151	8.01	<.0001
Rep	3	0.00132	0.00044	0.00	1.0000
Location	3	76.01223	25.33741	17.13	<.0001
Time	3	55059.58745	18353.19582	12405.00	<.0001

Appendix 11: ANOVA for the plant-parasitic nematode populations in soils treated with various soil fertility management practices in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	915.1	305.0	1.47	
Location	3	116454.2	38818.1	187.56	<.001
Time	3	712653.4	237551.1	1147.79	<.001
Treatment	3	262037.8	87345.9	422.04	<.001
Location.Time	9	43190.7	4799.0	23.19	<.001
Location.Treatment	9	255712.6	28412.5	137.28	<.001
Time.Treatment	9	55235.3	6137.3	29.65	<.001
Location.Time.Treatment	27	53497.2	1981.4	9.57	<.001
Residual	189	39116.1	207.0		
Total	255	1538812.4			

Appendix 12: ANOVA for the plant-parasitic nematodes population in soils treated with various soil fertility management practices in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	391.0	130.3	0.89	
Location	3	107070.7	35690.2	243.44	<.001
Time	3	500689.5	166896.5	1138.38	<.001
Treatment	3	630452.0	210150.7	1433.41	<.001
Location.Time	9	20630.1	2292.2	15.64	<.001
Location.Treatment	9	128739.5	14304.4	97.57	<.001
Time.Treatment	9	26658.2	2962.0	20.20	<.001
Location.Time.Treatment	27	61022.3	2260.1	15.42	<.001
Residual	189	27709.0	146.6		
Total	255	1503362.1			

Appendix 13: ANOVA for the *Pratylenchus spp.* population in soils treated with various soil fertility management practices in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	319.53	106.51	2.20	
Location	3	25177.34	8392.45	173.72	<.001
Time	3	77855.47	25951.82	537.20	<.001
Treatment	3	61571.88	20523.96	424.84	<.001
Location.Time	9	6790.62	754.51	15.62	<.001
Location.Treatment	9	40074.22	4452.69	92.17	<.001
Time.Treatment	9	7077.34	786.37	16.28	<.001
Location.Time.Treatment	27	4639.06	171.82	3.56	<.001
Residual	189	9130.47	48.31		
Total	255	232635.94			

Appendix 14: ANOVA for the *Pratylenchus spp.* population in soils treated with various soil fertility management practices in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	164.06	54.69	1.23	
Location	3	14011.72	4670.57	105.11	<.001
Time	3	129527.34	43175.78	971.64	<.001
Treatment	3	52600.78	17533.59	394.58	<.001
Location.Time	9	1939.06	215.45	4.85	<.001
Location.Treatment	9	40790.62	4532.29	102.00	<.001
Time.Treatment	9	3784.38	420.49	9.46	<.001
Location.Time.Treatment	27	10777.34	399.16	8.98	<.001
Residual	189	8398.44	44.44		
Total	255	261993.75			

Appendix 15: ANOVA for the plant non-parasitic nematodes population in soils treated with various soil fertility management practices in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	341.41	113.80	1.17	
Location	3	24421.88	8140.62	83.47	<.001
Time	3	622954.69	207651.56	2129.06	<.001
Treatment	3	174028.91	58009.64	594.77	<.001
Location.Time	9	16317.19	1813.02	18.59	<.001
Location.Treatment	9	337583.59	37509.29	384.58	<.001
Time.Treatment	9	28660.16	3184.46	32.65	<.001
Location.Time.Treatment	27	106202.34	3933.42	40.33	<.001
Residual	189	18433.59	97.53		
Total	255	1328943.75			

Appendix 16: ANOVA for the plant non-parasitic nematodes population in soils treated with various soil fertility management practices in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	189.36	63.12	1.44	
Location	3	37055.76	12351.92	282.39	<.001
Time	3	479569.82	159856.61	3654.69	<.001
Treatment	3	521960.45	173986.82	3977.73	<.001
Location.Time	9	59285.25	6587.25	150.60	<.001
Location.Treatment	9	54472.75	6052.53	138.37	<.001
Time.Treatment	9	32096.19	3566.24	81.53	<.001
Location.Time.Treatment	27	43147.17	1598.04	36.53	<.001
Residual	189	8266.89	43.74		
Total	255	1236043.65			

Appendix 17: ANOVA for the plant-parasitic nematodes populations in soils under beans treated with biocontrols in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	120.8	40.3	0.35	
Location	3	40225.1	13408.4	117.04	<.001
Time	3	277293.1	92431.0	806.83	<.001
Treatment	7	294785.5	42112.2	367.60	<.001
Location.Time	9	3773.9	419.3	3.66	<.001
Location.Treatment	21	70747.9	3368.9	29.41	<.001
Time.Treatment	21	29933.1	1425.4	12.44	<.001
Location.Time.Treatment	63	31176.5	494.9	4.32	<.001
Residual	381	43647.9	114.6		
Total	511	791703.9			

Appendix 18: ANOVA for the plant-parasitic nematodes populations in soils under beans treated with biocontrols in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	815.2	271.7	2.08	
Location	3	37009.0	12336.3	94.34	<.001
Time	3	742092.6	247364.2	1891.64	<.001
Treatment	7	563039.8	80434.3	615.10	<.001
Location.Time	9	5879.7	653.3	5.00	<.001
Location.Treatment	21	83948.0	3997.5	30.57	<.001
Time.Treatment	21	17686.3	842.2	6.44	<.001
Location.Time.Treatment	63	23294.5	369.8	2.83	<.001
Residual	381	49822.3	130.8		
Total	511	1523587.5			

Appendix 19: ANOVA for the *Meloidogyne spp.* populations (number/100cm^3) in soils under beans treated with biocontrols in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1308.40	436.13	6.88	
Location	3	21692.38	7230.79	114.12	<.001
Time	3	75283.40	25094.47	396.04	<.001
Treatment	7	113434.96	16204.99	255.75	<.001
Location.Time	9	1502.15	166.91	2.63	0.006
Location.Treatment	21	49231.84	2344.37	37.00	<.001
Time.Treatment	21	8968.95	427.09	6.74	<.001
Location.Time.Treatment	63	5445.51	86.44	1.36	0.043
Residual	381	24141.60	63.36		
Total	511	301009.18			

Appendix 20: ANOVA for the *Meloidogyne spp.* populations (number/100cm^3) in soils under beans treated with biocontrols in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1706.84	568.95	10.93	
Location	3	39673.63	13224.54	254.08	<.001
Time	3	422697.85	140899.28	2707.05	<.001
Treatment	7	117720.90	16817.27	323.10	<.001
Location.Time	9	2314.26	257.14	4.94	<.001
Location.Treatment	21	78108.40	3719.45	71.46	<.001
Time.Treatment	21	23499.80	1119.04	21.50	<.001
Location.Time.Treatment	63	19197.46	304.72	5.85	<.001
Residual	381	19830.66	52.05		
Total	511	724749.80			

Appendix 21: ANOVA for the plant non-parasitic nematodes populations in soils under beans treated with biocontrols in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	353.32	117.77	1.85	
Location	3	15118.95	5039.65	79.15	<.001
Time	3	660994.73	220331.58	3460.39	<.001
Treatment	7	108516.99	15502.43	243.47	<.001
Location.Time	9	3975.20	441.69	6.94	<.001
Location.Treatment	21	94784.18	4513.53	70.89	<.001
Time.Treatment	21	20961.52	998.17	15.68	<.001
Location.Time.Treatment	63	36481.05	579.06	9.09	<.001
Residual	381	24259.18	63.67		
Total	511	965445.12			

Appendix 22: ANOVA for the plant non-parasitic nematode populations in soils under beans treated with biocontrols in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	251.8	83.9	0.72	
Location	3	41696.3	13898.8	119.95	<.001
Time	3	772725.2	257575.1	2222.88	<.001
Treatment	7	266732.6	38104.7	328.84	<.001
Location.Time	9	8967.4	996.4	8.60	<.001
Location.Treatment	21	55170.9	2627.2	22.67	<.001
Time.Treatment	21	17770.1	846.2	7.30	<.001
Location.Time.Treatment	63	21068.6	334.4	2.89	<.001
Residual	381	44148.2	115.9		
Total	511	1228531.1			

Appendix 23: ANOVA for *Bacillus subtilis* counts in soil under beans treated with selected biocontrols in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	3.692E+10	1.231E+10	0.67	
Location	3	9.953E+13	3.318E+13	1798.73	<.001
Time	1	7.010E+12	7.010E+12	380.06	<.001
Treatment	7	1.188E+15	1.697E+14	9202.11	<.001
Location.Time	3	1.900E+11	6.333E+10	3.43	0.018
Location.Treatment	21	4.936E+14	2.350E+13	1274.21	<.001
Time.Treatment	7	8.033E+12	1.148E+12	62.22	<.001
Location.Time.Treatment	21	9.015E+12	4.293E+11	23.27	<.001
Residual	189	3.486E+12	1.845E+10		
Total	255	1.809E+15			

Appendix 24: ANOVA for *Bacillus subtilis* counts in soil under beans treated with selected biocontrols in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	3.036E+10	1.012E+10	0.32	
Location	3	1.234E+14	4.114E+13	1313.53	<.001
Time	1	1.324E+13	1.324E+13	422.67	<.001
Treatment	7	2.064E+15	2.948E+14	9413.12	<.001
Location.Time	3	4.023E+11	1.341E+11	4.28	0.006
Location.Treatment	21	2.791E+14	1.329E+13	424.29	<.001
Time.Treatment	7	9.667E+12	1.381E+12	44.10	<.001
Location.Time.Treatment	21	5.626E+12	2.679E+11	8.55	<.001
Residual	189	5.919E+12	3.132E+10		
Total	255	2.501E+15			

Appendix 25: ANOVA for the bean nodule counts under selected biocontrols in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	167.31	55.77	1.42	
LOCATION	3	15369.81	5123.27	130.03	<.001
TREATMENT	7	125751.75	17964.54	455.95	<.001
LOCATION.TREATMENT	21	20389.81	970.94	24.64	<.001
Residual	93	3664.19	39.40		
Total	127	165342.88			

Appendix 26: ANOVA for bean nodule counts under selected biocontrols in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	59.15	19.72	0.29	
LOCATION	3	18371.46	6123.82	91.43	<.001
TREATMENT	7	184990.30	26427.19	394.56	<.001
LOCATION.TREATMENT	21	21572.73	1027.27	15.34	<.001
Residual	93	6229.10	66.98		
Total	127	231222.74			

Appendix 27: ANOVA for bean haulm dry weights under selected biocontrols in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	3008.7	1002.9	6.52	
Location	3	46112.4	15370.8	99.99	<.001
Treatment	7	60473.1	8639.0	56.20	<.001
Location.Treatment	21	12359.9	588.6	3.83	<.001
Residual	93	14296.8	153.7		
Total	127	136251.0			

Appendix 28: ANOVA for bean haulm dry weights under selected biocontrols in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4291.3	1430.4	2.86	
Location	3	41260.5	13753.5	27.49	<.001
Treatment	7	35094.3	5013.5	10.02	<.001
Location.Treatment	21	9498.3	452.3	0.90	0.586
Residual	93	46524.3	500.3		
Total	127	136668.6			

Appendix 29: ANOVA for bean grain yields under selected biocontrols in the short rains of 2005

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	40551.7	13517.2	13.70	
Location	3	122275.9	40758.6	41.32	<.001
Treatment	7	289440.2	41348.6	41.92	<.001
Location.Treatment	21	22288.2	1061.3	1.08	0.387
Residual	93	91738.7	986.4		
Total	127	566294.7			

Appendix 30: ANOVA for bean grain yields under selected biocontrols in the long rains of 2006

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	50325.1	16775.0	10.24	
Location	3	136089.8	45363.3	27.70	<.001
Treatment	7	275712.5	39387.5	24.05	<.001
Location.Treatment	21	27408.8	1305.2	0.80	0.717
Residual	93	152306.6	1637.7		
Total	127	641842.8			