ASSESSING THE ROLE OF ORGANIC SOIL AMENDMENTS IN CONTROL OF ROOT-KNOT NEMADODES (*Meloidogyne* spp.) AFFECTING COMMON BEAN (*Phaseolus vulgaris* L.)

By

DAVID MUCHINA MUIRU

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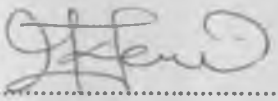
DECLARATION

This thesis is my original work and has not been presented to any other University.

23/4/2004

David Muchina Muiru **Date**

This thesis has been submitted with our approval as university supervisors.



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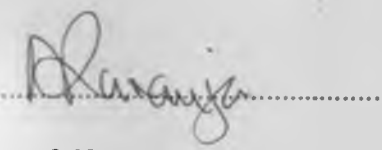

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Prof. N.K.Karanja **Date**

DEDICATION

To my late father Mr. Geoffrey Muiru Karanja whose quest for better education for his kin was relentless.

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Abstract

A study was undertaken to determine the role of organic amendments in supressing root-knot nematodes The organic amendments used in the study included chicken and cow manures and leaves of *Mucuna pruriens* (velvetbean), *Azadiracta indica* (neem) and *Tagetes minuta* (marigold).

An experiment was established to determine the effect of organic amendments on damage caused by nematode to beans. The materials were applied fresh (undecomposed) at the rate of 5% (w/w) in 5kg pots with untreated soil and soil treated with carbofuran as controls. The soil was infested with 6000 nematode eggs per pot. All the organic amendments reduced galling and reproduction of root-knot nematodes in beans. Galling indices ranged from1.5 to 3.8 (on a scale of 1-9) in soils amended with the organic materials compared to 6.3 in the control. Chicken manure was the most effective in reducing galling and reproduction of root-kot nematodes followed by *A. indca* and *T. minuta,* with cow manure being least effective. All the organic amendments increased plant growth, with chicken manure being superior to the other amendments. Carbofuran (non-fumigant nematicide) was the least potent of all the treatments.

A laboratory experiment was conducted to determine the effect of water extracts of the organic amendments on the mobility of second-stage (J_{2s}) *Meloidogyne* juveniles. The materials were decomposed in water and their extracts used to treat *Meloidogyne* juveniles. Counts of inactivated (immobile) J_{2s} were taken on an hourly interval for five hours. The extracts immobilized more than 90% of the J_{2s} treated, with the exception of

the extract from cow manure, which immobilized 36% in five hours. All the extracts immobilized a higher proportion of J_{2s} than carbofuran.

All the amendments increased the available NH_4-N, P ands pH with chicken manure giving the highest levels. Correlations between NH_4-N and nematode egg masses (r= -0.85) and juvenile population (r= -0.55) were negative suggesting the involvement of NH_4-N in nematode suppression. Phosphorous was negatively correlated to egg masses (r= -0.79) and juvenile population (r= -0.44) indicating that it plays a role in nematode suppression. Fertilizer-treated soil (galling= 3.3 at 45 DAI) had a better control level than the controls (galling = -5.8 at 45 DAI) indicating that nutrients have a role to play in nematode management.

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) and microbial activity were stimulated by all the amendments with chicken manure and *Tagetes* giving the highest level of stimulation. MBC and MBN were negatively correlated to nematode egg masses (r= -0.68 &-0.81), and juveniles (r= -0.66 &-0.23), respectively.

Most of the organic amendments encouraged a high population of *Bacillus* spp., which were used as the indicators of the presence of biocontrol agents with chicken manure and *Tagetes* being the best stimulants. *Bacillus* showed a high potential in reducing nematode egg masses since they were highly negatively correlated (r= -0.94).

CHAPTER ONE

1.0 INTRODUCTION

1.1 Bean production in Kenya

Common bean *(P. vulgaris)* is only second to maize in importance as a food crop in Kenya (MALDM, 1991). Beans are mainly grown for their dry seeds, green ripe seeds, green pods and tender leaves that are consumed as vegetables. They serve as a source of proteins providing 20-30% (Acland, 1971). Small-scale farmers mainly grow the crop as an inter-crop with maize, coffee, bananas, potatoes and others (Nderitu *et al.*, 1997; Wortmann *et al.*, 1998).

The area under bean cultivation is estimated at 700,000 ha mainly in Eastern, Central, Rift valley, Western and Coast provinces of Kenya (KARI, 1991). Mwang'ombe *et al*, (1994) reported that yields of up to 3,000kg/ha can be obtained on research stations, but most farmers only obtain 750 and 400kg/ha, in monocrop and in mixtures with maize respectively . The low yields are attributed to low soil fertility, diseases, insect pests and unfavourable climate (Allen and Edje, 1990; Nderitu *et al.*, 1997; Njuguna et al., 1997).

1.2 Plant parasitic nematodes associated with beans

Several genera of plant parasitic nematodes have been associated with legumes including beans (Mani *et al.*, 1982; Bridge, 1981; Luc *et al.*, 1990). Members of root-knot nematodes (*Meloidogyne* spp.), lesion nematodes (*Pratylenchus* spp.), sheath nematodes (*Hemicyclophora* spp.), stubby-root nematodes (*Trichodorus* spp.), stunt nematodes (*Tylenchorhnychus* spp.), *Criconemella* spp., *Tylennchus* spp., *Aphelenchus* spp., and others are associated with beans (Goodeyi *et al.*, 1965; Mani *et al.*, 1982; Bridge, 1981; Kimenju *et al* 1999). Kimenju *et al.* (1999) have shown that nematodes in the genera *Meloidogyne, Pratylenchus, Scutellonema,* and *Heicotylenchus* are widely distributed in bean growing

fields in Kenya. Root-knot nematodes are of considerable importance due to their wide distribution and polyphagous nature (Luc *et al.*, 1990).

Plant parasitic nematodes are reported to cause immense damage to beans. Hainsworth (1962) reported a 10% loss in agricultural produce in Kenya through nematodes. *Meloidogyne* spp. were reported to cause 50-100% yield loss to food crops including beans (Hollis, 1962), while Ngundo and Taylor (1974) estimated up to 60% yield loss in bean fields heavily infested with *Meloidogyne* spp. Variation in damage by nematodes to beans in different areas have been reported and can be attibutted to differences in bean cultiva grown, cropping systems adopted, enviromental factors and their interactive effects. Kimenju *et al.* (1999) reported varied nematode densities in bean growing areas in Kenya. This was due to differences in cropping densities and levels of manuring (Woomer *et al.*, 1998).

Apart from direct damage through root knotting, nematodes also affect nodulation potential in leguminous plants (Mani and Seth, 1987; Karanja, 1988; Sharma and Khurana, 1991; Siddiqui and Mahmood, 1994; Kimenju *et al.*, 1999). They also act as wounding agents creating entry avenues for other soil-borne pathogens leading to disease complexes (Hussey and McGuire, 1987; France and Abawi 1994).

1.3 Control of nematodes in beans

Several approaches have been developed for the management of nematodes with the ultimate aim of preventing population build-up (Netscher and Sikora, 1990). These include nematicides, resistant varieties, cultural practices, biological control, and organic amendments among others (Rodriguez-Kabana, 1986; Netscher and Sikora, 1990; Sikora 1992; Oka *et al.*, 1993; Sharma *et al.*, 1994; Bridge 1996).

Although nematicides have high efficacy against nematodes, their use by the small holder farmers is limited. They are too expensive for a low value crop like beans and too

sophisticated in use for the not-too-technical small holder farmers.

Resistant cultivars form the cheapest and most practised approach but these are either unavailable or the farmers do not recognize their potential. Cultural practices like crop rotation, fallowing and others are unviable due to scarcity of arable land and also the polyphagous nature of the nematodes. Although the potential for biological control of plant-parasitic nematodes appears great, several drawbacks such as slow manifestation, poor competitiveness and inadequacy of formulation relegates this approach to a non-preferred option (Jatala, 1986).

Organic amendments are widely used in nematode management on legumes including beans (Rodriguez-Kabana, 1986; Sikora 1992; Woomer *et a,l.* 1998). However, these are used mainly for nutrient supply in sub-optimal amounts to be effective in nematode control (Luc *et al.*, 1990; Oka *et al.*, 1993). In cases where they are used for nematode management information on their mode of action against nematodes is lacking. There is need to evaluate the mechanisms that come into play once organic materials are applied in the soil so as to exploit them for maximum use by the small-holder farmers.

1.4 Use of organic amendments in nematode management on beans

Incorporation of organic amendments into the soil has been shown to reduce root-knot nematode densities (Sikora, 1992; Oka *et al.*, 1993; Miano, 1999). Farmers incorporate amendments in form of animal manures and crop residues mainly for nutrient supply (Luc *et al.*, 1990; Oka *et al.*, 1993; Woomer *et al.*, 1998). This leads to build-up of soil organic matter. Decomposition of organic matter in the soil results in the accumulation of compounds with nematostatic effects and the stimulation of indigenous soil microbial biomass and activity some of which are antagonistic to nematodes (Rodriguez-Kabana, 1986; Rodriguez-Kabana and Morgan-Jones, 1987; Sayre and Starr, 1988). Organic amendments are a source

of plant nutrients, which improve plant growth and thus may lead to better nematode tolerance (Bridge 1996). They also change soil physical and chemical characteristics such as soil water holding capacity, pH, and infiltrability, some of which may be deleterious to nematodes. Although information on these changes is available, the link between some of the changes nematode suppression is lacking. The role of nutrients like phosphorous in nematode management is also lacking. The aim of this study was to quantify some of these changes and determine the role that they play in nematode suppression.

1.5 Justification

Since root-knot nematodes are widely distributed in bean growing areas and are associated with losses in crops, the need for the full scale embracement of organic amendments as a control strategy is paramount. Furthermore small-scale farmers need an enlightment on the efficacy of organic amendments for combating nematodes vis-a-vis provision of nutrients. Organic amendments give small-scale farmers the advantages of appropriateness, affordability, and acceptability and are relatively available. To maximise the advantages of their use there is need to understand their modes of action against nematodes and interactions with soil biological systems.

1.6 Objectives

The broad objective was to obtain an understanding of the mechanisms involved in nematode suppression in soils amended with organic materials.

Specific objectives were,

1. To assess the effect of organic materials on nematode damage to beans.
2. To determine the effect of water extracts from organic amendments on mobility of nematodes.
3. To determine the effect of organic amendments on soil physical, chemical and biological properties and their effect on nematodes.
4. To determine the effect of applying organic amendments on the dynamics of *Bacillus* spp as a nematode antagonist

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Common beans (*Phaseolus vulgaris* L.)

2.1.1 History and Classification of beans

Common bean is thought to have originated from Mexico, Northern America and Central America about 4300 years ago (Wilsei, 1962; Kaplan, 1965). The Spanish sailors introduced it to Europe, while the Portuguese brought it to the East African coast in the sixteenth century (Greenway, 1945). Beans are estimated to have been cultivated in Kenya for more than 300 years (Acland, 1971).

The common beans go by several names in different areas. Some of these are; French beans, kidney bean, haricot bean, salad beans, string beans, and/or frijoles (Wortmann *et al.*, 1998).

They are classified in Kingdom Plantae, Division Spermatophyta, Sub-division Angiospermae, Class Dicotylydonae, Sub-class Rosidae, Family Fabaceae/Papilionaceae and Genus *Phaseolus* (Holmes, 1986).

The genus *Phaseolus* includes 31 species, 10 of which are cultivated world-wide (Martin, 1984). *Phaseolus vulgaris* is most widely cultivated species of the genus *Phaseolus* (Allen, 1983). Common bean is an annual or perennial bushy or twinning herb that may sometime require support. It has large trifoliate leaves, stipules and stipels, with typical papillionaceaes flower in a terminal or auxiliary raceme (Purseglove, 1987).

2.1.2 Importance of beans

Beans rank second to maize in importance as a pulse and food crop in Kenya (MALDM, 1994). They provide essential proteins to the relatively poor Kenyan and world populations (Anonymous, 1996a). Beans form a cheaper and a readily available source of proteins compared to animal sources (Acland, 1971; Jalil, 1977; Kay, 1978). They contain 20-30%

proteins, 1.6% fat, 57.8% carbohydrates, 4% fibre with an accompanying high amount of essential amino acids such as lysine, tryptophan and methionine (Kay and Daisy, 1979). They also provide appreciable amounts of carbohydrates, vitamins B and C, calcium and iron. Beans are also produced as a cash crop in some areas, providing a source of income to a good percentage of Kenyans (Anonymous, 1996a). The crop improves soil fertility by fixing nitrogen biologically in association with *Rhizobium* bacteria (Walker, 1982; Nwokolo and Smart, 1996).

2.1.3 Bean cultivation

Beans are grown in a wide range of environments mainly by small-scale farmers in association with other crops (Gethi *et al.*,, 1997). The main bean intercrop is maize although coffee, sorghum, millet, potatoes, bananas and cassava form primary intercrops depending on locations of cultivation (Anonymous, 1990; Wortmann *et al.*, 1998). They require moist self-draining soils in altitude ranging from 800 to 2000 m above sea level, but some varieties do well at altitudes of up to 2700 m above sea level (Wortmann and Allen, 1994). Beans are annual legumes that grow determinately with a bushy canopy or indeterminately to a non-climbing semi bush (CIAT, 1994). They take about five weeks to flower and five weeks to mature and then dry off. They are self-pollinated (CIAT, 1994). Their dry seeds are harvested, but the green ripe seeds, pods and the green tender leaves may be consumed as vegetables. Residues are used as animal feeds and green manure (Nwokolo and Smart, 1996). The common varieties of beans grown in Kenya include Mwezi moja (GLP-1004), Rose coco (GLP-2), Mwitemania (GLP-X.92), Canadian Wonder (GLP-24), Red Haricot (GLP-585) and Zebra (GLP-806) (Anonymous, 1988).

Yields of about 750kg/ha have been realised by farmers but the potential is as high as 3000kg/ha on research stations (Mwangombe *et al* 1994).

2.1.4 Constraints to bean production

Bean production is affected by biotic, endaphic and climatic factors. The main constraints are diseases, insect pests, low soil fertility and weeds (Allen and Edje, 1990; Nderitu *et al.*, 1997; Njuguna et al., 1997). Lack of clean planting seeds is also a contributing factor (Anonymous, 1996a). The main bean diseases include anthracnose (*Colletotricum lindemuthianum),* angular leaf spot (*Phaeoisariopsis griseola),* rusts (*Uromyces appendiculatus*), halo blight (*Pseudomonas syringae pv. phaseolicola*), bean common mosaic virus, and root-knot nematodes (Mukunya and Keya, 1975; Nderitu et al., 1997). Root-knot nematodes (*Meloidogyne* spp.) are also a major constraint to bean farming, causing up to 60% yield losses in heavily infested fields (Ngundo and Taylor, 1974).

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

2.2.1 History, classification and distribution of root-knot nematodes

The first case of root-knot/gall symptom was observed by Berkley in 1885 on spinach as noted by Jensen (1972). By 1949 a worldwide distribution and 2500 plants host species for *Meloidogyne* spp. had been described (Dropkin, 1988). Until 1949 only one species, *Heterodera marioni,* was described but later other species were established (Chitwood, 1956). Other scientists have described other species, sub-species and pathotypes (Netscher and Sikora, 1990). Root-knot nematodes belong to the genus *Meloidogyne* with 51 species having been identified and their host range documented (Sikora, 1990). Of the 51 species described, *M.incognita, M.javanica, M.arenariae and M.hapla* are the most widely distributed and of particular importance to agriculture (Jepson, 1987).

Root-knot nematodes belong to Kingdom Animalia, Phylum Nematoda, Class Nemata, Subclass Secernentea, Order Tylenchida, Sub-order Tylenchina, Family Heteroderidae, and Genus *Meloidogyne.* Four Species are distingiushable namely *M. incognita, M. hapla, M. javanica and M. arenaria* (Luc *et al.*, 1990).

Root-knot nematodes have a worldwide distribution with different species predominating in different regions (Sikora, 1991). Out of 100 root-knot nematode populations collected in 75 countries, 53% were *M.incognita*, 30% *M.javanica*, 8% *M.arenaria*, 8% *M.hapla* and the rest were other species (Johnson and Fassuliotis, 1984). *M.incognita*, *M.javanica*, *M.arenaria*, and *M.hapla* are of economic importance in crop production. Except M. hapla, all the other species are more predominant in the warmer regions of the world (Luc *et al.*, 1990).

2.2.2 Life cycle and biology of *Meloidogyne* spp.

All *Meloidogyne* species have a similar life cycle (Agrios, 1988). Under average conditions a female produces 300 to 500 eggs in a sac-like gelatinous matrix. However under optimum conditions it can produce 2800 eggs and a new generation can arise within 25 days (Sherf and Macnab, 1986; Agrios, 1988). But when the conditions are unfavourable, only a few eggs are produced or production may cease. The life cycle is then prolonged to 30 or 40 days or even a stoppage of development may take place (Sherf and Macnab, 1986).

Development and moulting of the first-stage larvae takes place inside the egg to second-stage larvae (juvenile). The second-stage larva emerges from the egg to find a suitable host, being the only infective stage (Agrios, 1988). Once the parasite-host relationship is established, molting occurs to give rise to the third-stage larva that lacks a stylet. The third-stage larvae then molts into a fourth-stage larva. This larva can molt into a female, which remains embended in the root tissue, or into a free living male (Agrios, 1988).

Temperature, soil texture and structure, soil pH, aeration and water holding capacity influence nematode egg hatching, survival and disease severity (Sherf and Macnab, 1986). Egg laying and hatching occurs between 15 to 30 ^{0}C while larva and egg masses are killed by temperatures above 58 ^{0}C. Plant penetration occurs at 10 to 35 ^{0}C with an optimum of 27 ^{0}C depending on species. Except for *M. hapla* alternate thawing and freezing kills all the other

species (Sherf and Macnab, 1986).

Soil type and structure, and their relationship with water holding capacity and aeration affect nematode distribution (Sikora, 1990). Severe root damage is reported to occur in sandy soil while clayey soil appears to inhibit nematode activity (Sikora, 1989). This may be partly related to the effect of soil type on crop distribution (Prot and Van Gundy, 1981). The soil pH affects both egg production and hatching. Egg hatching is highest at pH 6.4 to 7.0 with inhibition occurring below pH 5.2. Reproduction and survival are at their best at pH 4.0 to 8.0 (Taylor *et al.*, 1982).

Root-knot nematode population consists of males and females, which are distinguishable morphologically. Mature females are pear shaped measuring 0.4-1.3mm long by 0.27-0.75mm diameter, while males are worm-like and measure about 1.2-1.5mm long by 30-60□m in diameter (Sherf and Macnab, 1986; Agrios, 1988). Second stage juveniles are vermiform in shape, while third and fourth stage juveniles are sausage shaped and microscopic in size (Sherf and Macnab, 1986). *Meloidogyne* females are distinguished by distinct patterns referred to as perineal patterns. They resemble human fingerprints (Williams, 1974; Machon and Hooper, 1991).

2.2.3 Plant invasion and disease development

The second stage juveniles enter the roots behind the tip and push their way between or through cells until they reach the zone of cell differentiation (Dropkin and Nelson, 1960). After the second stage juveniles reach behind the growing point in the growing root, they become permanently established with their head in the developing vascular cylinder (Agrios, 1997). In older roots the head is usually in the pericycle (Agrios, 1997). Two or three days after the juveniles have become established, some of the cells around its head begin to enlarge (Dropkin, 1980). The existing walls between the cells breakdown and disappear,

protoplasmic contents coalesce, giving rise to giant cells (Agrios, 1997). Enlargement of cells continues for 2 to 3 weeks, and the giant cells invade the surrounding tissues irregularly (Sasser and Carter, 1985). The enlargements of the cells seem to be brought about by substances in nematode saliva during feeding (Agrios, 1997).

The giant cells act as nutrient sinks serving as nurse cells for nematodes (Agrios, 1997). In the early stages of gall development the cortical cells enlarge in size, during the latter stages, they also divide rapidly (Agrios, 1997). Root swelling may also result from excessive enlargement and division of all types of cells surrounding the giant cells and from nematode enlargement (Agrios, 1997). As the females enlarge and produce their egg sacs, they push outward, split the cortex and may become exposed on the surface of the root or remain completely covered depending on the position of the nematode in relation to the root surface (Agrios, 1997). The giant cells degenerate when nematodes cease to feed or die. The galled root tissues eventually breakdown and rot (Agrios 1997).

2.2.4 Economic importance of Root-knot nematode

Root-knot nematodes are reported to cause immense damage to crops. Hainsworth (1962) reported a 10% loss in agricultural produce in Kenya through nematodes. In 1962 Hollis estimated a 50-100% yield loss to food crops due to *Meloidogyne* spp. Infected seedlings will die and most will not survive transplanting (Sherf and Macnab, 1986). In the few surviving plants, yields are greatly reduced (Netscher and Sikora, 1990). Infection at seedling stage may result in crop failure but for older crops, the effect is variable with yield losses ranging from slight to enormous (Agrios, 1988). Nematodes have also been found to suppress the nodulation potential of the leguminous plants (Karanja, 1988; Sharma and Khurana, 1991; Kimenju *et al.*, 1999; Sharma *et al.*, 2000).

2.2.5 Symptoms associated with root-knot nematode damage

The galls on the root system of affected plants are the primary symptoms of root-knot nematode damage (Agrios, 1988). Infected root tissues are stimulated to grow abnormally such that the invaded cells become 2-3 times larger than normal. Root tips are devitalised with subsequent stoppage of growth and/or excessive root production often-occurring (Sherf and Macnab, 1986). The root system is therefore reduced to a few galled roots with the vascular system becoming disorganised and root hairs are absent or reduced (Sherf and Macnab, 1986; Agrios, 1988). The greatest damage is caused by the knotting of roots and disruption of water and nutrient uptake (Agrios, 1988). Plants become stunted, leaves turn chlorotic and growth is generally retarded (Agrios, 1988). The weakened roots are also attacked by other root pathogens such as, *Rhizoctonia* and *Pythium*, which grow faster in the galled roots causing early tissue disintegration (Agrios, 1988). Nematode infection also breaks down host resistance to other pathogens (Kimenju *et al.*, 1999).

Field symptoms are often manifested as water, disease and/or nutrient stress signs except that they occur in patches (Agrios, 1988). The above-ground symptoms include wilting, pale green or chlorotic leaves and stunted growth. The flowers and pods are small and of poor quality (Dropkin, 1980). Below ground symptoms are confined to the roots which are malformed, and reduced in size and number (Bird, 1974; Sherf and Macnab, 1986; Agrios, 1988). Root hair differentiation is suppressed leading to decreased efficiency in water and nutrient uptake (Malakeberhan *et al.*, 1985; Wilcox and Loria, 1986).

2.2.6 Management of plant parasitic nematodes

An integrated approach where several methods and practices are used in harmonious combination is the best strategy for nematode control (Dropkin, 1980). The stategies incorporate chemical nematicides, cultural practises, biocontrol, host resistance and use of soil amendments (Bridge, 1996).

2.2.6.1 Chemical control

Nematicides are recommended in cases where other methods fail to combat nematodes and often for high value crops (CIAT, 1994). Fumigant nematicides such as D-D (1,3-dichlorpropene and related hydrocarbons), methylbromide, chloro-picrin, and vortex have been used successfully on beans and other crops (CIAT, 1994). In addition, control of nematodes and increase of bean yields have been obtained with the use of non fumigant nematicides such as aldicarb, phenamiphos, carbofuran, and oxamyl, applied as a broadcast or band and incorporated into the soil (CIAT, 1994).

Application of oxamyl to beans as a foliar spray has been effective against many nematodes (CIAT, 1994). However, its activity against the root-knot nematodes is limited and a combination of a soil treatment with foliar sprays of oxamyl is recommended (CIAT, 1994). There have been some encouraging results from the application of nematicides such as oxamyl, as seed treatment to beans (CIAT, 1994).

However, use of nematicides is expensive for a crop like beans and requires care in handling and often the use of special equipment for application. Further, nematicides are highly toxic, and hazardous to the environment. These limitations have resulted in banning of otherwise highly effective pesticides such as methyl bromide thus leaving growers without viable control alternatives. It is therefore important to continuously seek alternative control strategies for managing nematodes.

2.2.6.2 Physical control

Root-knot nematodes have been controlled using soil solarilisation (Katan, 1981). This involves raising temperatures to levels that are lethal to root-knot nematodes and other diseases (Overman and Jones, 1986). However, this technique is only adopted in regions where sufficient solar energy is available. *Meloidogyne* population densities drop significantly when soils are flooded for prolonged periods of time (Netscher and Sikora, 1990). However, this form of control is only applicable on level grounds and where water is not limited.

2.2.6.3 Host resistance

Use of bean cultivars tolerant to nematodes is the most efficient nematode management strategy, especially for resource poor small-scale farmers (Wilcox and Loria, 1986). Tolerance to *Meloidogyne* spp., has been reported in bean lines and cultivars (CIAT, 1994). Local resistant cultivars include GLP X-92 and GLP2 (Kimenju *et al.*, 1999). Resistant lines developed by Kimani *et al.*, (1993) include NOB, M30, L31, KK15 AND KK22. Ngundo (1977) reported nematode resistance in some local varieties namely, Kahuti, Red haricot, Rono, Saginaw and Kibuu. These were resistant to *M. incognita* and *M. javanica*. Despite the existence of resistant cultivars, their availability to farmers is limited and some still use susceptible lines such as GLP-24 and GLP-1004. Another constraint to widespread adoption of this strategy is the rejection of some of the resistant cultivars by farmers due to undesirable traits (Sharma *et al.*, 1994).

Although resistance against root-knot nematodes is stable (CIAT, 1994), resistance to one race or species of root-knot nematodes is often independent of other races or species. This makes the option of host resistance unfavourable and costly in developing a resistance line against each nematode species. Resistance to gall formation and to the build-up of nematode population in root system are characters independent of tolerance to yield reductions, they are

probably governed by separate genetic mechanisms (CIAT, 1994).

2.2.6.4 Biological control

Biological control has been defined as the action of parasites, predators or pathogens in maintaining nematodes at a lower than average population (De Bach 1964; Baker and Cook, 1974; Dropkin, 1980). Several microorganisms occur together with nematodes in a particular protosphere. These include predacious nematodes, obligate bacterial parasites, nematode trapping fungi, pathogenic fungi and viruses (Mankau, 1980a; Stirling, 1991; Sikora 1992). Symbionts such as fungal endophytes, vascullar-arbuscular mycorrhizal (VAM) and rhizobacteria have been shown to protect thier partner symbionts against nematode attack (Hussey and Rancadori, 1982; Sikora, 1992). Biological control is achieved through the action of nematode antagonists through such mechanisms as parasitism, predation, competation, and antibiosis (Stirling, 1991; Sikora 1992).

Several genera of nematodes in the orders Mononchida, Dorylaiminda and Diplogasteroidea are predacious on plant parasitic nematodes (Jatala, 1986). Although these nematodes exert certain natural control of plant-parasitic nematodes, their potential for biocontrol remains uncertain and warrants further study.

The bacterium *Peusteria penetrans* is probably the most specific obligate parasite of nematodes (Birchfield and Antanpolous, 1976). There is a synchronisation of this organism with the development and physiology of its host, *Meloidogyne* species (Mankau, 1995). The bacterium thus affects nematodes at all the stages (Sekhar and Gill, 1990; Davies *et al.*, 1991; Chen *et al.*, 1997c). This bacterium exerts various degree of nematode biocontrol under greenhouse and field conditions (Mankau, 1995; Chen and Dickson 1998; Giblin-Davis, 1990; Ciancio, 1995b). While many strains of P*eusteria* are nematode specific, emerging evidence shows cross-generic suppression of nematodes (Mankau and Prasad, 1972;

Bhattacharya and Swarup, 1988). A strain isolated in India parasitized both *Heterodera* spp. and *M. incognita* (Bhattacharya and Swarup, 1988). However, the obligate nature, lack of mobility and its dependence on water, man, animals, or cultivation practises for spread is its major drawbacks (Jatala, 1986).

Bacterial parasites produce metabolites that are inhibitory to nematodes (Jatala, 1986). Other modes of action by bacterial parasites are, interference with host finding process, modifying hosts' root exudates and hindering hatching of nematode eggs (Becker *et al.*, 1988; Oostendorp and Sikora, 1990; Spiegel *et al.*, 1991). *Bacillus thuringiensis* produce non-specific toxins deleterious to *M. incognita* (Oostendorp and Sikora, 1990). Many members of the genus *Bacillus* inhibit penetration of nematodes to roots (Becker *et al.*, 1988; Oostendorp and Sikora, 1990). Others produce antibiotic "bacitracin" (Brandbary, 1986). *Streptomyces avermitilis* was recently screened and "avermectins" isolated and proven to be potent, broad-spectrum nematicides. They are reported to be 10 times more effective than oxamyl or aldicarb in controlling *M. incognita,* (Garabedian *et al.*, 1983).

Many fungal species have been reported to trap and predate on nematodes in soil under natural and exprimental conditions (Sayre, 1971; Mankau, 1980a; Jatala 1986). These fungi capture nematodes using hypal networks and branches (Jatala 1986). Nematode pathogenic fungi have also been reported to colonise host nematodes (Barron, 1970; 1977) These produce spores that adhere or are ingested by nematodes where they germinate and caused death (Barron, 1970; 1977). Nematode egg parasites such as *V. chlamydosporium* and *Peacilomyces lilacinus* have been documented as potential biocotrol agents. These are effective on eggs and mature members of *M. arenaria* (De Leij and Kerry, 1991; Kerry, 1995; Al Raddad, 1995). Infection of *M. incognita* females by *V. chlamydosporium* has been observed (Jatala, 1986). This fungus is a facultative parasite of root-knot nematodes (Goodeyi *et al.*, 1965). This group of biocontrol agents is limited by their limited ability to colonise

eggs laid within root tissues and those in large egg masses (Stirling, *et al.*, 1979; De Leij and Kerry, 1991).

Although the potential for biological control of plant-parasitic nematodes appears great, several drawbacks such as slow manifestation, poor competitiveness and inadequacy of formulation relegates this approach to a non-preferred option (Jatala, 1986).

2.2.6.5 Cultural control

Cultural practices have been developed that often prevent or reduce pest/nematode outbreaks, but their results are often unseen and difficult to quantify (Bridge, 1996). These include crop rotation, prevention of the introduction and spread of nematodes, fallowing, use of nematode free planting materials, physical destruction of nematodes in planting materials, production of clean seedlings from nematode-free seedbeds, weed control, and escape cropping (Bridge, 1996).

Rotating crops in a sequential cropping system is ant appropriate practice but farmers rarely use it for several reasons. The basic principle of crop rotation for nematode management is to reduce initial populations of damaging nematode species to levels that allow the following crop(s) to become established and complete early growth before being heavily attacked (Nusbaum and Ferris, 1973). Interest has been largely concentrated on the rotation of plant crops, but rotations can also involve the sequential planting of plant crops with such others as green manure, cover and trap crops, antagonistic plants, grass and other fallows so as to effect better control (Bridge, 1996). The major constraints to the use of crop rotation are the wide host range of the plant parasitic nematodes, scarcity of arable land and lack of agronomically adapted cultivars (Noling and Becker, 1994). Most small-scale farmers do not follow these practises especially fallowing due to loss of productive season and land scarcity.

2.2.6.6 Control using antagonistic plants and trap crops

Plants belonging to 57 families have nematicidal properties (Sukul, 1992). The most widely reported antagonistic plants are *Tagetes* spp., mustard, asparagus, sesame, sunnhemp (*Crotalaria juncea*) and neem (*Azadirachta indica)* (Bridge, 1996). Trap crops are regarded as plants that reduce nematode populations by allowing invasion but only partial development of nematodes in their roots. The African marigold, *T.erecta*; the French marigold, *T. patula*; and the South American marigold, *T. minuta*, have been found to be effective in the control of root-knot nematodes (Antoon, 1999). Many of the antagonistic plants are used as inter-crops with the preffered crops, and the detrimental effects of weeds such as *Tagetes* on crop yield often outweigh the beneficial effects of nematode control (Bridge, 1996).

2.2.6.7 Control using soil amendments

Incorporation of organic materials into the soil is an old agricultural practice. Animal manures are perhaps the most widely used organic amendments with most farmers incorporating them into the soil for nutrient supply (Luc *et al.*, 1990; Oka *et al.*, 1993; Bridge, 1996). In Kenya for instance survey shows that 96-100% of farmers across four districts namely Kiambu, Machakos, Nyandarua and Thika use manure on their farms to improve soil fertility (Karanja et al., 1997). Other workers across the country have found simiar trends of manure use and/or compost making (Kapkiyai *et al.*, 1996; Woomer *et al*, 1998; Lekasi *et al.*, 1998; Wamuongo *et al.*, 1999). Researchers have however linked decreased nematode population and damage to crops in the fields incorporated with animal manures across the country (Woomer *et al.*, 1998; Kimenju *et al.*, 1999).

The suppresive ability of organic amendments tonematode damage was reported at the start of the century. Linford *et al.*, (1938) reported that incorporation of pineapple leaves into the soil resulted in suppression of *Meloidogyne* spp. Since then, many different types of organic amendments have been used most of which are agro based products such as oil cakes, animal

manures, plant-crop residues, plant composts, green manures and agro-industrial wastes (Thakur and Davekar, 1995; Bridge, 1996; Akhtar and Mahmood, 1997; Ringer *et al.*, 1997;Chavarria-Carvajal and Rodriguez-Kabana, 1998; Miano, 1999). Several workers have evaluated various animal manures such as poultry, cow, camel, goat and pig manures for nematode control in various crops (Thakur and Davekar, 1995; Bridge, 1996; Akhtar and Mahmood, 1997; Ismael and Youssef, 1997; Ringer *et al.*, 1997;Chavarria-Carvajal and Rodriguez-Kabana, 1998; Miano, 1999).

They have reported great potential by the manures in nematode management. The most promising of these is poultry manure, although most farmers use cow manure being more available. It was on the basis of effectiveness and wide spread use of poultry and cow manures, respectively that they were selected for evaluation in this study.

Green manures made from Crotalaria (*Crotalaria spectabilis*), Hairy Indigo (*Indigofera hirsuta*), neem (*Azadiracta indica*), jointvetch (*Aeschnomene americana*), pigeonpeas (*Cajanus cajan)*, velvetbean (*Mucuna* spp.) and marigold (*Tagetes* spp.) have been shown to reduce soil populations of root-knot nematodes (Reddy *et al.*, 1986). Neem has been utilised for nematode management in various forms, which include purified nematicides (Suneem G and Nimbin), cakes, oils, powders, leaf and kernel composts and green manures (Mishra and Prasad, 1974; Sitaramaiah, 1990; Akhtar and Mahmood, 1997; Miano, 1999). Most workers have demonstrated a high efficacy of neem based products against nematodes, but have failed to conclusively deduce the mechanisms involved in the nematode suppression by neem as a soil amendment. Green manure from neem was selected for evaluation due to its high efficacy and to attempt an understanding of the changes that occur in the soil that lead to nematode suppression.

The nematode suppressive effects of Tagates spp have been well-documented (Reddy *et al.*, 1986; Goldman, 1991; Oduor-Owino, 1993; Antoon, 1999; VanBiljon, 2001). Tagetes have

been utilised as a trap/antagonistic crop (Bridge, 1996; Antoon, 1999) green manure and compost (Oduor-Owino, 1993; VanBiljon, 2001). Since *Tagetes* grows readily as a weed colonising cultivated and previously cultivated sites, and its incorporation in the soil involves little or no costs, it can easily be adopted by the small holder farmers (Bridge 1987) as an amendment. This weed was selected for evaluation in this study in view of its obvious advantage.

The use of *Mucuna* spp. for nematode management has been documented with varied degree of success (Reddy *et al.*, 1986; Vicente and Acosta, 1987; Rodriguez-kabana, 1992; Chavarria-Carvajal and Rodriguez-kabana, 1998; Vargas-Ayala *et al.*, 2000). Chavarria-carvajal and Rodriguez-kabana (1998) reported a decline in root galling and population of root-knot nematodes as well as increased population of non-parasitic nematodes by *Mucuna* spp. *Mucuna* as a green legume intercrop has also been documented to reduce soil populations of root knot nematodes (Reddy *et al.*, 1986). Cultivation of *Mucuna* as a cover crop is being promoted by the Legume Research Network Project in Kenya with a high degree of adoption (Mureithi, 2001). This will provide the farmers with a cheap source of the amendment and thus its inclusion in this study was obvious.

Addition of organic matter in the soil improves soil fertility, water holding capacity, aeration as well as regulating other soil-borne pathogens (Bridge 1996). This results in a better crop that has nutriet-induced tolerance against nematodes as well as enhanced development allowing for escape from intense nematode build-up (Sikora, 1992). For instance cow manure with a percentage nutrient analysis of N (0.54), P (0.03), K (1.22) and a pH of (9.22) was reported to have appreciable nematode control (Dhliwayo, 1997). Miano (1999) expressed similar nematocidal activity while working with cow and chicken manures of C:N ratios 19.9 and 10.1, respectively. Amendments from plant materials such as neem are reported to contain appreciable amounts of plant nutrients e.g. N (5.5-7.1%), P (1.1-3.2%) and K (1.5-

2.1%), which are thought to contribute to nematode tolerance (Singh and Sitaramaiah, 1970; Ketkar, 1976; Vijayalakshmi *et al.*, 1985).

2.2.6.8 Modes of nematode control by organic amendments

Addition of organic matter stimulates the build-up of populations and activities soil organisms, some of which antagonise nematodes (Rodriguez-Kabana and Morgan Jones, 1987). For example nematode-trapping fungi (*Arthrobotrys dactyloides* and *Nematoctonus leisporus*) are reported to be higher in organically managed field plots. (Jaffee *et al.*, 1998). Microbial biomass (substrate induced respiration) is also reported to be greater in organic than in conventional plots (Jaffee *et al.*, 1998). Goswami and Battacharya, (1989) reported an increase in fungal population especially *Aspergillus* spp., in organically amended soil, which were thought to be responsible for nematode inhibition. Rodriguez-Kabana, (1992) suggested that bacteria in the velvetbean rhizosphere were antagonistic to cysts and root-knot nematodes. Organic amendments are also kwown to alter microbial communities of the rhizosphere, inducing soil conditions that suppress nematodes (Vargas-Ayala *et al.*, 2000). Proliferation of microorganisms results in increased enzymatic activities of the amended soil and accumulation of specific products some of which may be nematicidal (Badra *et al.*, 1979; Rodriguez-Kabana, 1992; Vicente and Acosta, 1987). Vicente and Acosta (1987) reported that decomposing velvetbean root exudates are nematicidal. The activities of enzyme catalase, esterase and urease, which are detrimental to nematodes, are reported to be greater in soils amended with velvet beans (Chavarria-Carvajal and Rodriguez-Kabana, 1998). Root galling and population of plant parasitic nematode were inhibited, while that of free-living nematodes was stimulated. Parasitism on *M. incognita* eggs was also increased and the suppression mechanism was deemed complex (Chavarria-Carvajal and Rodriguez-Kabana, 1998).

Some organic materials contain compounds that are toxic to nematodes, e.g. *Tagetes, Mucuna* and neem contain terpenoids (thienyls), aliphatic alcohol and esters, and azadirachtin and nimbin, respectively (Champagne *et al.*, 1992; Dhawan and Patnaik, 1993; Marisa *et al.*, 1996; Padma *et al.*, 1997).

In addition, a large group of high quality organic materials act against nematodes through release of ammonia (Rodriguez-Kabana, 1986; Miano, 1999). These materials have low C:N ratios and have high protein or amine content (Miano, 1999). Although ammonia and organic amendments with low C:N ratios are nematicidal, the quantities of these organic materials required to achieve consistent nematode control in the field are too large (Rodriguez-Kabana, 1986).

Amending the soil with fresh or composted organic matter also alters the physical, chemical and biological properties of the soil (Sikora, 1992). Soil temperatures, pH, oxygen and nitrogen status may also be changed (Brandy, 1984). This may result in conditions that are unfavourable to the growth and activity of nematode. The plants can develop good root system and increased host resistance when physical and chemical changes occur in the soil (Sikora, 1992). This can also lead to unfavourable osmotic relations (Singh and Sitaramaiah, 1970).

Organic amendments also contain appreciable amount of plant nutrients and promote good soil water relations (Bridge, 1996). This may give a crop the chance to grow fast and escape intense nematode pressure. The crop may also withstand nematode infestation through the good nutrient-enhanced nematode tolerance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Five organic amendments comprising of two animal manures and three plant based amendments were selcted for evaluation. Poultry and cow manures were selected due to their wide spread use by small holder farmers and their efficacy against root-knot nematodes. Green manure from *Mucuna* spp. (velvetbean), *Azadirachta indica* (Neem) and *Tagetes* spp. (marigold) were also selected according to Section 2.2.6.7.

A local bean line variety GLP-2 was used as the test crop due to its susceptibility to root-knot infection. The seeds were obtained from the Kenya Seed Company. Sub-soil (10-20 cm) was collected from Kabete field station, University of Nairobi. The soils are classified as humic Nitisol based on the FAO-UNESCO system (FAO, 1990), which is equivalent to Paleustalf in the USDA soil taxonomy system (Soil Survey Staff, 1990).

3.1.1 Collection and preparation of amendments

Chicken manure was obtained from a battery system of chicken rearing, while cow manure was collected from a beef unit at the University of Nairobi. The test materials were air-dried in a glasshouse and sieved through a 2-mm-aperture sieve.

Mucuna seeds were obtained from the Legume Research Network Project at K.A.R.I. (N.A.R.L.), and sown in small plots at the Field Station Upper Kabete Campus, University of Nairobi. The plots were regularly watered and weeded until flowering when the above-ground biomass was harvested. *Tagetes* was harvested from fields where it grew as a weed. Neem leaves were harvested from trees and transported to the green house for drying. All the leaves were chopped, air-dried, and then passed through a 2-mm aperture sieve.

3.1.2 Characterization of the soil and organic amendments

Three samples of each material were characterized for nitrogen, phosphorous, carbon, pH and C:N ratios calculated at the, Department of Soil Science, University of Nairobi.

The soil pH was determined in 1:2.5 soil in water suspension using a pH meter (Okalebo *et al.*, 1993). Total nitrogen in soil was determined by steam distillation after kjehldal digestion (Bremmer and Mulvany, 1982), while soil organic carbon was determined following the procedure described by Kurmies (Walinga *et al.*, 1992). Total elements contents in the soil (phosphorous, pottasium, calcium, magnesium and sodium) were determined following the wet ashing technique described by Okalebo *et al*, (1993). The pH of the amendments was determined in 1:5 manure/amendment in water using a pH meter. Total nitrogen was determined by steam distillation after kjeldal digestion (Bremmer and Mulvany, 1982), while organic carbon was determined following the procedure described by Kurmies (Walinga *et al.*, 1992). Total phosphorous in the amendments was determined using the wet ashing method (Okalebo *et al.*, 1993).

The soil properties after analysis were pH in water (6.4), total nitrogen (0.12%), carbon (3.0), available phosphorous (0.23 mg kg^{-1}), CEC (19.8 Cmol/kg soil), exchangeable potassium (1.6 Cmol/kg soil), exchangeable calcium (11.3 Cmol/kg soil), exchangeable sodium (0.12 Cmol/kg soil) and exchangeable magnesium (3.02 Cmol/kg soil) The soil C:N ratio was 25. According to the nutrient sufficiency ranges used at Kenya Soil Survey (Mehlich *et al.*, 1962), the soil was near neutral, had adequate levels of organic carbon and low levels of nitrogen and phosphorous. Magnesium and potassium were high, while sodium and calcium were moderate.

The chemical properties of the organic amendments are summarized in Table 1. All amendments had neutral to slightly basic reaction, were high in organic carbon, moderate amounts of nitrogen, and medium levels of phosphorous. Their C:N ratios ranged from 12.6

to 20.5 which were within mineralizable range owing to their high nitrogen content, although this depends with the quantity of lignin and polyphenolics (Weeraratna, 1979; Berg and McClaugherty, 1987; Palm and Sanchez, 1990). The quality of the materials was good with manures mainly composed of animal excreta and the plant materials composed of leaves, flowers, seed kernels and soft twigs which are usually low in both lignin and polyphenols (Jamaldheen and Mohan, 1999).

Table 1. Chemical characteristics of the amendments

Amendments	pH	% P	% C	% N	C: N
Poultry manure	8.2	3.9	34.5	2.6	14
Cow manure	8.2	0.1	36.9	1.8	21
Mucuna spp.	7.5	0.1	57.3	2.9	20
Tagetes spp.	7.2	0.2	34.2	2.7	13
Neem (*A. indica*)	7.8	0.1	36.9	2.7	14

3.1.3 Multiplication of root-knot nematodes

Root-knot nematodes were multiplied on tomato cv. Moneymaker in sterile sand/soil mixture in a glasshouse. Two-kilogram plastic pots were filled with steam sterilized potting mixture and four week old tomato seedlings (raised in sterile sand beds) transplanted into them. Nematode inoculum was obtained from galled tomato roots using the technique developed by Hussey and Barker (1973). One week after transplanting, 10ml of nematode suspension containing about 6000 eggs and juveniles was added into the root zone of the tomato seedling. The nematodes were allowed to multiply on tomatoes for eight weeks.

3.1.4 Preparation of root-knot juveniles

Second-stage juveniles were obtained from galled roots using the method by Omwega *et al.* (1988). The roots were washed free of soil using tap water and then rinsed using distilled water. The roots were immersed in sterile tap water and aerated using an aquarium pump. Second-stage juveniles were obtained in about 5 to 10 days by filtration and sieving technique (Hooper, 1990).

3.2 Methods

3.2.1 Effect of organic amendments on damage caused by nematode on beans

A greenhouse experiment was conducted to determine the effect of organic soil amendments on damage by nematode on bean crop. The amendments included, chicken and cow manures, and leaves of *Mucuna. Tagetes* and neem which, were applied at the rate of 5% (w/w) basis to 5-kg soil filled in pots. A nematicide (carbofuran) applied at the rate of 1g/kg soil and untreated soil were included as controls. The pots were arranged on benches in the greenhouse in a completely randomised block design with 10 replicates.

Each pot was sown with four bean seeds variety GLP-2 which were later thinned to 2 plants per pot at seedling emergence. The soil was infested with root-knot nematode eggs and juveniles at the rate of 6000 eggs/pot. The inoculum was suspended in10 ml of water, which was pipetted in 2cm deep indentations made around the plants. Light watering was carried out within the next 10 days to avoid washing the inoculum beyond the rrot zone. Fouty five and seventy days after soil infestation with nematodes, sampling was carried out by gently uprooting the plants. Their roots were washed free of soil using tap water.

The roots were then soaked in a biological stain (phloxine-B), rinsed with tap water and blotted dry using paper towels (Hooper, 1986c; Meyer *et al.*, 1988). The roots were rated for galling, and egg mass indices using a scale of 1-9 where 1=no gall, 2=1-5, 3=6-10, 4=11-20, 5=21-30, 6=31-50, 7=51-70, 8=71-100 and 9=>100 galls/plant (Sharma *et al.*, 1994). Second

stage juveniles were extracted from 200cm^3 soil samples using the sieving and filtration technique (Hooper, 1990). Plant dry shoot and root weights were also determined. This experiment was repeated once.

3.2.2 Effect of water extracts from organic amendment on mobility of *Meloidogyne* juveniles

The organic amendments (chicken and cow manure, and leaves of *Mucuna, A. indica* and *Tagetes*) were air-dried in a glasshouse, pulverized and passed through a 2-mm sieve. Water extracts were prepared using the method described by Chindo and Khan (1990) whereby 5 g of each amendment were added into 100-ml sterile distilled water held in 500-ml Erlenmeyer flasks and allowed to decompose for 5 days at room temperature. The extracts were passed through a piece of cheesecloth. Second-stage *Meloidogyne* juveniles (J_2) were obtained from galled tomato roots following the method by Omwega *et al.* (1988). The juveniles that emerged were harvested after 5-10 days and the concentration adjusted to 1000 J_2/ml.

One milliliter of the juvenile suspension was pippeted into 9 ml of extract held in McCartney bottles. Treatments were replicated three times and arranged on a laboratory bench in a completely randomized design. Sterile distilled water and carbofuran (nematicide) were included as controls. One milliliter was drawn at hourly intervals, pippeted into a counting slide and the J_2 counted under a compound microscope. Juveniles that showed no movement and lay straight were considered immobile (Patric *et al.*, 1965). The percentage of immobile juveniles was calculated out of 100 observed.

3.2.3 Effect of organic amendments on soil chemical and biological properties and their effects on nematodes

A greenhouse experiment was conducted using the materials as soil amendments. Fertilizer (diammonium phosphate) was applied at the rate of 36kg N/ ha, non-amended soil and a nematicide (carbofuran) were also included as controls. The treatments were applied following the procedure described in section 3.2.1 above. The experiment was replicated eight times but during sampling random mixtures of two samples each were combined to make up a final number of 4 replicates.

Soil samples were taken from the pots using microaugers at the 1st, 3rd, 5th, 7th, 9th and the 10th weeks after soil infestation with nematodes. The soils were analyzed for, nitrogen (NH_4^+-N), available phosphorous, pH, microbial biomass nitrogen and carbon, and carbon dioxide evolved using standard methods (see Anderson and Ingram 1993). All the analyses were carried out at the International Center for Research in Agroforestry (ICRAF), Nairobi.

At 45 and 70 days after soil infestation with nematode inoculum, two plants were taken at random and rated for galling and egg mass indices (Sharma *et al.*, 1994). Juvenile count/200cm^3 soil and shoot and root dry weights were also carried out.

3.2.3.1 Effect of organic amendments on available soil NH_4^+-N

Soil samples were transported to the laboratory in a cool box and stored in a refrigerator (4 ^{0}C). Extraction was carried out within 5 days of sampling. The method described by Dorich and Nelson (1984) was used for the analysis. This involved extracting soil using 2 N KCl and the sample (extract) reduction of NO_3-N to NO_2-N in a reduction column packed with copperized cadmium (Cd) granules. The nitrite produced was determined by diazotizing with sulphanilic acid (4-aminobenzene sulphonic acid) and coupling with 5-amino-2-naphthalene sulphonic acid (5-2 ANSA) solution to form a higly colored azo dye that was measured calorimetrically. The intensity of the reddish-purple dye produced, was proportional to the

amount of nitrate and nitrite in the sample.

The soil water was determined by labelling beakers and taking their weights before adding a sample of soil from each treatment. The weights of the beakers with the soil were then taken. The soil was then dried in an oven at 105°C for 24 hours. After cooling the beakers with the soil were weighed and their weights recorded.

Immediately after collecting the soil for moisture determination, a sample for extraction was also collected. A labeled 150-ml plastic bottle was tarred on a top loading balance. The soil sample was added to the bottle to a final weight of 20-21g and the actual weight was recorded. A 100-ml aliquot of 2N KCl was added to the bottle. The bottles were placed horizontally on an end-end shaker and shaken for 1 hour at 150 reciprocations per minute (rpm). Whatman No. 5 filter papers were folded and washed with deionized water to leach off ammonium from the paper. A small portion (about 5 ml) of the soil extract was first filtered through the paper, and discarded before transferring filter papers to filter bottles. Then the papers were transferred to labeled, clean and dry 50-ml filter bottles and the remainder of the soil extract filtered. The extracts were analysed for ammonium-nitrogen (NH_4^+-N).

Determination of NH_4^+-N

The soil extracts prepared in section 3.2.3.1 above were analysed for extractable NH_4^+-N. Using a macroset pipettor, 2ml of each standard (prepared to contain 0, 0.25, 0.5,1 and 2mg N/L in 2 N KCl) and sample were pipetted into labelled test tubes. Five milliliters of reagent N1 (containing 68g sodium salicylate, 50g sodium tartrate, 50g sodium citrate and 0.24g sodium nitroprusside in 2 litres of deionized water) were added. The solutions were well mixed with a vortex mixer and left for 15 minutes. Five milliliters of reagent N2 (containing 60g sodium hydroxide and 20ml sodium hypochlorite in 2 litres of deionized water) were added, mixed, and left to stand for one hour for full color development. The

absorbance/concentration of the standards and samples were read on a spectrophotometer at 655nm. The samples were thoroughly mixed and the spectrophotometer switched on 30 minutes before taking the readings.

Calculations

1. Gravimetric soil water content (%) (EXNGWC):

$$\frac{(EXNWCWS-EXNWCDS)\ (100)}{(EXNWCDS-EXNWC)}$$

Where EXNWCWS= Weight of container plus wet soil (g)

EXNWCDS= Weight of container plus dry soil (g)

EXNWC= Weight of container (g)

2. Dry weight of extracted soil (g) EXNSDSWT):

$$\frac{(EXNSLWT)*100}{(100+EXNGWC)}$$

Where EXNSLWT= Field-moist weight of extracted soil (g)

EXNGWC= Gravimetric soil water content (%)

3. NH_3-N concentration (dry soil basis) (EXANMGKG) (mg N/kg)

$$\frac{(EXACONC-EXABLNK)\ [EXVOL+\ (EXSLWT-EXNDSWT)]}{EXNDSWT}$$

Where EXACONC= NH_3-N concentration for sample (mg N/L)

EXABLNK= NH_3-N concentration for blank (mg N/L)

EXVOL= Extractant volume (ml).

3.2.3.2 Effect of organic amendments on extractable inorganic phosphorous

The sampled soil collected from the experiment described in sec 3.2.3 above was air-dried to constant moisture content before determining their phophorous content. Sodium bicarbonate was used to extract inorganic forms of phosphorous from the soil, which was then determined calorimetrically as the conventional bicarbonate extractable (Olsen) P.

Soil samples were weighed (2.5 ±0.01g) into labeled 125-ml polyethylene bottles. Fifty milliliters of 0.5 M $NaHCO_3$ extracting solution were added. The bottles were shaken on a flat bed shaker at 150 rpm for 30 minutes before filtering through Whatman No.5 paper into 50-ml plastic bottles.

Three milliliters of the extract and standards (prepared to contain 0, 1,2,3,4 and 5 mg P/l) were pipetted into labeled test tubes, and 4ml of ascorbic acid solution added. 3N H_2SO_4 (0.5 ml) solution was added to each tube, before adding 3 ml of molybdate reagent and mixing well. They were then left to stand for one hour for full color development. After 1 hour the samples and standards were read for absorbance and concentration at 880nm on a spectrophotometer.

Calculation

Bicarbonate extractable organic phosphorous in soil:

BIPMGKG= (BIPCONC-BIPBLNK) (20)

Where BIPMGKG= Phosphorous concentration in the soil (mg P/kg)

BIPCONC= Phosphorous concentration for sample (mg P/l)

BIPBLNK= Phosphorous concentration for blank (mg P/l).

3.2.3.3 Effect of organic amendments on soil pH

Soil samples collected in the experiment described in sec 3.2.3 above were analyzed for pH using the method described by Okalebo *et al.*, (1993). The pH meter was calibrated by immersing the electrode into pH 7 buffers and allowing the reading to stabilize for one minute, before adjusting the buffer knob to read 7.00. The electrode was removed, rinsed with distilled water and the remaining drop of water touched off with tissue paper. The electode was then immersed into pH 4 buffer and the reading allowed to stabilize for one minute before adjusting the slope (sensitivity) knob to read 4.00. The calibration process was repeated until the values obtained for the buffers aggress to within ± 0.02 pH unit of the theoretical values.

Ten milliliters of soil were scooped using a 10ml soil-scoop and placed in 60-ml bottles. Distilled water (25ml) was added to the soil using a dispenser to make up a soil: water ratio of 1:2.5. The soil-water mixture was placed on a mechanical stirrer and stirred for 10 minutes. The solution was allowed to stand for 20 minutes before stirring again for 2 minutes. Before measuring the pH each sample was stirred for 5 seconds with a glass rod and allowed to settle for 30 seconds before immersing the electrode. The pH measurements were recorded after the reading stabilized (after 1 minute). The electrode was removed, rinsed with distilled water and the drop touched off before continuing with other samples. After a batch of 11 samples one of the buffer solutions was re-checked to ensure instrument and electrode stability.

3.2.3.4 Effect of organic amendments on microbial biomass nitrogen and carbon

Soil samples collected in the experiment described in sec 3.2.3 above were mixed well and transported to the laboratory in a cool box. The samples were stored (when not extracted immediately) in a refrigerator (4 ^{0}C) but care was taken to extract them within five days of sampling. The chloroform fumigation-extraction method for soil microbial biomass

developed by Mueller *et al.* (1992) was used. This method involved the extraction of chloroform-fumigated and non-fumigated soil samples with 0.5 M K_2SO_4 followed by determination of organic carbon and nitrogen in the extracts. On the same day that a non-fumigated sub sample was extracted, a second sub sample was taken and incubated with chloroform for one day. The fumigated sub sample was extracted in the same way as the non-fumigated sub sample. Microbial biomass carbon and nitrogen were calculated as the difference in concentrations of carbon and nitrogen between extracts of fumigated and non-fumigated soil samples.

Soil water determination was carried out following the same procedure as described in section 3.2.3.1 above.

Immediately after taking the soil sample for moisture determination, a 30-ml labeled glass bottle was weighed before adding a soil sample of about 25g. Blank bottles were included at 2 bottles per 24-sample batch. The bottles were then placed in vacuum desiccator containing 50ml alcohol-free chloroform in a 100ml beaker with 10-15 boiling chips. The lid was closed and the desiccator evacuated until the chloroform started boiling. The tap of the desiccator was then closed and the desiccator placed in a dark place at 25 ^{0}C. After 1 day the desiccator was opened in a fume cupboard and the chloroform allowed to escape, any remaining chloroform was evacuated with a vacuum pump. The fumigated sample was transferred to labeled 125-ml polyethylene bottles for extraction.

Immediately after taking the soil sample for moisture determination, a 125-ml labeled plastic bottle was tarred before adding a soil sample of about 25g. Blank bottles were included at 2 bottles per 24-sample batch. 100ml of 0.5 M K_2SO_4 were added to the bottles with both the fumigated and the non-fumigated soil samples. The bottles were well stoppered before placing them on an end-end shaker in horizontal position. They were shaken for 1 hour at 150 reciprocations per minute. Whatman No. 5 filter papers were folded and washed with distilled

water before using them for filtering the samples into labeled 50-ml bottles. The filtrates were stored in a refrigerator before analyzing them for total nitrogen and carbon.

Determination of soil microbial biomass nitrogen

The extracts prepared in the procedure described in section 3.2.3.4 above were analyzed for microbial biomass nitrogen. Ten milliliters of the sample or standard were pipetted into test tubes and 10 ml of oxidizing agent containing, 50g $(NH_4)_2S_2O_8$ into 500ml of 1.2 M H_2SO_4, added. The mixture was well shaken. The tubes were placed on a rack, covered with an aluminum foil and autoclaved at 121°C and 17 lb pressure for 30 minutes. The samples were then cooled to room temperature before analyzing for nitrogen.

The samples were analyzed using the cadmium reduction method as detailed in section 3.2.3.1a above except that the standards were prepared in 0.5M K_2SO_4, and 3ml aliquots of sample or standard were used for this analysis.

Calculation

1. Gravimetric water content (%) (MBGWC)- calculated as in section 3.2.3.1 above.
2. Dry weight of extracted soil (g) (MBNDSWT)-calculated as in section 3.2.3.1 above.
3. Nitrogen concentration (dry soil basis):

$$MBNNMGKG = \frac{(MBNNCONC-MBNNBLNK)\,(100 + (MBNSLWT-MBNDSWT))}{MBNDSWT}$$

$$MBFNMGKG = \frac{(MBFNCONC-MBFNBLNK)\,(100 + (MBFSLWT- MBFDSWT))}{MBFDSWT}$$

Where

MBNNMGKG= Nitrogen extracted from non fumigated soil (mg N/kg)

MBFNMGKG= Nitrogen extracted from fumigated soil (mg N/kg)

MBNNCONC= Nitrogen concentration for non fumigated sample (mg N/L)

MBNNBLNK= Nitrogen concentration for non fumigated blank (mg N/L)

MBFNCONC= Nitrogen concentration for fumigated sample (mg N/L)

MBFNBLNK= Nitrogen concentration for fumigated blank (mg N/l)

4. Microbial biomass nitrogen (mg N/kg soil) (MBNMGKG):

(MBFNMGKG- MBNNMGKG).

Determination of soil microbial biomass carbon

The extracts prepared in the procedure described in section 3.2.3.4 above were analyzed for microbial biomass nitrogen. A10ml aliquot of the sample or standard (prepared in the concentrations of 0, 1,2, 3 and 4 mg C/ml) was transferred to a labeled digestion tube using an oxford Macroset pipettor. To each tube, 2 ml of 0.16-M potassium dichromate solution was added using a bottle-top dispenser and mixed well. Concentrated H_2SO_4 (10ml) was slowly added from an Eppendorf pipette while gently swirling the mixture on a vortex mixture. The tubes were heated in a digestion block for 30 min at 150°C. The samples were allowed to cool before determining carbon content. The absorbance/concentration of each sample and standard were read at 600nm on the spectrophotometer. The spectrophotometer was switched on 30 minutes before running samples and standards.

Calculation

1. Gravimetric water content (%) (MBGWC)- calculated as in section 3.2.3.1 above.
2. Dry weight of extracted soil (g) (MBNDSWT)-calculated as in section 3.2.3.1 above.

3. Carbon concentration (dry soil basis) (MBNCMGKG)

$$= \frac{(100)\,(MBNCCONC-MBNCBLNK)\,(100 + (MBNSLWT-MBNDSWT)}{MBNDSWT}$$

$$MBFCMGKG= \frac{(100)\,(MBFCCONC-MBFCBLNK)\,(100+(MBFSLWT-MBFDSWT)}{MBFDSWT}$$

Where

MBNCMGKG= Carbon extracted from non-fumigated soil (mg C/kg).

MBFCMGKG= Carbon extracted from fumigated soil (mg C/kg)

MBNCCONC= Carbon content of non-fumigated sample (mg C)

MBNCBLNK= Carbon content of non-fumigated blank (mg C)

MBFCCONC= Carbon content of fumigated sample (mg C)

MBFCBLNK= Carbon content of fumigated blank (mg C)

4. Microbial biomass carbon (mg C/kg soil) (MBCMGKG):

(MBFCMGKG-MBNCMGKG)

3.2.3.5 Effect of organic amendments on soil microbial activity

Soil samples collected in the experiment described in sec 3.2.3 above were analysed for the activity of microorganisms. The soil was incubated and the CO_2 evolved trapped in a standardized NaOH, forming sodium carbonate. The carbonate produced by the reaction of NaOH and CO_2 was precipitated as barium carbonate, and the remaining base titrated with standardized HCl. The volume of NaOH and the concentration of both the NaOH and the HCl to be used were standardized before the soil incubation.

Standardization of hydrochloric acid

Standardization involved drying 5g of THAM [Tris (hydroxymethyl) methylamine] at 103 ^{0}C for one hour. Triplicate samples of the dry THAM were weighed to about 0.5g into 100ml conical flasks and the weights recorded to 4 decimals. The HCl to be standardized was placed in a 25-ml burette. Fresh CO_2-free water (prepared by hard boiling deionized water) (50 ml) was added to the THAM and swirled to dissolve the THAM. A blank with Co_2-free water only was included. Six drops of bromocresol green indicator was added to the THAM solution and titrated with HCl from the burette while stirring constantly. The end point was reached with a color change from blue to yellow (just as the green color disappeared. The normality of the HCL was calculated as shown below.

Calculation

$$N_{HCl} = \frac{W_{THAM}}{0.1211\ (V_{HCl} - V_B)}$$

Where N_{HCl} = Normality of HCl

W_{THAM} = Weight of THAM used in titration (g)

V_{HCl}= Volume of HCL (ml) used to titrate THAM

V_B= Volume of HCL (ml) used to titrate water blank

Standardization of NaOH solution

The NaOH to be standardized was placed in a burette. Triplicate-10ml aliquots of the standard HCl solution were added into 50-ml conical flasks. CO2-free water blanks were also included. Two drops of phenolphthalein indicator were added to the flasks. The standardized acid was titrated with NaOH, while stirring constantly, until a faint pink color developed and persisted for 15-30 seconds.

Calculation

$$N_{NaOH} = \frac{N_{HCl}\,(10)}{(V_{NaOH} - V_B)}$$

Where N_{NaOH} = Normality of NaOH

N_{HCl} = Normality of HCL used to titrate NaOH

V_{NaOH} = Volume of NaOH (ml) used to titrate HCl solution

V_B = Volume of NaOH (ml) used to titrate water blank

Soil incubation

The soil to be incubated was air dried to a constant weight and its gravimetric water content determined before standardizing the moisture content to 55% by adding distilled water. Ten grams of the soil were placed in a 50-ml plastic bottle. The standard NaOH (10 ml) at 0.5N was placed in a 20-ml autoclave bottle. Both bottles, one containing the soil and the other, containing NaOH were placed in a 500-ml glass jar and made airtight immediately. Blank samples containing NaOH only were included at the rate of 1blank for every 20 samples. The soil and the blanks were incubated at 25 ^{0}C for 7 days.

Carbon dioxide estimation

Using CO2-free water for rinsing, the NaOH solution was quantatively transferred from the traps to 60-ml wide mouth bottles marked at 25-ml volume. A magnetic stir bar, 8 ml of 3 N $BaCl_2$ and 2 drops of phenolphthalein indicator were added and the volume made up to 25 ml mark with CO2-free water. A violet colour developed plus a white $BaCO_3$ precipitate. Titration was conducted immediately with the standard HCl. At the end point the violet colour turned transparent. Care was exercised not to uncover the samples before titration.

Calculations

$$CO2EVOL = \frac{(BLNKTIT - SAMTIT)\,(E)\,(NHCL)}{W}$$

Where CO2EVOL= Evolved CO_2 (mg CO_2–C/kg)

BLNKTIT= Volume of standard HCl used to titrate the NaOH in blanks (ml)

SAMTIT= Volume of standard HCl used to titrate the NaOH in samples (ml)

E= Carbon equivalent weight=6

NHCL= Normality of standard HCl

W= Weight of incubated soil (g)

3.2.3.6 Effect of organic amendments on the population of nematode antagonists, *Bacillus* spp. in amended soil

Soil samples were taken from experiment 3.2.3 above at the 1st, 3rd, 5th, 7th, 9th and the 10th weeks after soil infestation with nematodes. The soils were analyzed for the changes in the population of *Bacillus* spp as an indicator/signature of the presence and population dynamics of nematode antagonists in amended soils. Serial dilutions, then the plating of the soil samples in petri dishes and the colony counts were used to enumerate the population of *Bacillus* spp.

Preparation of water blanks

The method described by Keya (1975) and Clark (1965) was used to enumerate the population of the biocontrol agent. Ninety milliliters of tap water was dispensed into 500-ml conical flasks together with 10 spherical glass beads. The flasks were well stoppered using cotton wool and aluminum foil. Eighteen milliliters of water was also dispensed into McCartney's bottles using an automatic syringe calibrated to ± 0.01-ml accuracy. Both the conical flasks and McCartney's were autoclaved at 121°C and 1 atm. for 15 min. The bottles

were stored at room temperature after removal from the autoclave.

Preparation of serial dilutions

One conical flask and 5 McCartney's bottle were used for every sample for making serial dilutions to 10^{-6}. Ten grams of each of the soil samples were weighed aseptically into the conical flasks with 90 mls of sterile water. The contents were shaken for 10 min on a mechanical rotary shaker. Upon removal and settling the suspensions were further shaken by hand before drawing 2ml using 2-ml sterile pipettes and transferring them to the first McCartney's to make 10^{-1} dilution. Subsequent dilutions were made up to the 10^{-6}.

Preparation of the media

Nutrient agar (Difco Laboratories Detroit Mi USA) containing 3g beef extract, 5g peptone and 15g agar suspended in 1000 mls distilled water was used for culturing *Bacillus* spp. the agar was boiled to dissolve at 121-124 ^{0}C for 15 minutes. The final pH was checked to ensure its stability at 6.8 ± 0.02 at 25 ^{0}C.

Preparation of pour plates, incubation and counting of the colonies

The media was autoclaved at under standard conditions (120 ^{0}C and 15lb/in^2) and then removed to a water bath at 42 ^{0}C. Petri dishes that had been sterilized in metal canisters at 200 ^{0}C for 60 min were used. The soil suspension was heated at 80 ^{0}C for 10 minutes in a water bath to eliminate other mesophillic spores and cells. The soil suspensions were allowed to cool to room temperature before plating. Using sterile 2-ml pipettes, 1 ml of the soil suspension was dispensed into each of the sterile plates in duplicates for each sample at the 10^{-4}, 10^{-5} and 10^{-6} dilution in a laminar flow hood.

In each of the plates 15-20 mls of the media at 42 ^{0}C was poured and rotated to distribute the inoculum uniformly in the media. The plates were left to stand for 20- 30 minutes on the

hood for the media to solidify. The plates were then inverted and incubated at 28 °C for 7 days. A colony counter (Scientifica and Cook electron, ICS Ltd) was used for the enumeration of the colonies that developed on the plates. The numbers obtained were expressed per gram of dry soil.

CHAPTER FOUR

4.0 DATA ANALYSIS

The data collected was cleaned, edited and entered into excel work sheets. Data from section 3.2.1 was analysed using Genstat 5.2 release, for variance at P=0.05. The treatment means were recorded and their least significance differences were used to draw conclusions. The means were used to draw tables or figures where applicable.

Data from section 3.2.2 was converted to percentages. The percentages were then analysed for variance at P=0.05. Their treatment means at every hour upto five hours were recorde and their least significant differences used to draw conclusions.

The data from section 3.2.3 was analysed as described above, further the soil parameters data was subjected to Pearsons correlation analysis with the nematode stages data at $P \leq 0.05$. Their correlation coeficients were recorded and their corresponding levels of significance.

CHAPTER FIVE

5.0RESULTS AND DISCUSSION

5.1 Effect of organic amendments on root-knot nematode damage on beans

The organic amendments caused a reduction in galling index, egg masses and J_2 counts, and enhanced bean shoot and root dry weights (Table 4). Galling ranged from 1.5-5.1 in amended soil compared to 6.3 in the control. This was in agreement with other researchers working with various amendments who showed their potential in reducing galling, egg masses and population of root-knot nematodes (Kaplan and Noe, 1993; Ali, 1995; Akhtar and Mahmood 1997; Marull *et al.*, 1997; Chavarria-carvajal and Rodriguez-Kabana, 1998; Miano, 1999).

Significant (p=0.05) differences were observed at 45 D.A.I. in galling, egg masses, and J_2 counts between organic amendments and the controls at 45 D.A.I. There were no differences in dry shoot and root weights at this sampling time. At 70 D.A.I. two groups emerge, one comprising of chicken manure, *Tagetes, Mucuna* and *A. indica* on one hand and cow manure fertilizer and carbofuran on the other. Significant (p=0.05) differences in galling, egg mass and J_2 counts were observed between the groups with the "chicken manure group" causing a higher reduction. Significant (p=0.05) differences also occurred in shoot weights with "chicken manure group" promoting growth. This was possible due to high nutrient content especially nitrogen and the ability of manure to stimulate microbial population and activity some of which are antagonistic to nematodes (Kaplan and Noe, 1993; Ali, 1995; Akhtar and Mahmood 1997; Marull *et al.*, 1997; Chavarria-Carvajal and Rodriguez-Kabana, 1998; Miano, 1999).

The potential of applying *Tagetes* as a soil amendment in the management of nematodes has been demonstrated by various workers (Reddy *et al.*, 1986; Oduor-Owino *et al.*, 1993; Odour-Owino and Waundo, 1994; Antoon, 1999). The chemicals, thienyls, terpenoids and

flavonoids contained in the amendment have toxic effects and are responsible for the decline in nematode activity and population. Several forms of *A. indica* products including cakes, leaves, bark kernels and purified extracts have been used with various degrees of success in nematode management (Goswami and Bhattacharya, 1989; Agbakli *et al.*, 1992; Thakur and Davekar, 1995; Akhtar and Mahmood 1997). The main causes for nematode reduction by neem products are cited as nematostatic derivatives, released nutrients and altered microbial population and activity in the soil (Mojumder and Mishra, 1991b,c; Goswami and Bhattacharya, 1989; Thakur and Davekar, 1995). *Mucuna* has also been used as a soil amendment in the management of plant parasitic nematodes (Redyy et al., 1986; Chavarria-Carvajal and Rodriguez- Kabana, 1998). There is evidence that M*ucuna* produces nematicidal compounds (Vicente and Acosta, 1987) as well as altering soil microbial populations, inducing conditions that suppress nematodes (Vargas-Ayala *et al.*, 2000).

Carbofuran, a commonly used nematicide showed least effect and was not difference from the control. Sikora (1990) also noted that in most cases non-fumigant nematicides do not control nematodes in concentrations recommended for use today.

Table 2.Effect of organic amendments on nematode galling and egg mass indices, juvenile counts and bean growth, 45 and 70 days after soil infestation with nematodes (D.A.I.)

Treatment	Galling index		Egg mass index		J_2 numbers/200 cm^3 soil		Root dry weight (g)		Shoot dry weight (g)	
	45 DAI	70 DAI	45 DAI	70 DAI	45 DAI	70 DAI	45 DAI	70 DAI	45 DAI	70 DAI
Chicken manure	1.5	2.7	2.0	2.0	91	55	0.2	0.2	2.3	4.2
Cow manure	4.4	5.1	7.5	6.5	1474	1848	0.3	0.2	1.2	2.3
Neem (*A. indica*)	2.7	3.8	5.9	1.9	145	95	0.3	0.2	1.2	2.0
Mucuna spp.	2.6	3.1	5.4	4.9	432	150	0.2	0.3	1.3	3.4
Tagetes spp.	1.7	2.8	4.4	4.3	618	697	0.2	0.4	2.3	4.9
Fertilizer	3.3	4.8	4.9	7.5	623	696	0.2	0.3	1.5	1.7
Carbofuran	4.7	5.1	6.5	6.6	1213	1466	0.1	0.1	0.9	0.8
Control	5.8	6.3	8.8	7.4	1474	1511	0.2	0.2	0.7	1.3
$L.S.D_{0.05}$	1.2	0.9	1.2	1.5	314	61	0.1	0.1	0.4	0.9
%CV	36.9									

5.2 Effect of water extracts from organic amendments on mobility of Meloidogyne juveniles

Water extracts from organic amendments caused a reduction on mobility of *Meloidogyne* juveniles (J_2) (Table 3). This finding is in agreement with previous reports indicating that substances released by decomposing amendments have nematostatic effects (Singh and Sitaramaiah, 1977; Patric *et al.*, 1965; Miano, 1999). Extracts from chicken manure, *Tagetes*, and *Mucuna* inactivated 100% of the *Meloidogyne* juveniles after 5 hours of exposure. These extracts appear to have strong nematostatic properties. Several workers have reported this aspect (Padma *et al.*, 1997; Vijayalakshmi, 1976; Vijayalakshmi *et al.*, 1979; Kaplan and Noe, 1993). Padma *et al.* (1997) reported that leaves and flowers of the African marigold contain terpenoids (thienyls), which are nematicidal, leading to loss of activity of treated juveniles. These may also be responsible for the reduced galling, reproduction and population of *Meloidogyne* spp. in amended soils. Extracts from *Mucuna spp.* caused paralysis in *Meloidogyne* juveniles Marisa *et al.*, (1996) attributed this to aliphatic alcohol and esters released during decomposition.

Table 3. Percentage of immobile second-stage juveniles *Meloidogyne* (J_2) after treatment with water extracts from the organic amendments

Water extract	Exposure Time (hrs)				
	1	2	3	4	5
Chicken manure	69.2	77.7	82.5	100.0	100.0
Cow manure	6.0	11.4	20.3	31.3	35.8
Neem (A. indica)	39.3	57.1	64.5	80.0	92.6
*Mucuna*spp.	46.0	52.0	67.2	87.0	100.0
Tagetes spp.	43.2	70.8	83.4	91.6	100.0
Carbofuran	2.0	2.0	6.6	12.0	13.6
Control	0.0	0.0	0.0	0.0	0.0
$L.S.D_{0.05}$	7.84	8.21	8.04	8.57	3.7
% CV	15.2	12.1	10.4	8.5	3.3

Neem extracts inactivated more than 90% of *Meloidogyne* juveniles after 5 hours of exposure. Extracts from neem leaves are known to be highly toxic to second-stage juveniles of *M. incognita.* (Vijayalakshmi *et al.*, 1979; Mojumder and Mishra, 1991b,c; Miano, 1999) demonstrated the loss of activity by nematode juveniles after treatment with extracts from neem leaves. According to Mojumder and Mishra, (1991a) the toxicity of neem extracts vary with nematode species, with *Meloidogyne* juveniles being the most sensitive.

Water extracts from chicken manure immobilized a significantly higher (P=0.05) number of juveniles after 1 hour of exposure when compared to extracts from other organic amendments, including the nematicide (carbofuran) which immobilized only 35% of the (J_2) in five hours. The two groupings observed earlier in section 4.2 continue with the same trend.

5.3 Effect of organic amendments on soil chemical and biological properties

5.3.1 Effect of organic amendments on available soil NH_4^+-N

Soils treated with chicken manure and fertilizer had significantly (p=0.05) higher amounts of NH_3-N than the other amendments and the controls at 1st to 5th week (figure 1). These two treatments had a similar pattern of NH_4^+-N release. The pattern was characterised by an initial rapid release followed by a steady decline, which is typical of fertilizer and animal manures with high nitrogen content (Palm and Sanchez, 1990; Kaboneka, 1993).

From table 2 chicken manure is highly suppressive to nematode damage and also had the highest amount of NH_4^+-N released (figure 1). Rodriguez-Kabana and Morgan Jones (1987) reported that most organic materials with high nitrogen content have relatively higher nematocidal effect. This fact is further supported by the negative correlation between NH_4^+-N and nematode egg masses, galling indices and juvenile population (see table 6). Rodriguez-Kabana (1986) attributed this to the stimulation of specialized soil micro flora capable of decomposing the organic material resulting in accumulation of NH_4^+-N. Huebner *et al.* (1983)

and Eno *et al.* (1995) observed that high NH_4^+-N was toxic to nematodes through its disruption of their cell membranes.

Although the other organic amendments did not have significantly higher amounts of NH_4^+-N than the controls, they had better nematode control (see table 2). Further the fertilizer treated soil showed high NH_4^+-N content but a lower level of nematode suppression than the rest of the amendments. This suggests that there are other properties other than NH_4-N responsible for nematode suppression by organic amendments. Most authors attribute this to high nutrient content and the ability of amendments to stimulate changes in microbial population and activity some of which are deleterious to the nematodes (Kaplan and Noe, 1993; Ali, 1995; Akhtar and Mahmood 1997, Marull *et al.*, 1997; Chavarria-Carvajal and Rodriguez-Kabana, 1998; Miano, 1999). The NH_4^+-N release pattern for the plant based amendments was typical of low lignin/polyphenol plant materials, where an initial rapid release is encountered followed by a steady slow release (Palm and Sanchez, 1990; Lefroy *et al.*, 1995; HSRDC, 1997).

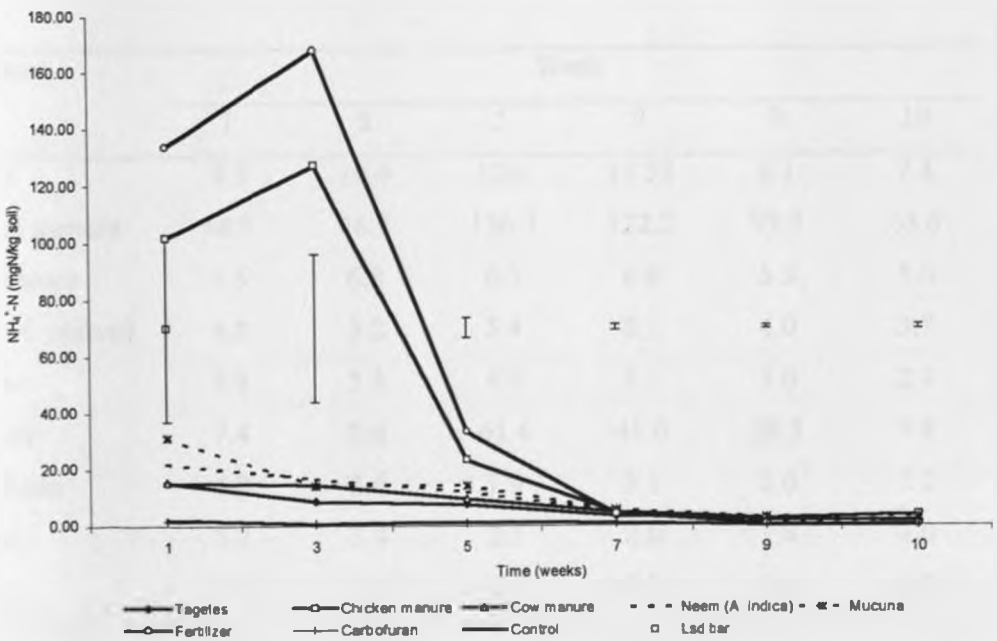

Fig 1.Effect of organic amendments on available soil NH_4^+-N (mg-N/kg soil) in amended soil

5.3.2 Effect of organic amendments on extractable inorganic phosphorous

The soil amended with chicken manure had significantly (p=0.05) higher amount of available P than the other amendments as well as the controls. Although not much work has been regarding the relationship between P and nematodes, evidence from this study indicates a negative correlation (see table 6). Serigne *et al*, (2000) suggested that P enhanced crop growth through better nutrition, which leads to increased nematode tolerance. Zhou and Paulitz (1994) and Zhang *et al*, (1996) advanced the theory that nutrient induced nematode tolerance or resistance as well as systemic acquired resistance could be aided by good P supply.

Although the soil treated with fertilizer had a significant (p=0.05) difference in the amounts of available P than all the other amendments except chicken manure, some had better nematode control, indicating the presence of other nematocidal factors in the amendments other than nutrients.

Table 4. Available inorganic Phosphorous (mg-P/kg soil) collected from the amended soil

Treatment	Week					
	1	3	5	7	9	10
Tagetes	9.3	10.4	10.6	11.52	8.1	7.4
Chicken manure	68.3	76.2	136.7	122.2	95.9	65.6
Cow manure	5.6	6.8	6.5	6.8	5.3	5.6
Neem (*A. indica*)	4.5	5.2	5.4	5.1	4.0	3.7
Mucuna	3.9	5.5	4.0	4.1	3.0	2.9
Fertilizer	7.4	5.8	61.4	41.0	38.3	9.8
Carbofuran	4.0	2.6	1.9	3.1	2.0	2.2
Control	3.2	3.4	2.7	2.0	1.8	2.0
Lsd $_{0.05}$	2.0	2.1	12.4	6.7	6.2	4.2

5.3.3 Effect of organic amendments on soil pH

Except for soils treated with *Tagetes* all other amended soil had significantly (p=0.05) higher pH in the 1st week than the controls and fertilizer treatment. High pH (beyond 7) is reported to be detrimental to nematode reproduction and egg hatching (Taylor *et al.*, 1982). The negatively correlated pH data and nematode egg masses observed in this study (see table 6) support this factor. Further, high pH is reported to aid in NH_4^+-N build up (Lazarovitis, 1999). This is evident in this study where a rise in pH from 1st week to 3rd week corresponds to a rise in NH_4^+-N amounts. This is clear in the soils treated with chicken manure.

Table 5. Effect of organic amendments on soil pH of amended soils

Amendments	Week 1	Week 3	Week 5	Week 7	Week 9	Week 10
Chicken manure	7.3	7.6	6.3	6.9	7.0	7.1
Cow manure	7.4	7.5	6.5	7.2	7.3	7.1
Neem *(A. indica)*	7.7	7.7	7.5	7.4	7.5	7.5
Mucuna	6.8	6.9	5.8	6.5	6.6	6.8
Tagetes	6.6	6.6	6.3	6.5	6.6	6.7
Fertilizer	6.5	6.3	6.6	5.6	5.9	6.1
Carbofuran	6.5	6.5	6.5	6.6	6.6	6.4
Control	6.4	6.4	6.5	6.5	6.7	6.4
$L.S.D_{P=0.05}$	0.3	0.3	0.8	0 2	0.2	0.1

5.3.4 Effect of organic amendments on microbial biomass nitrogen and carbon

Soil treated with organic amendments supported significantly (p=0.05) higher microbial nitrogen and carbon than the control and carbofuran (figure 2 & 3). A similar pattern of microbial biomass carbon and nitrogen was exhibited by organic amendments there was an initial rapid increase followed by a steady decline. This is in agrement with patterns described by other researchers (Rodriguez-Kabana *et al.*, 1981; Badra *et al.*, 1979). The two groupings

described in sections 4.1 and 4.2 with "chicken manure" group being more effective are also consistent here. Carbofuran had the lowest microbial biomass nitrogen at all the sampling times except at the 5th week where a slight increase was encountered.

The amendments tested stimulated the build up of microbial biomass. This aspect was reported earlier by several researchers (Badra *et al.*, 1979; Rodriguez-kabana and Morgan Jones 1987; Jaffe *et al.*, 1998). The increased microbial population is reported to suppress nematodes in various ways. Rodriguez-kabana and Morgan Jones (1987) demonstrated that the build up of microbial population may lead to build up of nematode antagonists in the soil. For example nematode trapping fungi are reported to be higher in organically managed plots (Jaffe *et al.*, 1998). Further proliferation of microorganisms results in increased enzymatic activity and accumulation of specific metabolites some of which are nematicidal (Badra *et al.*, 1979; Mian and Rodriguez-kabana, 1982; Rodriguez-kabana and Morgan Jones 1987; Stirling, 1991).

The inability of carbofuran to stimulate both the microbial biomass and their activity was evident from the study. Weischer and Mueller (1985) and Crump and Kerry (1986) also noted this in their studies and suggested that this reduction in activity and population could lead to reduced potency of the pesticide through microbial degradation.

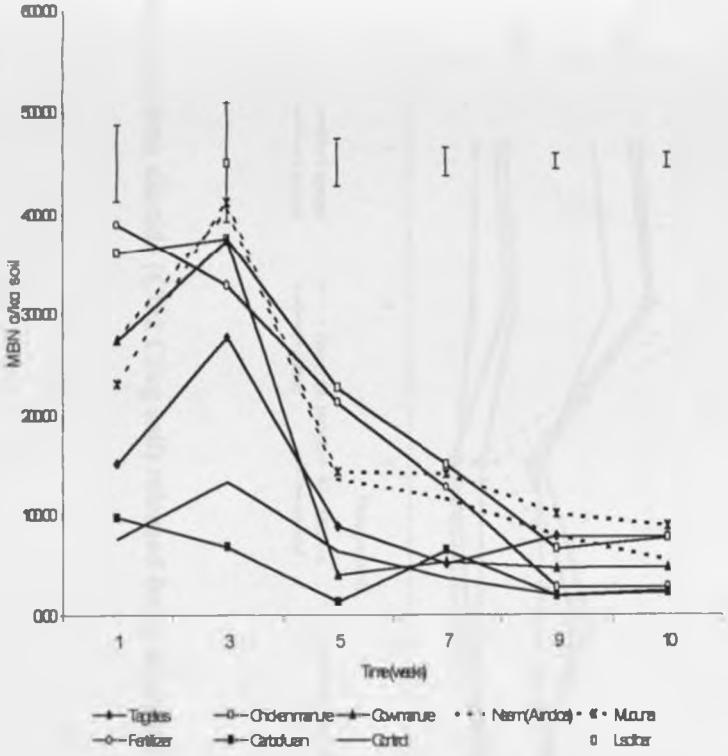

Figure 2. Microbial biomass nitrogen (MBN) (g/kg soil) collected in amended soil

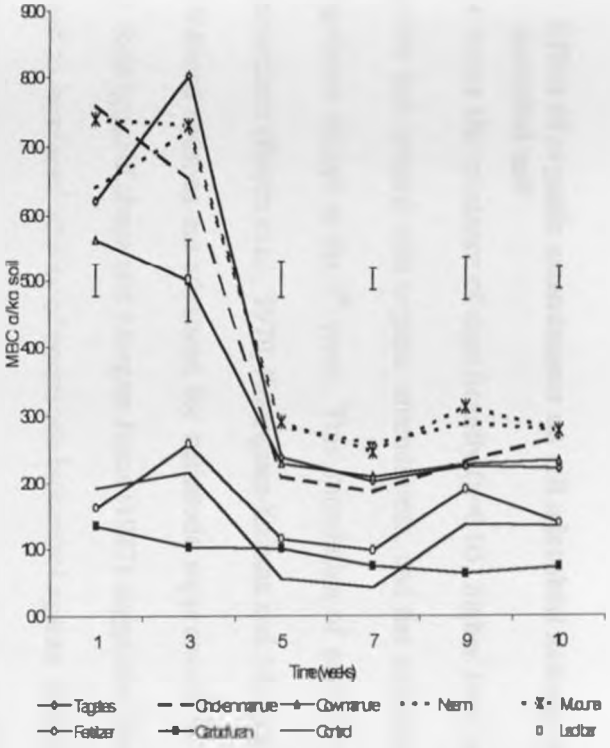

Figure 3. Microbial biomass carbon (MBC) (g/kg soil) collected in amended soil

5.3.5 Effect of organic amendments on soil microbial activity (CO_2) released from amended soil

Figure 4 shows the existence of significantly ($p=0.05$) higher level of microbial Co_2 released between the soil treated with organic amendments, and the controls and fertilizer at all the sampling times except at the 5th week. This stimulation of activity was earlier observed by other researchers (Badra *et al.*, 1979; Rodriguez-Kabana and Morgan Jones 1987; Jaffe *et al.*, 1998). Various reasons are advanced for nematode suppression by the increased microbial activity. Rodriguez-Kabana and Morgan Jones (1987) suggested that high microbial activity cuold lead to increased activity of nematode biocontrol agents. High microbial activity results in increased enzymatic activity and accumulation of specific metabolites that are nematocidal (Badra *et al.*, 1979; Mian and Rodriguez-Kabana, 1982; Stirling, 1991).

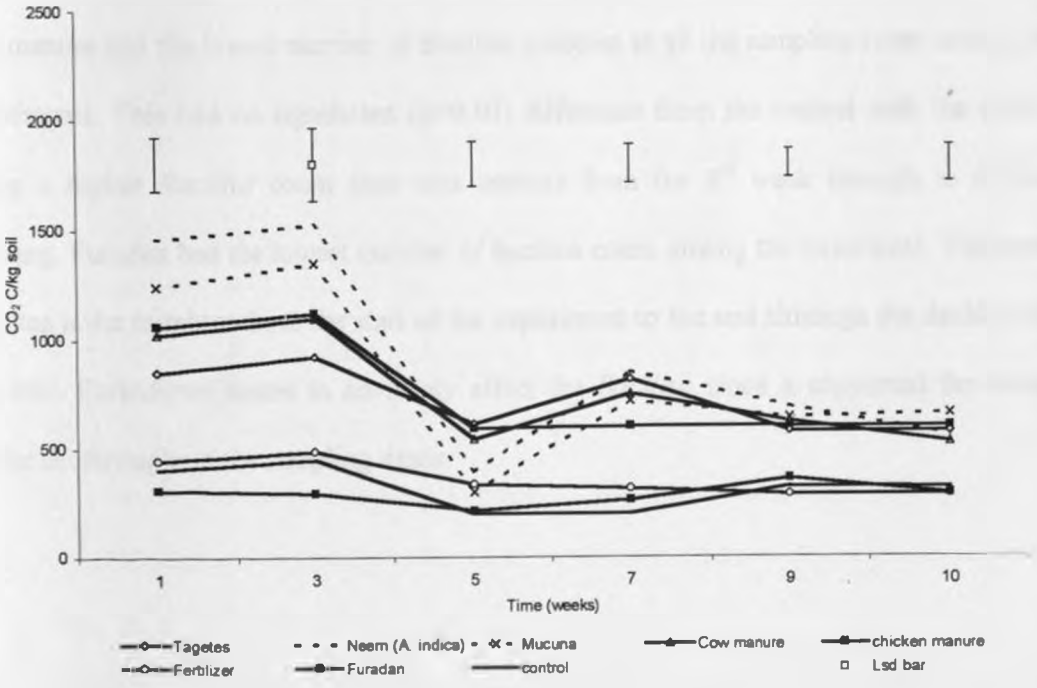

Figure. 4 Carbon dioxide (CO2 C/kg soil) released from amended soil

5.3.6 Effect of organic amendments on the population of nematode antagonists *Bacillus* spp. in amended soils

The population of *Bacillus* spp was significantly (p=0.05) higher in soils treated with chicken manure and *Tagetes* than in soils treated with other amendments at all sampling times except the last (figure 5). This indicates the potential of organic amendments to support high populations of natural nematode bio-control agents, which was reported by Goswami and Bhattacharya (1989) and Jaffe *et al.* (1998). These amendments had better control of nematodes than thecontrols. This could have been contributed by the higher population of the biocontrol agent, *Bacillus* whose presence in the rhizosphere is known to alter root exudates and nematode host recognition (Oostendorp and Sikora, 1990). This may have lead to the reduction of the initial nematode eggs/inoculum as shown by the strong negative correlation between nematode egg masses and the *Bacillus* population (section 4.4.7)

Cow manure had the lowest number of *Bacillus* colonies at all the sampling times among the amendments. This had no significant (p=0.05) difference from the control with the control having a higher *Bacillus* count than cow manure from the 3rd week through to the last sampling. Furadan had the lowest number of bacillus count among the treatments. There was a decline n the numbers from the start of the experiment to the end although the decline was very low. Carbofuran seems to adversely affect the *Bacillus* since it supported the lowest population throughout the sampling times.

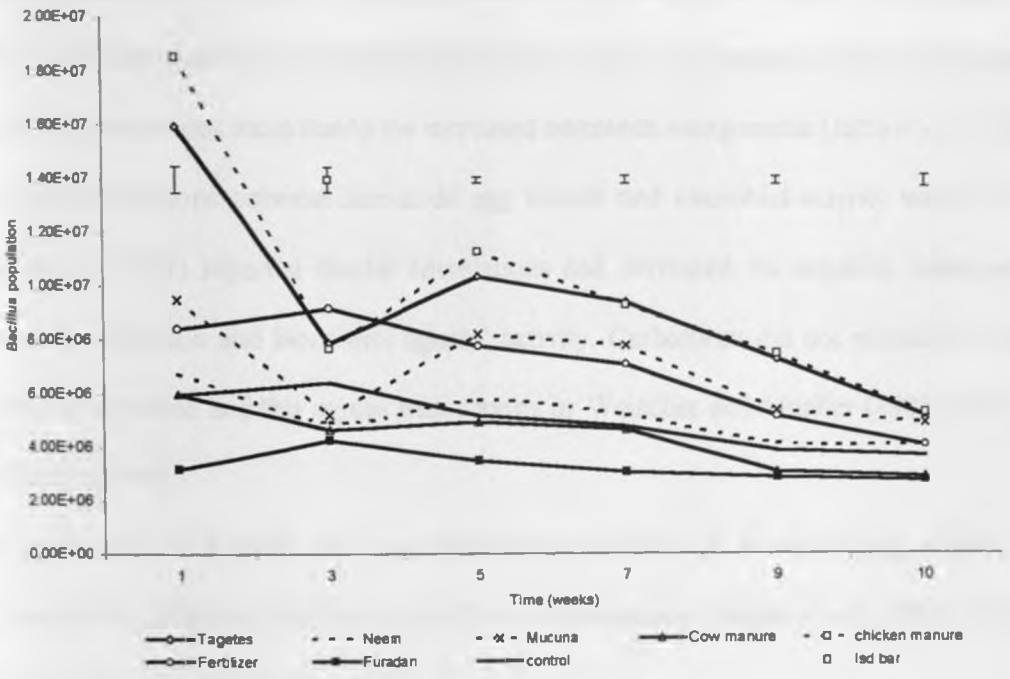

Figure 5. Population of Bacillus spp assessed in the amended soil

5.3.7 Correlation between nematode stages and soil parameters in amended soils.

Negative correlations were observed between the soil characteristics and the nematode stages (Table 6). Significant but negative correlations were exhibited between NH_4^+-N and egg masses at 45 and 70 DAI. Such negative correlation was reported by Rodriguez-Kabana *et al.*, (1981) who noted a direct negative relationship between NH_4^+-N content and nematode suppression. Huebner *et al.* (1983) and Eno *et al.* (1995) reported that high NH_4^+-N was toxic to nematodes through its disruption of their cell membranes.

Available phosphorous was also significantly negatively correlated with egg masses. This may also suggest toxicity against nematodes although this aspect has not been studied extensively.

There were negative correlation between nematode damage and egg masses, and microbial biomass and activity. The microbial biomass was more strongly correlated to the nematode

parameters than their activity. This aspect was noted earlier by Jaffe *et al.* (1998), Badra *et al.* (1979) and Rodriguez-kabana and Morgan Jones (1987). The increased microbial population may affect nematodes more due to the increased nematode antagonistic (Jaffe *et al.*, 1998). Negative correlations between nematode egg masses and microbial activity were observed. Jaffe *et al*, (1998) reported similar correlations and attributed the negative relationship to increased enzymatic and biocontrol agents' activity. Carbofuran did not stimulate microbial activity as expected and this agrees with reports by Weischer and Mueller (1985) and Crump and Kerry (1986).

The population of *Bacillus* spp. was negatively correlated to nematode egg masses. They have antibiotic inhititory and parasitic effects on nematodes (Becker *et al.*, 1988; Brandbary 1986; Oostendorp and Sikora, 1990).

Table 6. Correlation coefficients between nematodes stages and soil parameters

Nematode stages	MBC	MBN	CO_2	*Bacillus* cuont	P	pH	NH_4^+-N
J2 at 45 DAI	-0.20	-0.23	-0.03	-0.12	-0.22	-0.48	-0.47
Egg masses at 45 DAI	-0.68	-0.81*	-0.06	-0.94**	-0.79*	-0.15	-0.39
J2 at 70 DAI	-0.66	-0.19	-0.03	-0.28	-0.44	-0.38	-0.55
Egg masses at 70 DAI	-0.26	-0.34	-0.23	-0.74**	-0.78*	-0.28	-0.85**

**Correlation significant at 0.01 level, *Correlation significant at 0.05 level.
DAI-days after soil infestation with nematodes, MBC-Microbial biomass carbon, and MBN-Microbial biomass nitrogen,.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The organic amendments tested reduced nematode population, reproduction and root damage on beans. Chicken manure was the most effective of the amendments with cow manure being the least effective. Carbofuran, a chemical used for nematode control on beans was very poor.
2. Water extracts from organic amendments immobilized *Meloidogyne* juveniles (J_{2s}) with chicken manure being the most potent. All the amendments showed high nematostsic activity thus suggesting their ability to immobilize root-knot nematodes.
3. The organic amendments tested enhanced soil NH_4^+-N, phosphorous and soil pH. NH_4^+-N, phosphorous and soil pH released were negatively correlated to egg masses, juveniles (J_{2s}) and galling indices. This indicated their involvement in nematode reduction. The nutrients were also thought to induce systemic nematode resistance or tolerance since the fertilizer treatment offered a better nematode control level than the control and carbofuran.
4. Addition of organic materials resulted in increased microbial biomass nitrogen, carbon and CO_2. They were negatively correlated to nematode stages. The increased microbial biomass and activity were thus directly involved in nematode suppression.
5. The population of nematode antagonists (*Bacillus* spp.) in the soil was stimulated by addition of organic materials in the soil. Their population was also negatively correlated to nematode reproductive potential and population. It is possible that they play a role in nematode suppression.

5.2 Recommendations

(a) There is need to standardize the amendments where possible e.g. through nutrient supplementation during composting to increase their potency against nematodes.

(b) Biocontrol agents need to be encouraged in the amendments either through amendment mixtures and/or isolation and introduction.

(c) There is also need to study further the interactions between nematodes, plant health and soil parameters in order to enhance the understanding of the mechanisms.

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Appendices

Appendix 1.Analysis of variance for the dry root weight at 70 DAI

Source of variation	DF	SS	MS	F
Dry root weight	7	0.141	0.02	3.74^{NS}
Error	21	0.113	0.005	
Total	31	0.29		

NS not significant at P=0.005

Appendix 2. Analysis of variance for dry shoot weight at 70 DAI

Source of variation	DF	SS	MS	F
Dry root weight	7	58.43	8.34	18.47**
Error	21	9.49	0.45	
Total	31	69.05		

**significant at P=0.001

Appendix 3. Analysis of variance for egg mass indices at 70 DAI

Source of variation	DF	SS	MS	F
Egg mass index	7	144.00	20.57	20.95**
Error	21	20.62	0.98	
Total	31	167.00		

**significant at P=0.001

Appendix 4. Analysis of variance for galling indices at 70 DAI

Source of variation	DF	SS	MS	F
Galling indices	7	85.00	12.14	18.3**
Error	21	13.93	0.66	
Total	31	102.00		

**significant at P=0.001

Appendix 5. Analysis of variance for juvenile count at 70 DAI

Source of variation	DF	SS	MS	F
Juveniles	7	14166236	2023748	1175.01**
Error	21	36169	1722	
Total	31	14208562		

**significant at P=0.001

Appendix 6. Analysis of variance dry root weights at 45 DAI

Source of variation	DF	SS	MS	F
Dry root weight	7	0.099	0.014	4.54^{NS}
Error	21	0.066	0.003	
Total	31	0.210		

NS not significant at P=0.005

Appendix 7. Analysis of variance for dry shoot weights at 45 DAI

Source of variation	DF	SS	MS	F
Dry shoot weight	7	9.52	1.36	14.37**
Error	21	1.98	0.09	
Total	31	11.53		

**significant at P=0.001

Appendix 8. Analysis of variance for egg mass indices at 45 DAI

Source of variation	DF	SS	MS	F
Egg mass indices	7	117.718	16.81	24.2**
Error	21	14.593	0.69	
Total	31	134.21		

**significant at P=0.001

Appendix 9. Analysis of variance for galling indices at 45 DAI

Source of variation	DF	SS	MS	F
Galling indices	7	37.2	5.31	16.26**
Error	21	7.82		
Total	31	47.73		

**significant at P=0.001

Appendix 10. Analysis of variance for juveniles at 45 DAI

Source of variation	DF	SS	MS	F
Juveniles	7	9233002	1319000	28.8**
Error	21	961656	45793	
Total	31	10289423		

**significant at P=0.001

Appendix 11. Analysis of variance for microbial biomass nitrogen from 1st to the 10th week

Source of variation	Wk 1	Wk 3	Wk 5	Wk 7	Wk 9	Wk 10
F	51.64**	10.23**	10.23**	108.79**	39.28**	72.26**
Error	2102	6192	6192	350.2	95.6	5.016

**significant at P=0.001

Appendix 12. Analysis of variance for microbial biomass carbon from 1st to the 10th week

Source of variation	Wk 1	Wk 3	Wk 5	Wk 7	Wk 9	Wk 10
F	31.01**	31.36	22.99**	6.44**	16.55	72.26**
Error	92.13	71.34	13.65	1.05	19.95	5.16

**significant at P=0.001

Appendix 13. Analysis of variance ammonium nitrogen from 1st to the 10th week

Source of variation	Wk 1	Wk 3	Wk 5	Wk 7	Wk 9	Wk 10
F	25.39**	52.28**	21.71**	6.66**	7.84*	7.41**
Error	2012	1249	22.25	2.13	0.16	1.00

**significant at P=0.001

Appendix 14. Analysis of variance for pH from 1st to the 10th week

Source of variation	Wk 1	Wk 3	Wk 5	Wk 7	Wk 9	Wk 10
F	19.71**	31.72**	3.81^{NS}	101.4**	35.39**	152.4**
Error	0.04	0.03	0.26	0.01	0.02	0.05

**significant at P=0.001
^{NS} not significant at P=0.001

Appendix 15. Analysis of variance for immobilized juveniles from the 1st to the 5th hour

Source of variation	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
F	107.43**	183.76	183.02**	217.38**	1315.87**
Error	13211.05	217.35	23452.99	31576.38	35478.5

**significant at P=0.001

Appendix 16. Analysis of variance for phosphorous released from 1st to the 10th week

Source of variation	Wk 1	Wk 3	Wk 5	Wk 7	Wk 9	Wk 10
F	9.73**	11.23**	130.27**	315.94**	243.29**	220.4**
Error	168.21	229.3	66101.11	48754.77	31136	135551

**significant at P=0.001

Appendix 17. Analysis of variance for *Bacillus* population from 1st to the 10th week

Source of variation	Wk 1	Wk 3	Wk 5	Wk 7	Wk 9	Wk 10
F	244.23**	141.71*	69.76**	1042.96*	279**	102.77*
Error	8.034 E+14	9.262E+13	2.222E+14	1.522E+14	7.824E+13	2.720E+13

**significant at P=0.001

Appendix 18. Analysis of variance for carbon dioxide relaeased from 1st to the 10th week

Source of variation	Wk1	Wk3	Wk5	Wk7	Wk9	Wk10
F	25.32**	16.04**	6.39**	25.17**	13.43**	5.55**
Error	672140	958681	574802	323532	162147	780403

**significant at P=0.001