PLANT PARASITIC NEMATODES ASSOCIATED WITH CABBAGE IN DIFFERENT AGRO-ECOLOGICAL ZONES IN NYANDARUA AND EMBU COUNTIES, KENYA

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DECLARATION

I, James Maina Mwangi, declare that this thesis is my original work and has not been presented for the award of a degree in any other University or any other award.

James Maina Mwangi

Signature……………......................Date...........................…………

Supervisors Approval

We confirm that the work reported in this thesis was carried out by the candidate under our supervision as university supervisors.

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DEDICATION

This work is dedicated to my dad Joseph Mwangi, mom Mary Wangari and my wife Evalyne for their love, support and encouragement during the entire period of study.

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ABBREVIATIONS AND ACRONYMS

AEZ	Agro-ecological zones
ANOVA	Analysis of variance
FLN	Free-living nematodes
GR	Genera richness
HCDA	Horticultural Crop Development Authority
IPM	Integrated pest management
J2	Stage two juvenile
JICA	Japan International Cooperation Agency
LSD	Least significant difference
PPM	Parts per million
PPN	Plant parasitic nematodes
RKN	Root knot nematodes
TAF	Triethanolamine-formaline
UH	Upper highland
UM	Upper midland

ABSTRACT

Cabbage (*Brassica oleracea* L. Var. *capitata*) is an important vegetable crop in Kenya. It is widely used as human food and animal feed. Owing to findings that cabbage is a poor host to root knot nematode (RKN), this crop has been incorporated in plant parasitic nematode (PPN) management as a rotation crop. As a result very little has been done to study nematodes associated with this crop, yet there are reports of nematodes infecting it. A survey was therefore conducted in Nyandarua and Embu Agro-ecological Zones (AEZs), of Kenya to determine the types, abundance and frequency of occurrence of PPNs associated with cabbage. A total of 60 soil and root samples were collected from six AEZs. Nematodes were extracted from 100 g of soil per sample using the modified Baermann extraction tray technique while nematodes within the roots were extracted using modified maceration and filtration technique. Roots were rated for galling index using a scale of 0-5, then stained for egg masses and presence of endoparasitic nematodes. Extracted nematodes were enumerated and identified to genera level. A total of nineteen (19) genera of PPNs belonging to ten (10) families of the Order Tylenchida, Dorylaimida and Triplonchida were identified from both soil and roots. Lesion nematodes (*Pratylenchus* spp.) were detected in 58% of the root samples, followed by *Helicotylenchus* spp. (27%) and root-knot nematode (RKN) *Meloidogyne* spp. (23%). The RKN galling index ranged from 1 to 2 on a scale of 1-5. The lesion nematodes had the highest frequency of occurrence (A/F) in soil (87%) followed by *Helicotylenchus* spp. (82%) and *Tylenchorhynchus* spp. (67%). Other PPNs genera were identified at varying absolute frequencies. The population densities of nematodes differed significantly ($P<0.05$) between AEZs. The population density of PPNs recorded from cabbage roots in UH1b was significantly ($P<0.05$) higher than all other AEZs. The highest nematode population in soil was recorded in UH1a while UM1 had the lowest for both root and soil samples. Various genera of PPN were found to differ ($P<0.05$) significantly. *Pratylenchus* spp. was present in a higher population density compared to other nematodes in both roots and soil, *Helicotylenchus* spp. and RKN were second in density in soil and root respectively. There was no significant difference ($P>0.05$) between RKN galling index across AEZs. Soil physical-chemical properties, farmer practices and cabbage varieties cultivated may have significantly influenced occurrence and population densities of various PPN. Pathogenicity tests are necessary to determine the damages caused by nematodes reported herein. There is also need to screen various cabbage cultivars for resistance and tolerance to various PPNs.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

White cabbage (*Brassica oleracea* L. Var. *capitata*) is widely grown in Kenya. In 2007, it was ranked first in terms of production ahead of tomato and kale according to a report by Ministry of Agriculture, (2007). Cabbage is a source of food and income to most Kenyans (Kungu, 2005). It is a high source of fibre, protein, calcium, vitamins such as A, B_6, C, K, folic acids and minerals. It is used as vegetable by human and fodder to animals in addition to having medicinal values to human (Fahey *et al*., 2001). According to the Ministry of Agriculture (2007), the common varieties of cabbage grown in Kenya include Victoria, Riana, Pructor, Rinda, Gloria, Golden Acre, and Amigo. In 2007 alone, 609,292 metric tonnes of cabbage were produced in Kenya compared to 493,376 metric tonnes produced in 2006. However cabbage production is faced with various constraints including bacterial diseases such as bacterial soft rot and black rot, fungal diseases such as ring spot and downy mildew among others. Major pests of cabbage include Aphids, cutworms and diamond blackmoth among others (Kungu, 2007).

Cabbage has been used as a rotational crop in management of root-knot nematodes (RKN) especially in vegetable and cereal production systems (Bello *et al*., 2004; Pattison *et al*., 2006). This has led to the assumption that cabbage is a poor or non-host to all other plant parasitic nematodes (PPNs). However, studies conducted in different parts of the world reveal that cabbage is a host to some nematodes of high economic importance (Potter and Olthof, 1993 and Waceke, 2007). Continuous use of cabbage as a rotational crop in PPN management, therefore, may lead to emergence of serious nematode pests. Survey of PPN associating with cabbage was therefore necessary to avert any danger arising from the practice. Information on PPN associated with cabbages is lacking in Kenya.

1.2 Problem statement and justification

Cabbage is a poor host to RKN (Bafokuzara, 1983; Carneiro *et al*., 2000) a characteristic that is attributed to production of compounds by cabbage which have bio-fumigant properties in the soil (Clark, 2007). Reports of cabbage as a poor host to RKN has been interpreted to mean resistance to all PPNs, thus, it has been widely used in nematode management as a rotational, intercrop, cover crop or as soil amendment against all PPNs (Manfort *et al*., 2007). As a result, very little research work has been done to study PPNs associating with cabbage while the crop continues to be used in nematode management (Bello *et al.*, 2004; Pattison *et al.,* 2006).

Research has revealed that some PPNs associate with cabbages in Kiambu and Kajiado-Kenya (Waceke, 2007), in Uganda (Bafokuzara, 1996) and other parts of the world (Mennan and Handoo, 2006; Potter and Olthof, 1993). Reports of

PPNs associating with cabbage raise concern over cabbage use in nematode management.

Resistance of cabbage to RKN does not necessarily imply that it is resistant to all other PPN species (Crittenden, 1955). Therefore, persistent use of cabbage by farmers as rotational crop in PPN management might allow nematodes hosted by cabbage to increase beyond the economic threshold level leading to emergence of serious nematode pests initially unknown to be important. It has been found that crop rotation designed to manage a specific plant nematode species often does so at the expense of increasing other species hosted by the crop to damaging levels (Daulton, 1963; Brodie *et al.,* 1970; Minton and Donnelly, 1971). As such, repeated use of species specific non-host cultivars may select nematodes species able to overcome the resistance (Netschers, 1976). In addition, overreliance on a single crop in nematode management may break crop resistance to nematode (Bost and Triantaphyllou, 1982).

As such, cabbage may become a host to non-target PPNs. For instance, the lesion nematode (*Pratylenchus zeae)* is listed among the common nematodes associated with maize production (Swarup and Sosa-moss, 1990) and has also been reported in cabbage (Waceke, 2007). Rotation of maize with cabbage could lead to build up of this nematode raising its population above the injurious threshold thus limiting cabbage production. Continued use of cabbage may lead to emergence of a new serious crop pest hitherto unknown thus limiting vegetable production.

There was need therefore, to carry out an extensive survey of plant parasitic nematodes associating/infecting cabbage with an aim of understanding any risk that may arise owing to over reliance on cabbage as a tool in PPN management. The study will provide information on the viability of cabbage as a rotation crop in nematode management as well as providing information on PPN of cabbages that could have serious implication to cabbage production.

1.5 Hypotheses

i. Plant parasitic nematodes do not associate with cabbages in Nyandarua and Embu agro-ecological zones.
ii. Population densities of plant parasitic nematodes associated with cabbage do not differ in Nyandarua and Embu agro-ecological zones.
iii. Population densities of various plant parasitic nematode genera associated with cabbages in Nyandarua and Embu AEZs do not differ.
iv. Farming practices and soil factors do not influence plant parasitic nematode population in Nyandarua and Embu agro-ecological zones.

1.6 Objectives

1.6.1 General objective

To understand the population structure of plant parasitic nematodes associated with cabbages in different agro-ecological zones of Nyandarua and Embu and the potential impact of continued use of cabbage as a tool in nematode management for vegetable production in Kenya.

1.6.2 Specific objectives:

i. To determine plant parasitic nematodes associated with cabbage in Nyandarua and Embu agro-ecological zones.
ii. To compare population densities of plant parasitic nematodes associated with cabbages between various agro-ecological zones of Nyandarua and Embu.
iii. To determine the difference in population densities of various plant parasitic nematode genera associated with cabbage, in Nyandarua and Embu agro-ecological zones.
iv. To determine the influence of selected farming practices and soil factors on plant parasitic nematode population in Nyandarua and Embu agro-ecological zones.

CHAPTER TWO

LITERATURE REVIEW

2.1 Production of cabbage and its economic importance

Cabbage grows well in cool and moist climate 16-20^{o} C or even lower, with an ability to adapt to a wide range of soil and climatic factors and hence it is grown in various parts of the world. It does well in altitude ranging from 800 to over 2,000 m, well drained soil with high organic matter content and pH of 6 to 6.5 (Ministry of Agriculture and JICA, 2000). Cabbage is a major horticultural crop in Kenya, grown for both local and export market in European market (Kungu, 2005). In the year 2007, cabbage was leading in the country in terms of production ahead of tomatoes and kales (Ministry of Agriculture, 2007) with more than six thousand metric tonnes produced.

Figure 1.1 Cabbages production in UH1, growing (A) and mature (B) cabbages

Cabbage is a high source of fibre, protein, calcium, vital vitamins such as A, C, B_6, K and folic acid (HCDA, Ministry of Agriculture and JICA, 2003; Kungu, 2005). Besides being used as human food and feed to animals, cabbage has been found to have medicinal value associated with glucosinolates (Fahey *et al*., 2001) with the potential of preventing or lowering risks of some cancers in human (Zhang and Talalay, 1994; Zasada and Ferris, 2003). This makes it a good constituent of human diet. Consumption of Brassica vegetables contributes most of our glucosinolate compounds intake (Fenwick *et al.*, 1983; Cartea *et al*., 2008).

2.2 Cabbage and nematode interaction

Brassicas posses nematode suppressive effects attributed to glucosinolate compounds contained in their residues (Guerana, 2006). During decomposition glucosinolates breakdown to active sulphur containing compounds such as isothiocynates (Gardiner *et al*., 1999; Petersen *et al*., 2001) giving Brassicas their natural fumigant potential against some nematodes, bacteria, fungi, insect and weeds (Clark, 2007). Several thiocyanates and isothiocyanates are known to have toxicity to certain nematode species (Zasada and Ferris, 2003).

Brassicas have been reported to have nematicidal properties when incorporated as green manure and suppressive ability when used as cover crop (Manfort *et al*., 2007). High levels of this nematoxic-glucosinolate compound, has been found in cabbage (Cartea *et al*., 2008). It is on these grounds that cabbage has been considered as a good rotation crop in control of RKN. For instance, addition of Brassica amendment was shown to significantly reduce RKN (*Meloidogyne incognita*) gall on tomato by between 38-100% (Stapleton and Duncan, 1998).

2.2.1 Plant parasitic nematodes

Plant parasitic nematodes are obligate parasites that complete their life cycle partially or entirely in the soil environment closely associated with plant roots and destroy plant tissue during feeding. They are wide spread and adapted to nearly every ecological area. They exploit all parts of vascular plants but the most economically significant species infect roots (Bird and Kaloshian, 2003).

2.2.2 Factors influencing distribution of PPN

The population density and the distribution of PPNs are influenced by various biotic and abiotic factors. Such factors include farming practices such as application of organic amendment in form of manure. According to Wachira *et al* (2009), addition of organic matter in the soil suppresses PPN by generating toxic compounds in the soil that suppress nematodes. On the other hand, soil texture influences the population density of nematodes. According to Norton (1979) loose soil, that has high porosity, support high population of PPN as a result of improved aeration and mobility of nematodes in the soil (Sultan and Ferris, 1991).

Environmental factors that influence nematode distribution include soil moisture. Plant nematodes are able to multiply in wet soil (but not water logged). Moisture enables mobility and infection of appropriate host by PPN (Sultan and Ferris, 1991). According to Norton (1979) temperature may also influence nematodes. Most PPN are active between 25-30° C. As such there is high nematode density of PPN along the tropical region. However these factors do not work independently to influence nematode population.

2.2.3 Economic importance of PPN

Plant parasitic nematodes affect crop production and cause yield losses (Sasser, 1980) estimated at US$ 125 billion worldwide annually (Chitwood, 2003). Some nematodes such as, root-knot (*Meloidogyne)* and cyst nematodes *(Heterodera* and *Globodera)* cause enormous losses every year by attacking a wide range of economically important horticultural and field crops as well as forest systems (Ibrahim and Traboulsi, 2009). Despite PPN destructive nature, they are often over looked (Bridge, 1996) or rarely perceived to be pests by many agricultural scientists. As a result, farmer's awareness of the nematodes as pests is very limited especially in Sub-Saharan Africa (Bridge, 1996) while they continue to halt crop production.

Plant parasitic nematode attack and damage to plant occurs over a period of time and interacts dynamically with plant growth, as well as environment in which the plant is grown. Nematodes harm the plant by causing direct mechanical injury using the stylet during penetration and/or by secretion of enzymes into the plant cells while they utilize plant's photosynthates (Luc *et al.*, 1990). The physical presence of PPNs blocks transport in the vascular system (Hussey and Williamson, 1997) making the roots less efficient in absorbing water and minerals from the soil as well as transporting them to the plant for use in photosynthesis. Nematodes also expose crops to secondary infections by soil-borne fungal and bacterial pathogens (Agrios, 1978; Sikora and Carter, 1987). Some transmit plant viruses (Brown *et al*., 1995). Some nematodes such as RKN are important worldwide, infecting nearly every plant species (Sasser, 1980; Potter and Olthof, 1993) including cabbage (Waceke, 2007).

2.2.4 Feeding behaviour of PPN

Plant parasitic nematodes exhibit different feeding mechanisms (Luc *et al.,* 1990). Some are ectoparasites where they feed on root tissue by inserting their stylet from outside the root. Such nematodes which include *Tylenchorhynchus* spp., and *Trichodorus* spp., are migratory ectoparasites. Some other nematodes are semi-endoparasitic where the anterior section of the nematode penetrates the root while the posterior section of the nematodes remain in the soil, they include *Helicotylenchus* spp. and *Scutellonema* spp. (Orbin, 1973). Endoparasitic nematodes invade the root tissue by their entire body. They possess a robust stylet, which they use to enter the root and periodically feed as they migrate intracellularly through the root tissue. Nematodes such as *Pratylenchus* spp. cause extensive destruction of root tissue along the feeding path (Hunt *et al.*, 2005). Sedentary endoparasites feed from a single cell or a group of cells leading to a formation of specialized feeding cells that become permanent source of nutrients for the nematodes (Luc *et al*., 1990). The invasive juveniles of these sedentary endoparasites penetrate plant cells and lose their mobility and, if they are to develop into adult females, they transform the cells on which they feed to remain alive and change in ways that improve the supply of food by formation of a single multinucleate giant cell (Trudgill, 1991). They include *Meloidogyne* spp., *Heterodera* spp. and *Globodera* spp. These nematodes lose their mobility and become obese. They are damaging particularly to vegetables in tropical and subtropical countries (Sikora and Fernandez, 2005). Some of the common symptoms of nematode infection are the formation of root galls which results in growth reduction, reduction in nutrient and water uptakes, increased wilting, mineral deficiency, weak and poor yielding plants (Abad *et al*., 2003).

2.2.5 Control and management of PPN

Control of PPNs is quite challenging and relies heavily on the use of soil chemical fumigants especially in commercial production systems. However, concerns have arisen out of findings relating their use to human health and environmental problems. Some pesticides contain active ingredients that have been shown to act as hormone disruptors, possibly causing loss of fertility, carcinogenesis and inducing mutagenesis. Because of this some broad spectrum nematicides have been restricted or withdrawn from the market and also due to their adverse effect to the environment (Lilley *et al*., 2007).

As a result, integrated pest management (IPM) has been adopted in nematode management. Integrated pest management integrates different practices in order to manage nematodes. They include use of cultural practices which involve the use of nematode free nurseries or seedlings to prevent introduction of nematode into the field, crop rotational practices which are designed to reduce the impact of nematode in cropping systems and use of organic amendments. Other PPN control methods include use of resistant and tolerant varieties, physical methods such as solarisation and use of naturally occurring biological control agents (Oka *et al*., 2007; Bridge, 1996).

Cabbage is said to possess some vital attributes thus making it a potential crop in nematode management (Pattison *et al*., 2006). It is has been proposed as a good choice in crop rotation systems in nematode management. A number of rotation systems exist which are predominantly composed of cruciferous crops which are said to be moderately resistant or tolerant to nematodes (Luc *et al*., 1990). This has led to persistence use of cabbage in nematode management.

2.3 Nematodes of cabbage

Different genera of PPNs have been found associating with cabbages in different countries (Radewald *et al*., 1971; Olthof *et al.,* 1974; Potter and Olthof, 1993). They include eleven genera identified by Mennan and Handoo (2006) in Turkey among them lesion (*Pratylenchus thornei*), spiral

(*Helicotylenchus* spp.) and cyst (*Heterodera cruciferae* and *Heterodera mediterranea*), while Waceke (2007) has identified various nematodes associating with cabbages in Kiambu and Kajiado counties of Kenya.

These findings showing presence of PPNs associating with cabbage raises concern over consistency of cabbage use as rotation crop in nematode management. In addition, the identity and population of nematodes that are associated with yield losses in the crop have not received the necessary attention and are largely unknown (Bafokuzara, 1996).

2.4 Taxonomy of plant parasitic nematodes

Plant parasitic nematodes are mainly divided into three groups; the Tylenchs, Longidorids and Trichodorids (Luc *et al.*, 1990).

2.4.1 Order Tylenchida

This Order has the most PPNs, and they include; Heterodoridae, Hoplolaimidae, Pratylenchidae, Meloidogynidae among others (Hunt *et al.*, 2005). Members of this group are vermiform nematodes though in some genera such as *Meloidogyne*, *Heterodera* and *Globodera* the female loses vermiform shape and become obese or even globose (sexual dimorphism). Their body length ranges between 0.2 to 1 mm, but occasionally over 3 mm. They possess a stomatostylet, a protrusible culticular tube generally swelling posteriorly to form a basal knob. The knobs may be rounded as in *Pratylenchus* or tulip shaped with anterior tooth-like projections as in *Hoplolaimus* (Siddiqi, 2000; Luc et al., 1990).

Tylenchina have a complete digestive system comprising of stylet, oesophagus, intestine and rectum all of which are of diagnostic value. The type of oesophagus and intestine overlap is vital in identification. In *Radopholus, Scutellonema* and *Hoplolaimus* spp. oesophagus overlaps intestine dorsally. In *Pratylenchus* and *Helicotylenchus* the overlap is ventral while oesophagus abuts intestine in *Tylenchorynchus, Tylenchus* and *Coslenchus* spp. (Hunt *et al*., 2005; Siddiqi, 2000).

Female possess a reproductive structure which comprises of ovary, oviduct uterus and vagina and a specialised spermatheca may be present. The female genital system may be didelphic or monodelphic. The position of vulva is significant is identification, for instance the vulva is posterior (60%-70%) in *Helicotylenchus* and median in *Radopholus* spp. The shapes of the tail tip tend to vary within members of this group of nematode. Some are dorsally convex-conoid or hemispherical as in *Helicotylenchus*, others are elongated as is the case with *Radopholus* while other are conical in shape. For instance *Heterodera* J2 has a conical tail with hyaline portion about 50% of the tail length while *Meloidogyne* J2 has conical tail with hyaline region starting near the tail tip (Jabbari and Niknam, 2006; Hunt *et al*., 2005). These features are significant in nematode identification.

2.4.2 Order Dorylaimida

Sub-order Dorylaimina (Longidorids) (Luc *et al.,* 1990)

This group of nematodes share much in common with Tylenchs, besides, they are much longer and range from 0.9-12 mm in size; they possess odontostylet which is needle like structure attached posteriorly to a cuticular extension-the odontophore. They include *Xiphinema* and *Longidorus* (Hunt *et al*., 2005). *Xiphinema* in identified by the presence of odontostylet with three prominent basal flanges and forked base. In addition, *Xiphinema* stylet has a guiding ring located at the posterior of the odontostyle. These features distinguish it from *Longidorus* whose amphid is poach-like, the stylet lacks flanges and the guiding ring is at the anterior half of odontostyle (Hunt *et al*., 2005).

2.4.3 Order Triplonchida

Sub-order Diphtherophorina (Trichodorids)

They are short in size ranging between 0.5-1.1 mm, cigar-shaped with bluntly rounded head and tail. They possess an onchiostyle which is a curved feeding structure. Males have slightly curved spicules and a weak bursa. They include *Paratrichodorus* spp. and *Trichodorus* spp. (Hunt *et al*., 2005). *Trichodorus* is characterised by strong vaginal scelerotization while the vagina extend into body for about half its diameter. *Paratrichodorus* has weak vaginal sclerotization that extends into body for about a third of its diameter (Hunt *et al*., 2005).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study sites

Soil and cabbage root samples were obtained from six agro-ecological zones in Nyandarua and Embu regions (Fig 3.1 and 3.2). The areas represented high altitude (Upper Highland Zones-UH) and mid altitude (Upper Midland Zone-M) in the two regions.

Sampling in Nyandarua was carried out in five agro-ecological zones where cabbage was being grown during the period of sampling. These AEZs included; UH1a at Mutarakwa in North-Kinangop Division; UH1b at Melangine in Ol-kalou Division; UH3 at Magumu in South-Kinangop Division, UH2 at Ngano in Ol-joro-orok Division and UH4 near Lake Ol-bolossat in Ndaragwa Division (Jaetzold and Schmidt, 1983).

The upper highland 1 (UH1) is also described as Sheep and Dairy Zone. It lies at an altitude of 2500-2740 m, and temperature ranging between 10.0-14.6^{o} C and an average annual rainfall of 1150-1600 mm. The zone has two long rain seasons in a year. It has good yield potential for crops such as oats, peas, potatoes, cabbages, carrots among others with very little maize production (Jaetzold and Schmidt, 1983). The UH2 is also described as the Pyrethrum-wheat Zone with a very long cropping season and intermediate rains divided into two variable cropping seasons. It lies at 2400-2500 m and temperature close to UH1 and it receives an average annual rainfall of 1000-1200 mm. The zone has good yield for crops such as vegetables and oats in addition to growing wheat, pyrethrum and pasture.

The UH3 is also referred to as Wheat-Barley Zone with a very long cropping season, which can be split into two variable cropping seasons perfect for vegetable, wheat, barley and maize production. The UH4 is also referred to as Upper Highland Ranching Zone. The zone has unimodal rainfall but with intermediate rains. It is not suited for agriculture due to low rainfall and frequent night frosts (Jaetzold and Schmidt, 1983). Cabbage production in this zone is done by supplementing rain fed agriculture with irrigation during drought. These AEZs in Nyandarua are leading in vegetable production contributing to 34% value share in all vegetables transported to Nairobi, (Tschirley and Ayieko, 2009).

In Embu, samples were collected in one AEZ (UM1) at Manyatta where cabbage was being grown during sampling. This zone is also described as Coffee-Tea Zone; located in altitude of 1600-1850 m and with temperatures ranging between 17.5-18.9^{o} C. It has a fully long cropping season with intermediate rains. It has two rainy seasons at mid-March and mid-October. The first season favour cabbage production amongst other vegetables (Jaetzold and Schmidt, 2006).

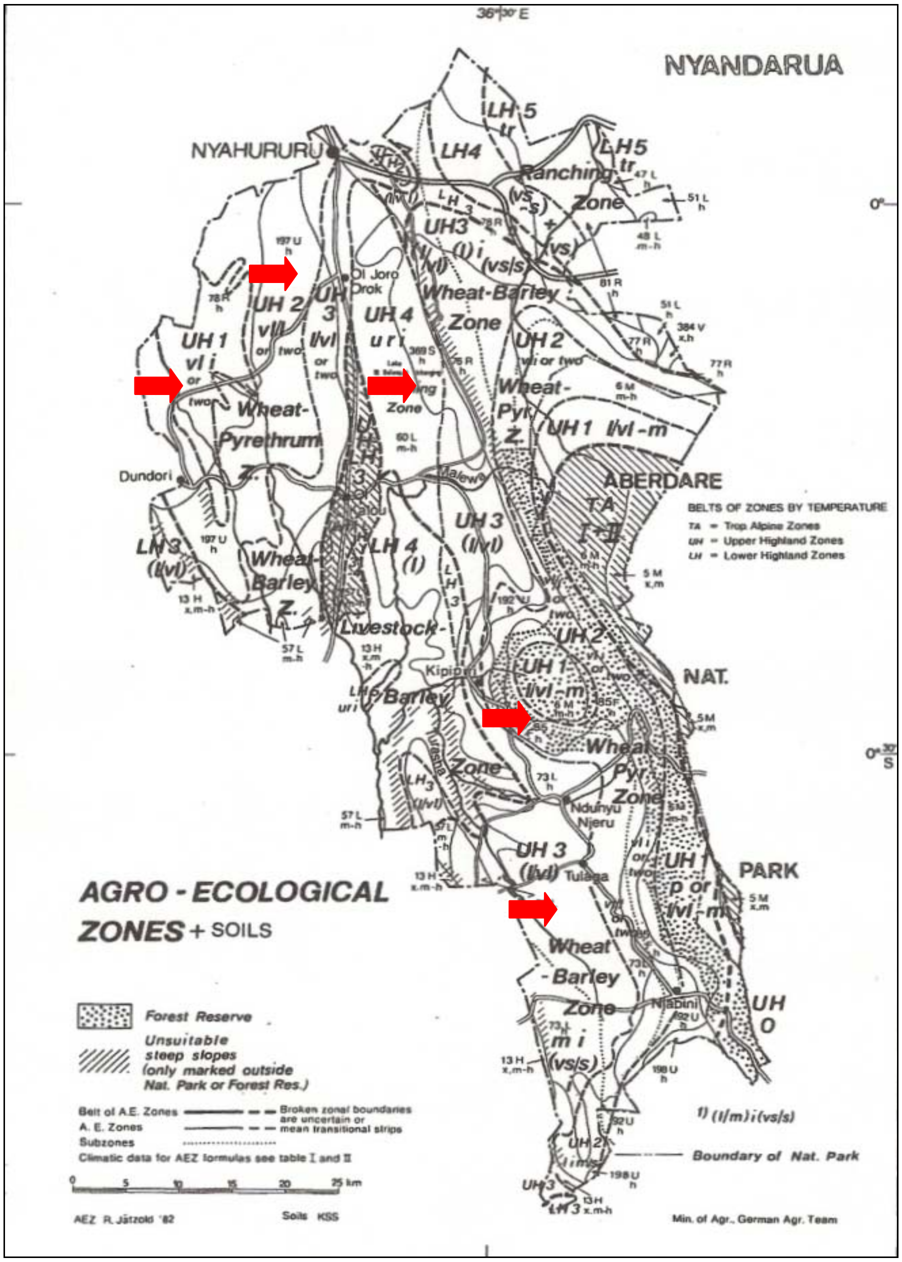


Figure 3.1 Map showing Nyandarua agro-ecological zones; sampled areas are indicated with the arrow. Adopted from Jaetzold and Schmidt (1983)

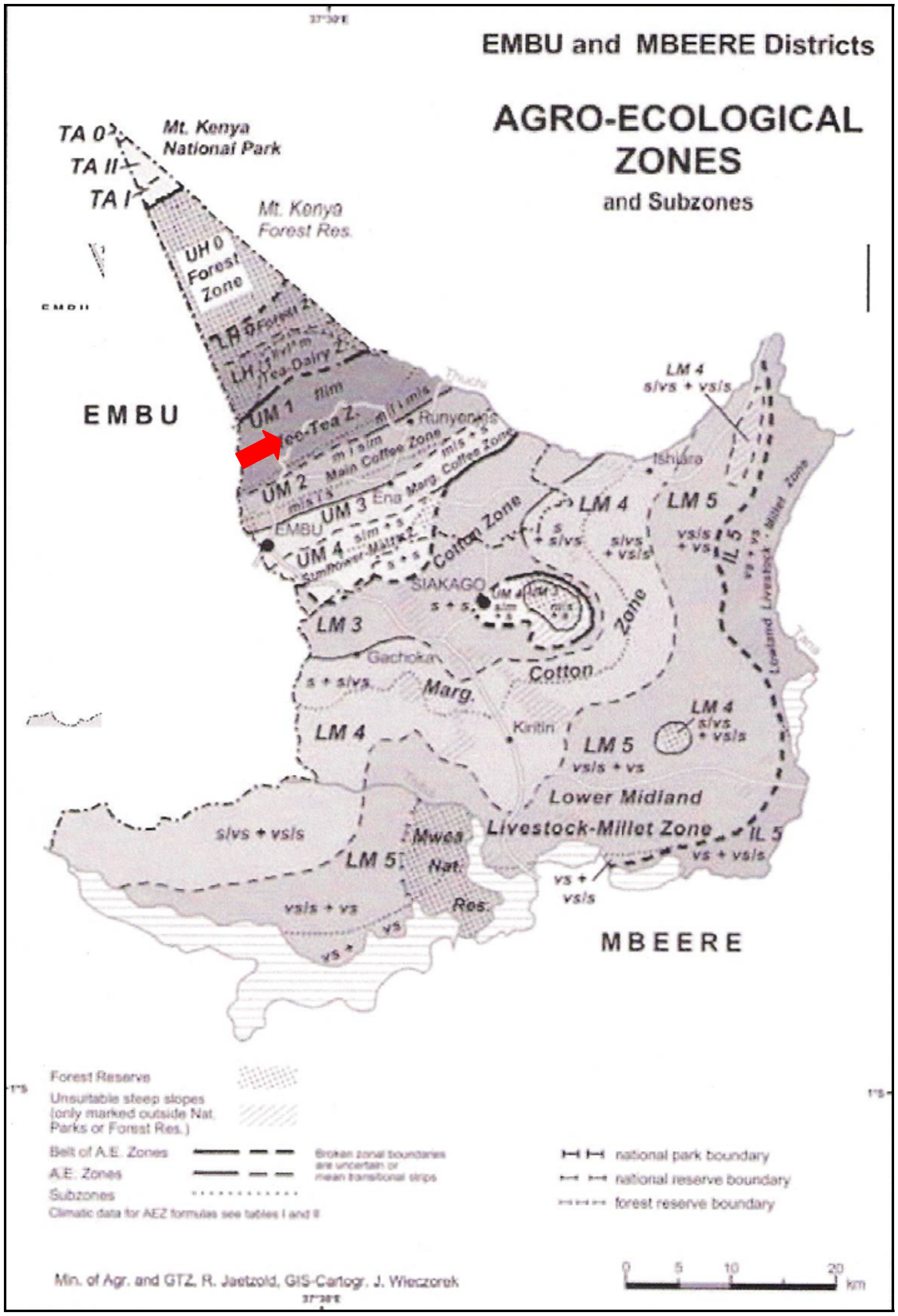


Figure 3.2 Map showing Embu agro-ecological zones; sampled areas are indicated with the arrow. Adopted from Jaetzold and Schmidt (1983)

3.2 Sampling

Sampling was done between September and November, 2009. This is the period that corresponded with the cabbage maturity in the two regions thus providing enough samples for the study. A total of ten soil and root samples were randomly taken in each AEZ from a list of cabbage farmers prepared per AEZ. Ten samples of cabbage roots and soil per farm were collected in a systematic, zigzag pattern to obtain a representative sample. Areas with roots of weeds or other species were avoided whenever possible to avoid sampling nematodes from non-target host (Knight, 2001).

The plants were gently dug out using a garden trowel to a depth of 20 cm taking care not to damage the fine roots. About 1 kg rhizosphere soil was taken after uprooting the plant. Approximately 10 kg of soil per farm was obtained by compositing 10 samples from locations within an area of one hectare at each field (Young, 1990). The composited soil was thoroughly but gently mixed before withdrawing a representative sub-sample of about 1 kg to be used for nematode assay. The roots together with soil subsamples were put in a plastic bag, sealed and labelled accordingly before being transported in a cool box to the laboratory and stored at 10º C until processing.

Information on farmer practices, the cropping history, pests, diseases, other crops grown in the farms, sources of planting material, frequency of cabbage cropping among others were collected from the field by observation and using a structured questionnaire administered to sixty farmers from whom samples were collected (Appendix I).

3.3.2 Soil analysis

A representative composite soil sample was obtained from each farm for soil physical-chemical analysis which was done at the National Agricultural Research Laboratories (NARL) in Kenya. Total nitrogen (N) in the soil was determined using Kjedahl method. Total organic carbon (C) was estimated through calometric method (Gallaher *et al*., 1975). Phosphorous (P) was analyzed by colomitery, potassium (K) and sodium (Na) by flame emission spectrophotometry, and calcium (Ca) and magnesium (Mg) by atomic absorption spectro-photometry (Gallaher *et al*., 1975). The soil characteristics are depicted in tables 3.1-3.3.

3.3.3 Staining of nematodes in plant tissues

Ten grams of roots system from each sample were stained to help visualize *Meloidogyne* spp. females and egg masses. Roots were immersed in phloxine B (0.15 g/l water) for 15 minutes rinsed and examined in water. Gelatinous matrix of the egg sac stained red (Holbrook *et al*., 1983). Egg masses were counted by use of stereomicroscope and scored using 0-5 egg mass rating index; where 0 = no egg masses;1 = 1-2; 2 = 3-10; 3 =11-30; 4 =31-100 and 5> 100 egg masses per root system (Taylor and Sasser, 1978).

Table 3.1 Physical-chemical properties of soils from UH1a and UH1b study sites

	Upper Highland 1a (UH1a)					**Upper Highland (UH1b)**				
Properties	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
pH	5.36	5.35	4.83	5.73	5.32	4.74	4.85	5.24	5.09	5.0
N (%)	0.17	0.17	0.16	0.16	0.16	0.16	0.20	0.17	0.16	0.17
K (%)	2.01	1.00	0.90	2.49	1.6	0.96	0.90	1.61	2.21	0.98
C (%)	1.70	1.65	1.58	1.62	1.60	1.65	2.01	1.71	1.71	1.72
P (p.p.m)	56	25	15	40	34	39	29	36	48	37
Ca (%)	7.90	4.80	5.0	9.7	6.4	3.80	3.60	6.0	9.7	4.10
Mg (%)	4.75	2.61	1.78	4.86	3.5	1.97	3.05	3.12	3.62	3.21
Mn (%)	0.34	1.05	0.80	0.99	0.94	0.94	1.13	1.13	1.58	1.14
Na (%)	0.3	0.20	0.26	0.30	0.2	0.2	0.14	0.16	0.16	0.15
Texture	C	CL	CL	CL	CL	L	L	CL	CL	CL

SL=Sandy Clay Loam **CL**=Clay Loam **C**=Clay **L**=Loam

Table 3.2 Physical-chemical properties of soils from UH2 and UH3 study sites

	Upper Highland 2 (UH2)					Upper Highland 3 (UH3)				
Properties	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
pH	5.67	5.56	6.26	5.69	5.56	5.27	5.22	5.93	5.32	5.22
N (%)	0.18	0.18	0.17	0.19	0.18	0.17	0.17	0.17	0.17	0.18
K (%)	3.09	2.25	2.21	2.41	2.33	1.49	1.75	2.25	1.61	1.82
C (%)	1.84	1.79	1.65	1.85	1.77	1.68	1.72	1.67	1.66	1.7
P (p.p.m)	41.0	39.0	44.0	31.0	37.0	38.0	35.0	183	40	42
Ca (%)	10.9	8.10	7.7	11.7	9.8	6.70	7.70	9.9	6.5	7.3
Mg (%)	6.09	4.27	4.25	4.69	4.30	2.22	2.24	4.31	3.36	2.32
Mn (%)	1.35	1.25	1.34	0.98	1.27	0.48	0.73	0.92	1.54	0.96
Na (%)	0.3	0.28	0.18	0.6	0.5	0.2	0.28	0.36	0.24	0.26
Texture	L	CL	C	CL	CL	CL	L	CL	L	CL

SL=Sandy Clay Loam **CL**=Clay Loam **C**=Clay **L**=Loam

Table 3.3 Physical-chemical properties of soils from UH4 and UM1 study sites

	Upper Highland 4 (UH4)					Upper Midland 1 (UM1)				
Properties	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
pH	6.11	5.99	6.19	5.58	5.98	6.19	6.43	5.80	6.04	5.16
N (%)	0.17	0.17	0.16	0.18	0.16	0.87	0.87	0.87	0.73	0.56
K (%)	1.16	2.01	2.41	0.54	1.86	0.92	0.41	0.46	0.06	1.24
C (%)	1.73	1.72	1.63	1.68	1.53	4.07	3.33	4.24	4.17	2.69
P (p.p.m)	133	92	145	41	70	52.73	51.85	33.90	35.02	41.31
Ca %	10.7	8.50	13.3	7.7	10.2	24.8	28.0	8.40	14.8	7.60
Mg (%)	5.53	5.56	5.96	6.27	5.66	6.09	3.53	0.97	3.83	2.05
Mn (%)	0.61	6.71	0.29	0.38	0.44	0.47	0.42	0.25	0.46	1.48
Na %	0.4	0.36	0.72	0.66	0.52	0.30	0.28	0.11	0.16	0.10
Texture	CL	SL	CL	CL	CL	SL	L	SL	CL	SL

SL=Sandy Loam **CL**=Clay Loam **C**=Clay **L**=Loam

To detect the presence of the endoparasitic nematodes, 10 grams of roots were cleared in dilute sodium hypochlorite bleach (5.25% NaOCL). The roots were then rinsed thoroughly on 100 µm sieve to remove all traces of the bleach and facilitate staining. They were then stained with acid fuchsin solution consisting of 875 ml of lactic acid, 63 ml of glycerol, 62 ml of water and 0.1 g of acid fuchsin as described by Hooper *et al.,* (2005). The stained nematodes were visible in largely unstained root tissues and subsequently enumerated.

3.3.4 Extraction of nematodes from roots

Ten grams per composite root sample per farm was taken for nematode extraction using the modified maceration and filtration technique, (Hooper *et al*., 2005). Roots were chopped into lengths of 1cm and then placed into 100 ml of tap water and macerated in a blender for five seconds. A tissue paper was placed on a supporting sieve and the chopped material placed on it. The sieve and the material were placed on a tray filled with tap water just to submerge the sieve and the materials and kept for 24 hours (Fig 3.3A).

The nematodes emerged from the roots, passed through the tissue paper into the dish. The nematodes suspension was then poured though 38 µm sieve and the nematodes were back-washed by a gentle stream of water and collected in a small beaker. Nematodes obtained were enumerated and expressed as number of nematodes per 10g dry weight before being processed further for identification.

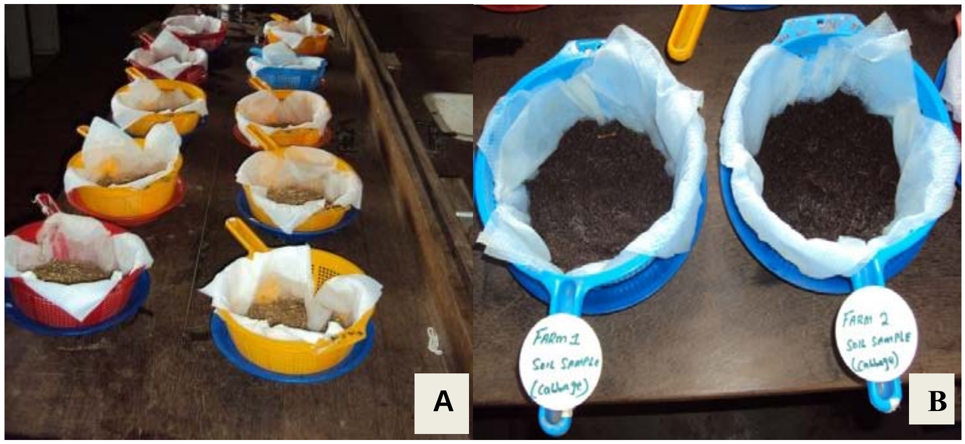


Figure 3.3 Extraction of PPNs from roots (A) and soils (B)

3.3.5 Extraction of nematodes from soil

A soil sub-sample weighing 100 g was used for extraction of nematodes using modified Baermann funnel technique as described by Hooper *et al*., (2005). A coarse plastic mesh was placed on a plastic tray and its bottom and sides covered with a layer of a strong tissue paper. One hundred grams of finely crumbed soil was spread out evenly over the tissue paper. Clean water was carefully added down the inside edge of the collecting tray until the soil layer looked wet and left for 24 hours (Fig 3.3B). Nematodes were harvested by pouring the nematode suspension out of the tray over 38 µm sieve size and backwashing the nematodes off into a small beaker with about 20-30 ml water.

3.3.6 Extraction of cysts from the soil

Cysts from the soil were extracted as described by Hooper (1990). Soil sample was air dried for four weeks, 100 ml sample of dried soil was placed into 1000 ml beaker and 750 ml of water added. The soil was thoroughly stirred and allowed to sediment for 4 minutes. Cysts were found floating to the surface with other organic debris. The suspension was poured on to a filter paper in a funnel. The water drained off, and the paper was air dried and examined for cysts (Hooper, 1990).

3.4 Quantative analysis of nematodes

Nematode suspension was concentrated to 30 ml in a glass vial by letting them settle for an hour and sucking excess water with a pipette. The suspension was shaken gently and 5 ml volume was sucked from the suspension with a pipette and poured into an open 50 mm plastic counting dish. This counting dish was prepared by scratching grids (2mm×2mm) on the inner side of the base of a plastic petri dish with a needle to act as a guide when counting and examined under the microscope (LEICA) at X10, and X20 magnification (Fig 3.4 A & B). A hand tally counter was used for counting nematodes. Counting was repeated three times to obtain the mean which was used to calculate the total nematodes in a sample suspension. The nematodes enumerated were expressed as number of nematodes in 100 g of dry soil.

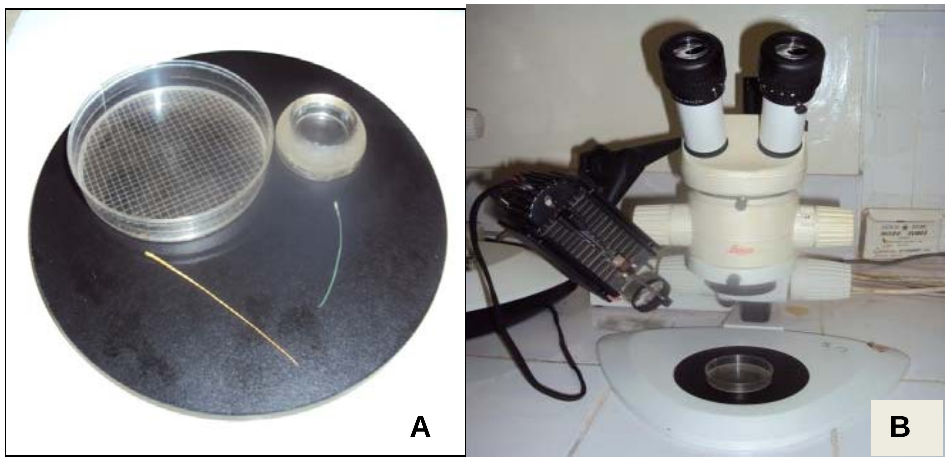


Figure 3.4 Tools used when handling nematodes, Counting dish and picking bristles (A) and a LEICA microscope used in enumeration (B)

3.4.1 Handling of nematodes

To facilitate identification, nematode suspension was further concentrated to 20 ml. The nematode suspension was shaken and small batches of 2 ml nematodes were sucked using a small pipette and transferred to a Hawksley counting dish. Nematodes for identification were selected randomly from the counting dish using an old curved brush bristle which had been tapered with a sharp scalpel to give it a fine edge. The process was repeated five times and the obtained mean was used to estimate the total PPNs per sample. The selected nematodes were mounted on a drop of water on a microscopic slide and placed on a hot plate at 60^{o} C (Hooper, 1990) until nematode suddenly straightened out. The specimens were then examined under a stereomicroscope and identified to genera level.

3. 4.2 Processing nematodes

Extracted nematodes were examined directly under a compound microscope. To allow for further identification and long term storage, nematodes were fixed. Nematodes were concentrated in glass vial by letting them settle and pipeting excess water. The triethanolamine-formalin (TAF) fixative was heated to about 70-75^{o} C and an excess 3 ml was quickly added to the nematodes to kill and fix them in one process (Seinhorst, 1966, Courtney *et al.*, 1955). Nematodes were then cleared with glycerol (Hooper, 1990) before being mounted on a slide and viewed under a compound microscope. Fixed specimens were then concentrated in glass vials and stored for further identification.

3.5 Nematode identification

Nematodes were identified to genera level basing the identification on morphological features as described by Siddiqi (1989), Luc, *et al*., (1990), Siddiqi, (2000) and the University of Nebraska Lincoln nematode identification website (http://nematode.unl.edu/konzlistbutt.htm). Identification was based on adult female nematodes, but in some occasion second stage juveniles (J2) were also used. Major morphological features used in identification are discussed in section 2.4.

3.6 Parameters of PPN analysed

Identified and enumerated PPN were subjected to various analyses. Absolute frequency, population density, richness, diversity and evenness of various genera of PPNs from the soil and root samples were calculated based on methods of community analysis (Norton, 1978; Beals, 1960) where:

$$1.\ \text{Absolute frequency} = \frac{\text{Number of samples containing genera}}{\text{Number of samples collected}} \times 100$$

2. Genera richness = number of genera in a given soil sample

3. Shannon-Weiner index was used to calculate nematode genera diversity, to determine genera variation in the areas sampled;

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

Where: ∑ refers to "the sum of"

s= the number of genera in the community

p_i = is the relative abundance (proportion) of the ith genera in the community

ln = natural log (Shannon and Weaver 1963)

4. Genera evenness was calculated using Simpson's index of diversity (Ds) to determine relative abundance with which each genus was represented in areas sampled. This was done using formula below;

$$(Ds)=\sum (ni/N)^2 \text{ (Simpson, 1943)},$$

Where Ds= Simpson's Diversity Index

ni= the number of individuals of genera i

N= the total number of genera in the sample

The Ds values obtained from different samples were compared for any statistical significance using ANOVA test.

Data collected were normalized using logarithmic transformation (Log_{10} [x+1]) prior to analysis of variance. Means that were considered significantly different ($P \leq 0.05$) were compared by LSD post ANOVA test. Descriptive statistics were used to summarise the data.

CHAPTER FOUR

RESULTS

4.1 Plant parasitic nematodes associated with cabbage in Nyandarua and Embu AEZs

Nineteen genera belonging to ten families of Order Tylenchida, Dorylaimida and Triplonchida were found associating with cabbages in the six agro-ecological zones of Nyandarua and Embu (Table 4.1).

Table 4.1 Plant parasitic nematodes genera isolated from soils and roots of cabbage in Nyandarua and Embu AEZs

Order	**Sub-order**	**Family**	**Genus**
Tylenchida	Tylenchina	Pratylenchidae	*Pratylenchus*
		Hoplolaimidae	*Helicotylenchus*
			Hoplolaimus
			Rotylenchulus
			Peltamigratus
			Scutellonema
		Heteroderidae	*Heterodera*
		Meloidogynidae	*Meloidogyne*
		Criconematidae	*Hemicycliophora*
		Tylenchulidae	*Tylenchus*
			Filenchus
			Coslenchus
		Tylenchorhynchidae	*Tylenchorhynchus*
			Quinisulcius
		Paratylenchidae	*Paratylenchus*
Dorylaimida	Dorylaimina	Longidoridae	*Longidorus*
			Xiphinema
Triplonchida	Diphtherophorina	Trichodoridae	*Paratrichodorus*
			Trichodorus

Six of the genera were present in both cabbage roots and soils, namely: *Pratylenchus* (lesion), *Helicotylenchus* spp. (spiral), *Meloidogyne* spp. (root knot), *Xiphinema* spp. (dagger), *Trichodorus* spp. (stubby), *Tylenchorhynchus* spp. (stunt) and *Longidorus* spp. (Needle). While *Quinisulcius* spp. was only extracted from the roots.

Seventy eight percent (78%) of the nematode genera identified belonged to the Order Tylenchida, while 22% belonged to the Order Dolylaimida and Triplonchida. Family Hoplolaimidae had the highest representative with five genera out of the nineteen. Family Tylenchulidae represented three genera of nematodes which are not considered to be of major economic importance in crop production. Occurrence of most of these PPN genera cut across various AEZs in Nyandarua and Embu.

4.1.1 Absolute frequency of occurrence (A/F) of PPN associated with cabbage in Nyandarua and Embu AEZs

Plant parasitic nematodes were detected in nearly every sample but at varying absolute frequencies of occurrence. *Paratrichodorus* spp. and *Filenchus* spp. were present in all AEZs at high frequencies (Table 4.2). *Pratylenchus* spp. (Fig 4.1 A and B) and *Helicotylenchus* spp. (Fig 4.1 C and D) occurred in high frequencies in five out of the six AEZs. *Peltamigratus* spp. was present in only one AEZ (UH4) while *Hemicycliophora* spp. and *Longidorus* spp. occurred in 50% of the AEZs, the rest were present in nearly all the other zones (Table 4.2).

Table 4.2 Absolute frequency of occurrence (A/F) of PPN in soil samples of cabbage in Nyandarua and Embu AEZs

Genera	**UH1a**	**UH1b**	**UH2**	**UH3**	**UH4**	**UM1**	**A/F %**
Coslenchus	+++	++	+++	+++	+++	0	60
Filenchus	+++	+++	+++	+++	+++	+++	88
Helicotylenchus	+++	++	+++	+++	+++	+++	82
Hemicycliophora	+	+	+++	0	0	0	15
Heterodera	+++	++	+	+++	+	0	33
Hoplolaimus	+	0	+	+	++	+	13
Longidorus	0	+++	0	+	0	++	20
Meloidogyne	++	+	+++	+	++	+++	42
Paratrichodorus	+++	+++	+++	+++	+++	+++	78
Paratylenchus	+++	+++	+++	+++	++	++	58
Peltamigratus	0	0	0	0	++	0	8
Pratylenchus	+++	+++	+++	+++	+++	++	87
Rotylenchulus	++	+	+	++	+	+++	27
Scutellonema	+	0	++	+	+++	+++	33
Trichodorus	+	+++	+++	+	+++	+++	57
Tylenchorhynchus	+++	++	+++	+++	+++	++	67
Tylenchus	+++	+	+++	0	++	+++	52
Xiphinema	+	+++	0	+	+	++	27

0= not recorded; + = present in low frequency; ++ present in moderate frequency; +++ present in high frequency

Although PPNs occurred across all sampled AEZs, cabbage roots from UH1b and UH4 had the highest PPNs occurrence (Table 4.3) while UH1a and UH4 recorded the highest occurrence of soil nematodes (Table 4.2).

The *Pratylenchus* spp. was present with an absolute frequency (A/F) of 58% and 87% in the roots and soil respectively. This lesion nematode occurred in high frequency in nearly all sampled areas. However, UM1 exhibited slightly lower frequency. Nearly 50% of the nematode genera isolated from soil samples (nine genera out of nineteen) had a high absolute frequency (50-100%). These were; *Filenchus* spp. (88%), *Pratylenchus* spp. (87%), *Helicotylenchus* spp. (82%), *Tylenchorhynchus* spp. (67%), *Paratrichodorus* spp. (78%), *Coslenchus* spp. (60%), *Paratylenchus* spp. (58%), *Trichodorus* spp. (57%) and *Tylenchus* spp. (52%) (Table 4.2).

Meloidogyne spp. (Fig 4.1 E and F), S*cutellonema* spp. (Fig 4.1 O and P), *Heterodera* spp. (Fig 4.1 J), *Xiphinema* spp. (Fig 4.1 K and L) and *Rotylenchulus* spp. were present in the soil at an absolute frequency of 42%, 33%, 33%, 27% and 27% respectively. The remaining four genera were recovered in less than 20% absolute frequency in the soil.

Fifty eight percent of the total root samples collected had *Pratylenchus* spp. This genus was present in cabbage roots at a high frequency in 50% of the AEZs. However, it was found in low frequencies in UH1a and UM1 (Table 4.3). *Helicotylenchus* spp. and *Meloidogyne* spp. were present in cabbage roots at a moderate frequency, of 27% and 23% respectively. *Tylenchorhynchus* spp., *Xiphinema* spp., *Longidorus* spp. and *Trichodorus* spp. occurred at low frequencies of 12%, 8%, 5%, and 3%, respectively (Table 4.3).

Table 4.3 Absolute frequency of occurrence (A/F) of PPN in cabbage roots from Nyandarua and Embu AEZs

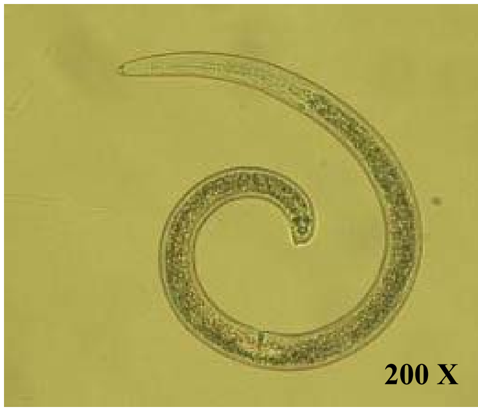
Genera	**UH1a**	**UH1b**	**UH2**	**UH3**	**UH4**	**UM1**	**AF %**
Helicotylenchus	+	+	+	+++	++	0	27
Longidorus	0	0	0	+	0	+	5
Meloidogyne	+	+	+	+++	+	+	23
Pratylenchus	+	+++	+++	+++	+++	+	58
Quinisulcius	+	++	0	0	+	+	10
Trichodorus	0	+	0	+	0	0	3
Tylenchorynchus	0	++	0	0	+	+	12
Xiphinema	0	++	0	+	0	0	8

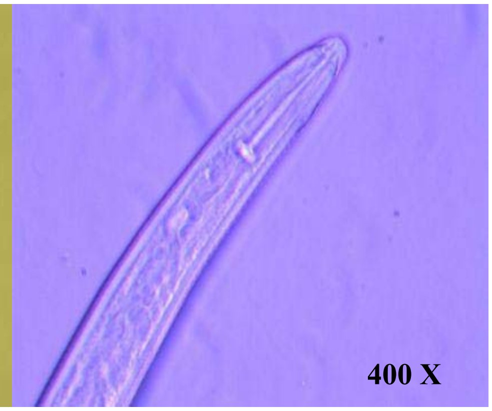
0= not recorded; + = present in low frequency; ++ present in moderate frequency; +++ present in high frequency

Figure 4.1 Plant parasitic nematodes found associated with cabbage in Nyandarua and Embu AEZs

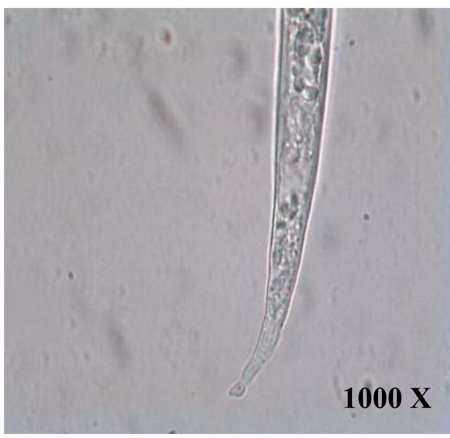
A: Female *Pratylenchus* spp. **B:** Male *Pratylenchus* spp.

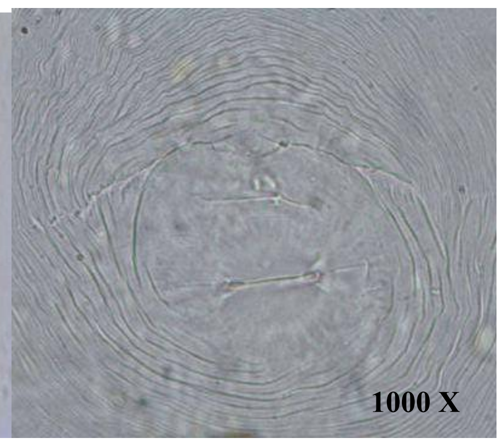
Figure 4.1 continued



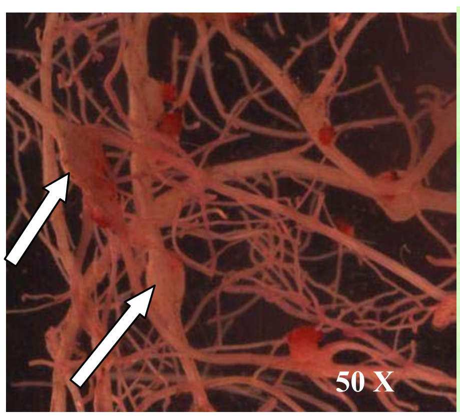


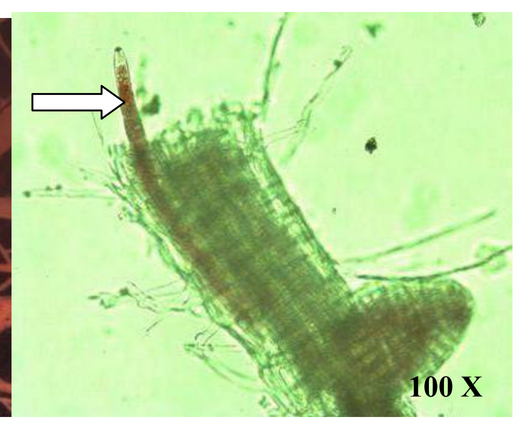
C: Female *Helicotylenchus* spp. **D:** Head region of *Helicotylenchus* spp.





E: Tail of RKN J2 **F:** Perineal pattern for *M. javanica*



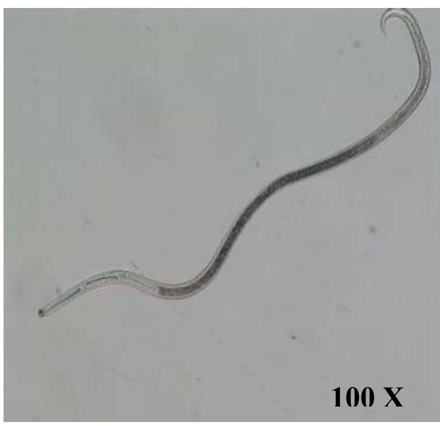


G: Galled cabbage roots (arrow) caused by RKN **H:** Endoparasitic nematode

Figure 4.1 continued

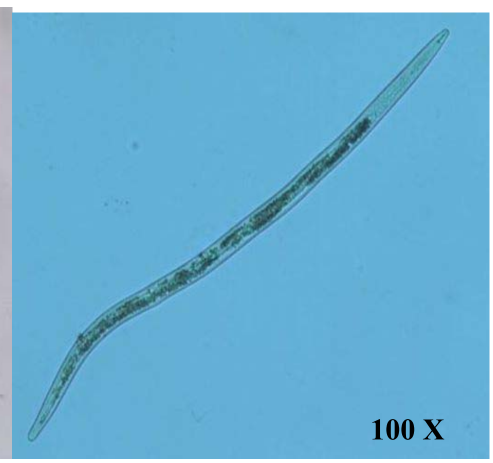
I: Cysts of *Heterodera* spp.

J: J2 of *Heterodera* spp.



K: *Xiphinema* spp.

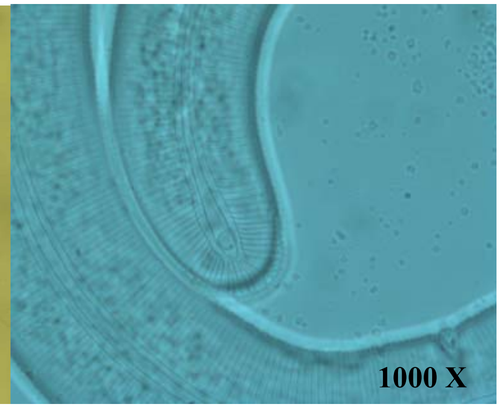
L: Head section of *Xiphinema* spp.



M: *Trichodorus* spp.

N: *Tylenchorhynchus* spp.

Figure 4.1 continued



O: *Scutellonema* spp. **P:** Tail of *Scutellonema* spp.

4.2 Populations of PPNs associating with cabbages in various AEZs of Nyandarua and Embu Counties

4.2.1 Plant parasitic nematode and free-living nematodes in the soil samples

The mean population densities of PPNs and free-living nematodes (FLNs) did not differ significantly (P>0.05). The population of PPNs was slightly higher than that of FLNs in UH4 and UH1a AEZs while in UH2, UH3 and UM1 the population of PPNs was lower than that of free-living nematodes. However, the population for both nematode groups was almost equal in UH1b AEZs (Fig 4.2).

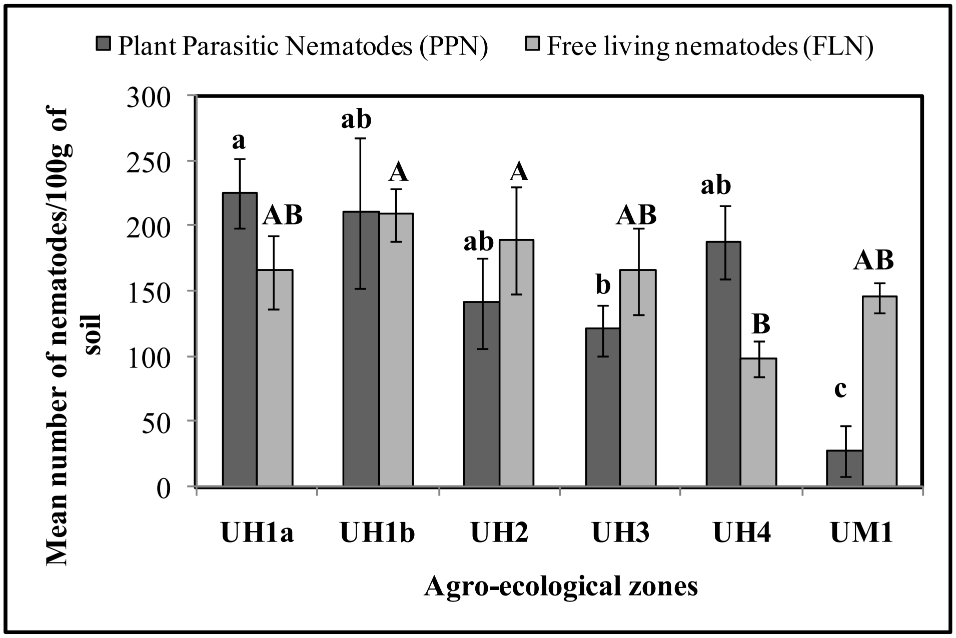


Figure 4.2 Number of plant parasitic nematodes (PPNs) and free-living nematodes (FLNs) associating with cabbages in Nyandarua and Embu AEZs. †Bars showing the same letters are not significantly different

4.2.2 Plant parasitic nematodes from cabbage rhizosphere

Mean population densities of PPNs associated with cabbage soil were significantly ($P<0.05$) different among the six AEZs in Nyandarua and Embu region. Among all AEZs, UH1a was the most heavily infested with an overall mean number of 225 nematodes per 100 g of dry soil (Fig 4.2) followed closely by UH1b with 211 nematodes but there was no significant ($P>0.05$) difference between the two. Number of nematodes in UH1a was significantly ($p<0.05$) different from that of UH3 and UM1 but not different from UH1b, UH2 and UH4. The lowest number of nematode was recorded in UM1 which had 28 nematodes per 100 g of dry soil, UH4, UH2 and UH3 had a mean population of 188, 141, and 121 nematodes per 100 g of dry soil respectively (Fig 4.2).

4.2.3 Plant parasitic nematodes from the cabbage roots

There was a significant (P<0.05) difference in the number of PPNs extracted from cabbage roots in the six AEZs of Nyandarua and Embu (Fig 4.3).

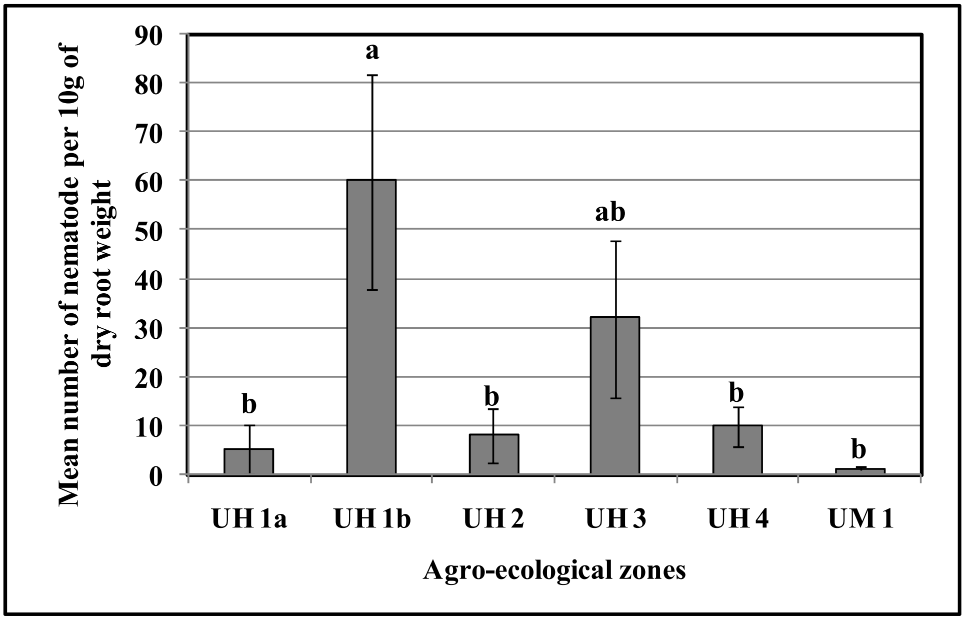


Figure 4.3 Mean number of plant parasitic nematodes population in cabbage roots in Nyandarua and Embu AEZs. †Bars showing the same letters are not significantly different

The highest number of nematodes in roots (60.2 per 10 g of dry roots), was recorded in UH1b. This was followed by UH3, UH4, UH2, UH1a and UM1 in decreasing order. The number of PPNs from UH1b differed significantly (P<0.05) from that of UH1a, UH2, UH4 and UM1. However there was no significant (P>0.05) difference between UH1b and UH3 (Fig 4.3). The lowest number of nematodes in roots was recorded in UM1 with 1.2 nematodes per 10 g of dry roots.

Further analysis revealed a significant positive correlation (P<0.05, r=0.56) between the population of PPN in the soil and those obtained from the roots (Fig 4.4).

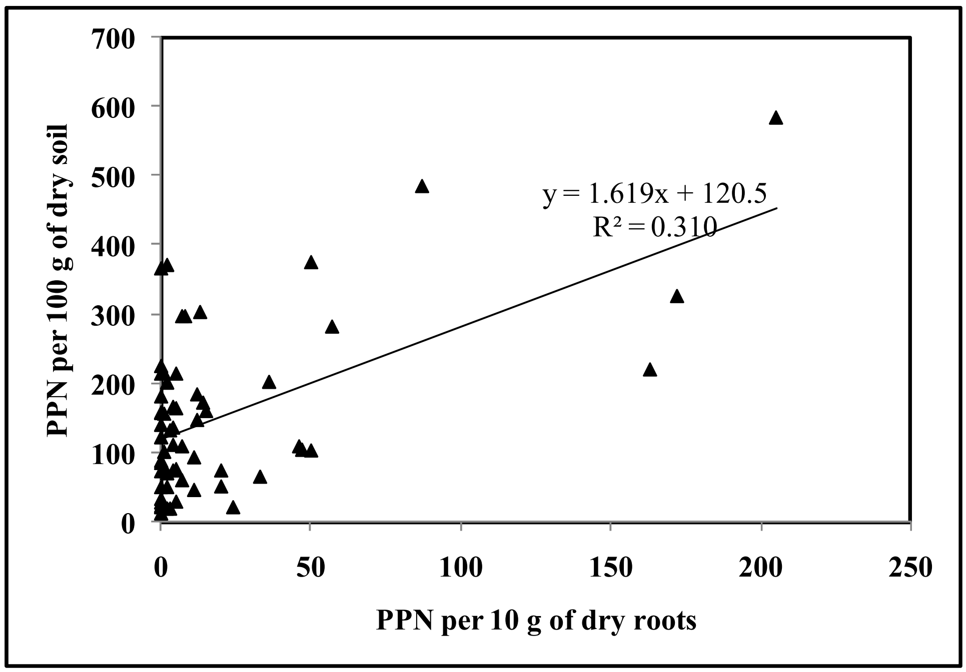


Figure 4.4 Relationship between soil and root plant parasitic nematodes in Nyandarua and Embu AEZs

The UH1b, which recorded the highest density of root nematodes, ranked second in soil nematodes while UM1 had the lowest population density of nematodes from both soils and roots. The UH3 ranked second in number of nematode within the roots but second last in soil nematodes.

4.3 Population densities of PPN genera associated with cabbage in Nyandarua and Embu AEZs

4.3.1 Population densities of PPNs from cabbage roots

The sampled cabbage roots were positive for endoparasitic nematodes. The general population density of PPN genera in roots of cabbage ranged from 0.1 to 16.4 per 10 g of dry roots (Fig 4. 5 and Table 4.4). *Pratylenchus* spp. was present in high population density compared to the other genera. A population density of 16.4 individual per 10 g of roots was recorded for *Pratylenchus* spp. This mean was significantly ($P<0.05$) high compared to other PPN genera isolated from cabbage roots. The root-knot nematode had a population density of 1.1 nematodes per 10g of roots (based on J2). This mean was not significantly different from other nematodes apart from *Pratylenchus* (Fig 4.5 and Table 4.4).

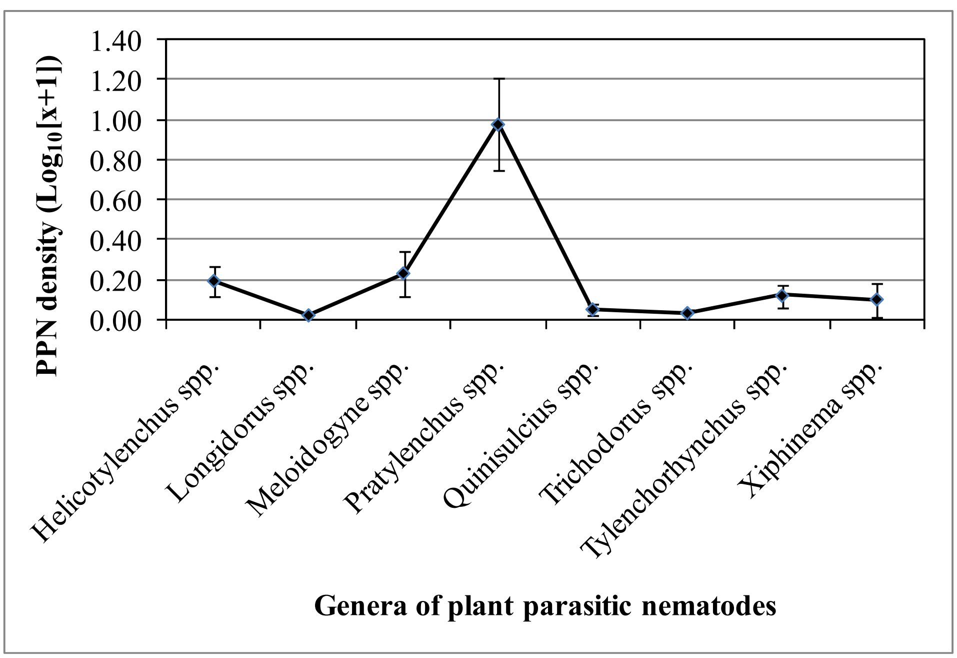


Figure 4.5 Population of PPN genera from cabbage roots in Nyandarua and Embu AEZs

Table 4.4 Population densities of plant parasitic nematodes (PPN) in cabbage roots in Nyandarua and Embu AEZs

PPN Genera	**PPN per 10g of dry roots ± SE†**
Helicotylenchus	0.70±0.35[b]
Longidorus	0.05±0.03[b]
Meloidogyne	1.10±0.76[b]
Pratylenchus	16.38±8.38[a]
Quinisulcius	0.13±0.08[b]
Trichodorus	0.08±0.07[b]
Tylenchorynchus	0.38±0.20[b]
Xiphinema	0.42±0.38[b]

Data are the mean ± standard error (SE) of nematodes per 10g of dry roots in sixty root samples collected in six AEZs.
†Means separated using Fisher's LSD test, means followed by the same letter are not significantly different.

Helicotylenchus, *Longidorus*, *Tylenchorhynchus*, *Quinisulcious*, *Trichodorus* and *Xiphinema* spp. were present in low number; an average density of less than one nematode per ten grams of roots and their means were not significantly (P>0.05) different.

Densities of PPN genera in roots differed significantly (P<0.05) across the AEZs (Table 4.5). The highest population of *Pratylenchus* spp. was recorded in UH1b. This differed significantly (P<0.05) with all other AEZs, *Pratylenchus* population density in UH2, UH3 and UH4 was not significantly (P>0.05) different, with the least population being recorded in UM1.

Table 4.5 Variations in densities of plant parasitic nematodes genera from cabbage roots in Nyandarua and Embu AEZs

	Mean nematodes density in 10 g of dry roots							
Nematode	**UH1a**	**UH1b**	**UH2**	**UH3**	**UH4**	**UM1**	**Overall Mean**	**F- Probability**
Pratylenchus spp.	4.8[c]	56.0[a]	7.6[bc]	20.2[b]	9.5[b]	0.2[c]	16.4	**
Meloidogyne spp.	0.2b	0.3[b]	0.5[b]	4.9[a]	0.1[b]	0.6[b]	1.1	*
Helicotylenchus spp.	0.3[b]	0.8[b]	0.1[b]	2.3[a]	0.7[b]	0.0[b]	0.7	*
Xiphinema spp.	0.0[b]	2.3[a]	0.0[b]	0.2[b]	0.0[b]	0.0[b]	0.4	*S
Longidorus spp.	0.0[a]	0.0[a]	0.0[a]	0.2[a]	0.0[a]	0.1[a]	0.1	NS
Quinisulcius spp.	0.1[a]	0.5[a]	0.0[a]	0.0[a]	0.1[a]	0.1[a]	0.1	NS
Tylenchorhynchus spp.	0.0[a]	0.2[a]	0.0[a]	0.0[a]	0.08[a]	0.10[a]	0.4	NS
Trichodorus spp.	0.0[a]	0.4[a]	0.1[a]	0.0[a]	0.0[a]	0.0[a]	0.1	NS

*Significant at $p<0.01$, ** Significant at $p<0.05$, NS: Not Significant, Means followed by the same letter(s) along rows are not significantly different

Similarly, population density of *Meloidogyne* spp. differed significantly ($P<0.05$) across the AEZs, with UH3 recording the highest population which was significantly different from all other AEZs. The UH3 also recorded significantly high levels of *Helicotylenchus* than any other AEZ. The UH1b recorded high population of *Xiphinema* than other AEZs. The population densities of other PPN genera isolated in cabbage roots did not differ significantly with AEZs (Table 4.5).

4.3.2 Population densities of PPN genera from cabbage rhizosphere

When the populations of different PPN genera in soil were evaluated for all AEZs, the population density ranged from 0.47 to 41 nematodes per 100 g of dry soil (Table 4.6). A significant difference ($P<0.05$) in the mean population densities of various nematode genera isolated from soils was noted. *Pratylenchus* spp. had a high mean density of 41 individuals per 100 g of soil. The predominant species present were *Pratylenchus brachyurus, P. zeae*, and *P. neglectus* which occurred across the AEZs.

Mean number of *Pratylenchus* spp. was significantly higher than all other genera but not significantly ($P>0.05$) different from that of *Helicotylenchus* spp. and *Filenchus* spp. which had a population of 26.3 and 16.2 per 100 g dry soil respectively. Likewise, means for *Tylenchorhynchus*, *Paratrichodorus*, *Meloidogyne, Paratylenchus* and *Coslenchus* spp. (11.9, 7.8, 6.4, 6.2, and 10.3, respectively) were not significantly different ($P>0.05$).

Table 4.6 Population densities of plant parasitic nematodes (PPN) in cabbage rhizosphere in Nyandarua and Embu

PPN genera	PPN per100 g of soil ± SE †
Pratylenchus spp.	41.04 ± 18.01[h]
Helicotylenchus spp.	26.28 ± 8.34[gh]
Filenchus spp.	16.24 ± 6.12[f-h]
Tylenchorhynchus spp.	11.94± 3.44e-h
Coslenchus spp.	10.34 ± 4.84[d-f]
Paratrichodorus spp.	7.78±1.72[e-g]
Trichodorus spp.	6.71±3.37[c-e]
Meloidogyne J2 spp.	6.36 ± 2.92[c-f]
Paratylenchus spp.	6.20 ± 3.01[c-f]
Heterodera spp.	5.72 ± 3.00[b-e]
Tylenchus spp.	4.62±2.26[b-e]
Scutellonema spp.	2.77 ± 1.42[a-d]
Xiphinema spp.	2.02±1.42[a-c]
Rotylenchulus spp.	1.58 ± 0.61[a-c]
Longidorus spp.	1.05 ± 0.71[ab]
Hemicycliophora spp.	0.73 ± 0.55[ab]
Peltamigratus spp.	0.51 ± 0.51[a]
Hoplolaimus spp.	0.47 ± 0.47[a]

Data are the mean ± standard error (SE) of nematodes per 100 g of soil and 10g of dry roots in sixty soil and root samples collected in six AEZs.
[†]Means separated using Fisher's LSD test, means within the column followed by the same letter are not significantly different

Table 4.7 Variations in densities of plant parasitic nematodes genera from cabbage rhizosphere in Nyandarua and Embu AEZs

Nematode	**Mean nematodes density in 100 g of dry soil**							
	UH1a	**UH1b**	**UH2**	**UH3**	**UH4**	**UM1**	**Overall Mean**	**F-probability**
Pratylenchus spp.	14.1^{b}	125.7^{a}	43.2^{b}	31.9^{b}	31.0^{b}	0.4^{c}	41.1	**
Meloidogyne spp.	6.9^{b}	4.5^{b}	20.2^{a}	1.2^{b}	0.8^{b}	4.7^{b}	6.4	**
Helicotylenchus spp.	53.1^{a}	7.6^{bc}	17.1^{bc}	31.1^{ab}	45.6^{a}	3.2^{c}	26.3	*
Filenchus spp.	43.8^{a}	13.9^{bc}	14.6^{bc}	3.6^{c}	19.1^{b}	2.7^{c}	16.2	*
Tylenchorhynchus spp.	24.1^{a}	7.5^{bc}	7.7^{bc}	12.7^{ab}	18.8^{ab}	0.9^{c}	11.9	*
Coslenchus spp.	29.3^{a}	1.8^{bc}	3.8^{bc}	6.5bc	20.5^{ab}	0.0^{d}	10.3	*
Trichodorus spp.	0.7^{c}	14.5^{ab}	3.3^{bc}	0.4^{c}	19.6^{a}	1.8^{bc}	6.7	*
Paratylenchus spp.	20.7^{a}	4.3^{b}	5.7^{b}	4.6^{b}	1.2^{b}	0.7^{b}	5.7	*
Heterodera spp.	7.9^{b}	4.7^{b}	1.3^{b}	19.3^{a}	1.2^{b}	0.0^{c}	5.7	*
Paratrichodorus spp.	4.1^{a}	11.7^{a}	11.1^{a}	3.6^{a}	12.1^{a}	4.4^{a}	7.8	NS

Data for the top ten PPN genera from cabbage rhizosphere
*Significant at $p<0.01$, ** Significant at $p<0.05$, NS: Not Significant, Means followed by the same letter(s) along rows are not significantly different

Hemicycliophora, *Hoplolaimus* and *Peltamigratus* spp. recorded the least population density; an average of less than one nematode per 100 g of dry soil and not significantly different from that of *Scutellonema*, *Xiphinema*, *Rotylenchulus*, and *Longidorus* (Table 4.6).

Population densities of various PPN genera from soils differed significantly ($P<0.05$) with AEZs (Table 4.7). The *Pratylenchus* was present in high density in UH1b. This was significantly higher than other AEZs, however, there was no significant difference ($P>0.05$) in *Pratylenchus* population in UH1a, UH2, UH3 and UH4 while UM1 had the least. Upper highland 2 (UH2) recorded population of *Meloidogyne* that was significantly higher ($P<0.05$) than other AEZs. The population of *Helicotylenchus* spp. also differed significantly with AEZs, UH1a and UH4 had significantly high population. Variations in population densities for other PPN genera with AEZs are as indicated in Table 4.7.

4.3.3 Root knot nematodes galling index

Cabbage root were found to have very tiny galls that were difficult to see with naked eyes. When the means of galling indices were compared, there was no significant ($P>0.05$) difference in galling index across AEZs. However, UM1 recorded a higher galling index (1.6) followed by UH2 with an index of 1.5 respectively. The UH1a had an index of 1.1 while UH3 and UH1b had galling indices of 0.7 and 0.6, respectively (Fig 4.6).

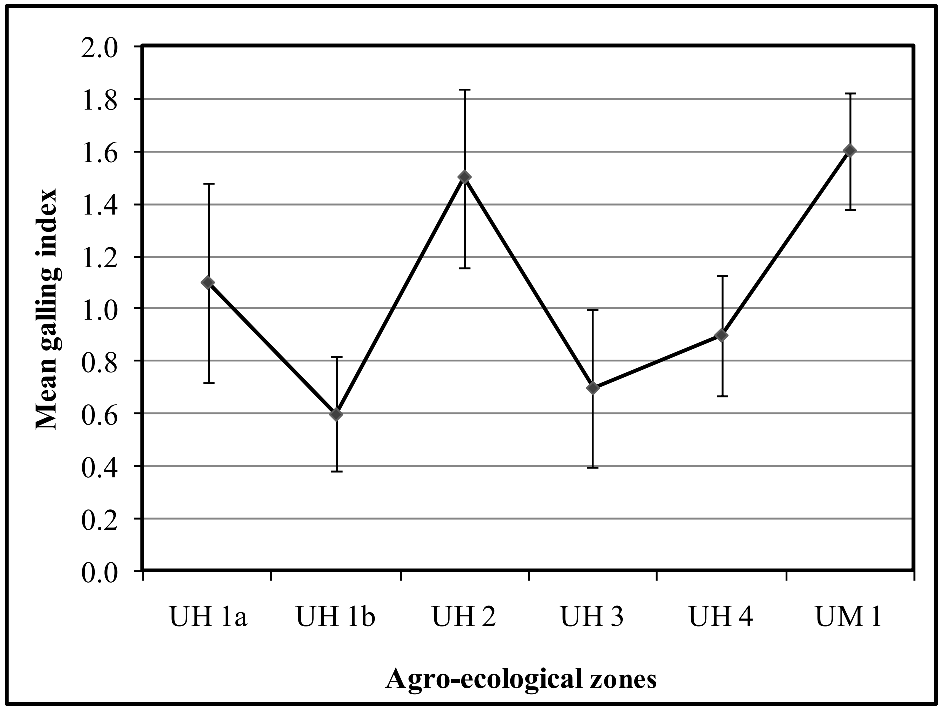


Figure 4.6 Galling indices for cabbage roots in Nyandarua and Embu AEZs

4.3.4 Cyst nematode-*Heterodera* spp.

Cyst nematodes were detected in 49 out of the sixty soil samples collected from cabbage fields. The number of cysts ranged from zero to 261 cysts per 100 cm^3 of dry soil. When mean number of cysts in various AEZs was compared, a significant ($P<0.05$) difference was noted. The highest mean numbers of cysts were recorded in UH2 which had 71 per 100 cm^3 of dry soil, this mean was significantly ($P<0.05$) higher than all other AEZs (Fig 4.7). Means for UH1b and UH4 were not significantly different (26 and 19 cysts per 100 cm^3 of dry soil, respectively). The lowest number of cysts was recorded in UH3 and UH1a while UM1 had absolutely no cyst.

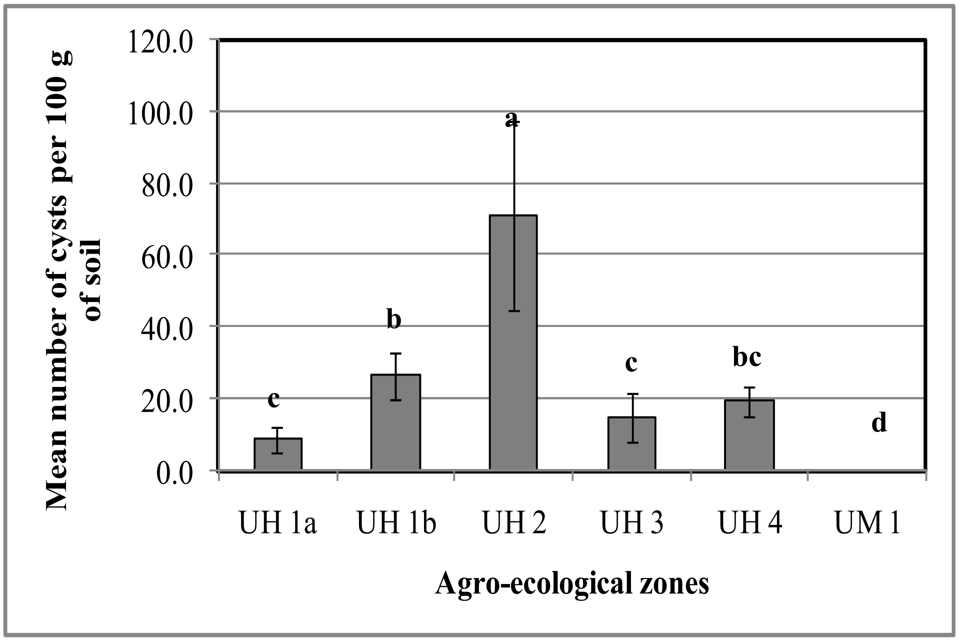


Figure 4.7 *Heterodera* cysts from soil samples from different agro-ecological zones in Nyandarua and Embu counties. †Bars showing the same letters are not significantly different.

4.3.5 Nematode diversity in agro-ecological zones

4.3.5.1 Plant parasitic nematode genera richness (GR)

The genus richness was computed and means for all AEZs compared. A highly significant ($P<0.05$) difference was found for GR in various AEZs. Genus richness was significantly ($P<0.05$) higher in UH4 which had an average of 10.3 nematode genera. However this mean was not significantly different from UH1a and UH2 AEZs. Genera richness was slightly low in UM1 and UH3 with UH1b recording the lowest number of genera. The genera richness was not significantly ($p>0.05$) different in UH1b, UH3 and UM1 (Fig 4.8).

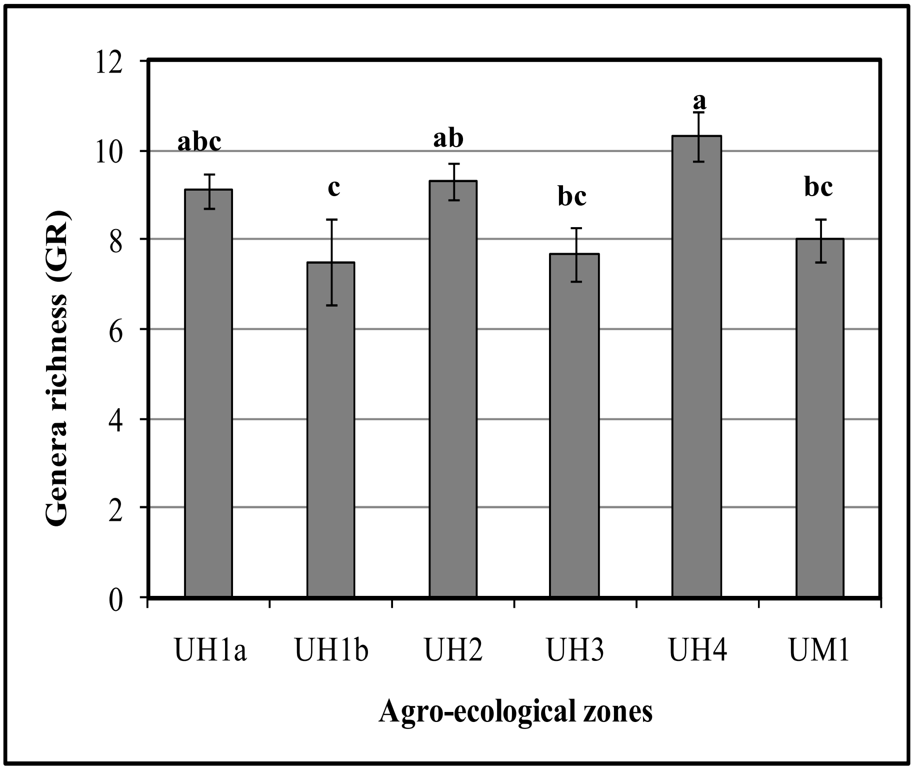


Figure 4.8 Genera richness for plant parasitic nematodes in Nyandarua and Embu AEZs. †Bars showing the same letters are not significantly different

4.3.5.2 Plant parasitic nematode genera diversity (H)

There was no significant (P>0.05) difference in the genera diversity in AEZs. However, Shannon diversity indices indicated slight variation among AEZs. The index was higher in UM1 (1.83). This was followed by UH1a and UH2 with 1.80 and 1.76, respectively. Other AEZs, UH1b, UH3 and UH4 had a diversity index of 1.42, 1.59 and 1.59, respectively (Fig 4.9).

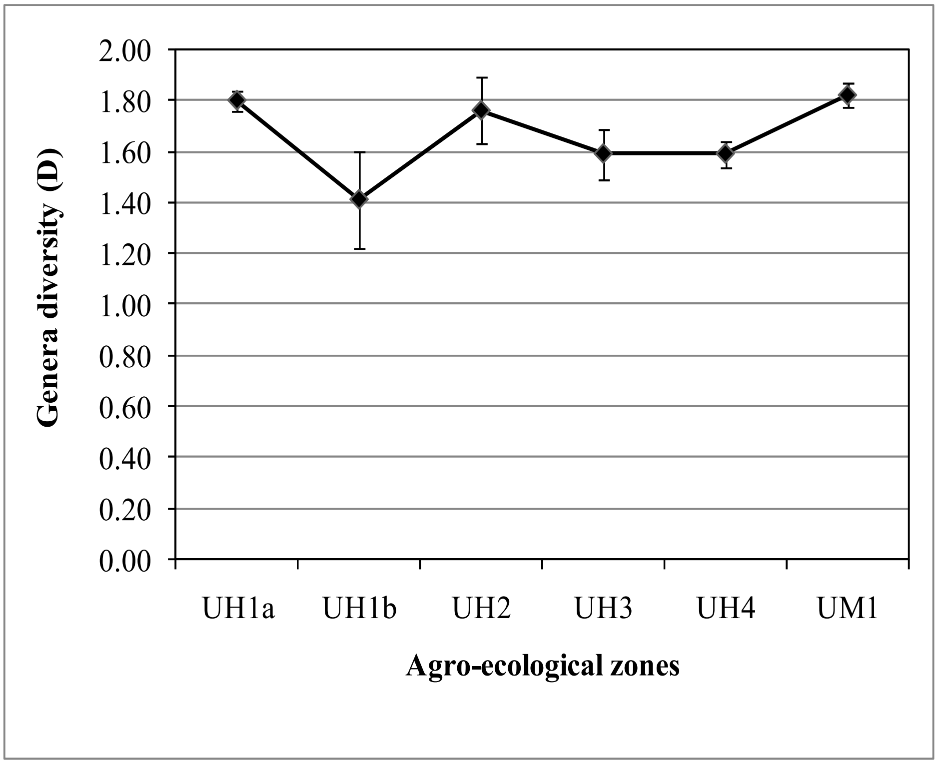


Figure 4.9 Genera diversity for plant parasitic nematodes in Nyandarua and Embu AEZs

4.3.5.3 Plant parasitic nematode genera evenness (D)

Simpson's evenness indices were variable among AEZs, although the trend was similar to the one shown by Shannon diversity index. Simpson's index varied significantly ($P<0.05$) in various AEZs. The index was higher in UM1 followed by UH1a although there was no significant difference between the two AEZs. However there was no significant ($P>0.05$) difference between the Simpson's index in UH1a, UH2, UH3 and UM1. The lowest Simpson's index was recorded in UH1b and it differed significantly ($P<0.05$) from other AEZs apart from UH2 and UH3 (Fig 4.10).

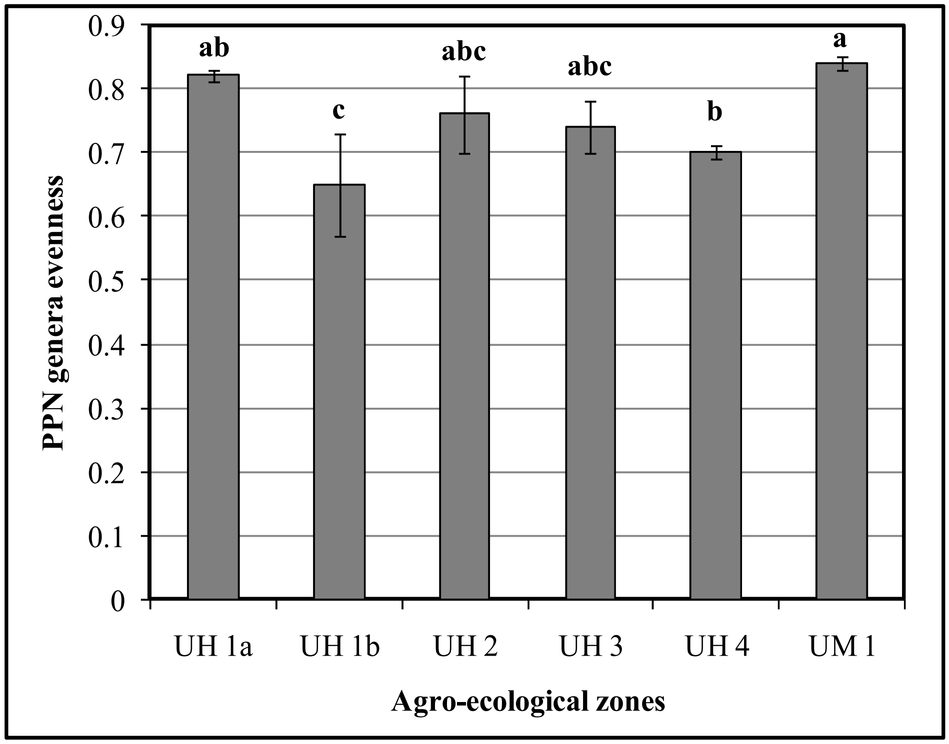


Figure 4.10 Genera evenness for plant parasitic nematodes in Nyandarua and Embu AEZs. †Bars showing the same letters are not significantly different

4.4 Influence of farming practices and soil factors on plant parasitic nematode population in Nyandarua and Embu AEZs.

4.4.1 Varieties of cabbage grown in Nyandarua and Embu and populations of associated PPNs

The survey revealed that a range of cabbage varieties are grown in Nyandarua and Embu AEZs. A total of ten white cabbage varieties were identified from the sixty sampled farms. Copenhagen market was the most widely grown variety (24% of the farmers) followed by Gloria (22%), Clobe master (13%), Riana F1 (11%) and Pruktor (9%) (Fig 4.11).

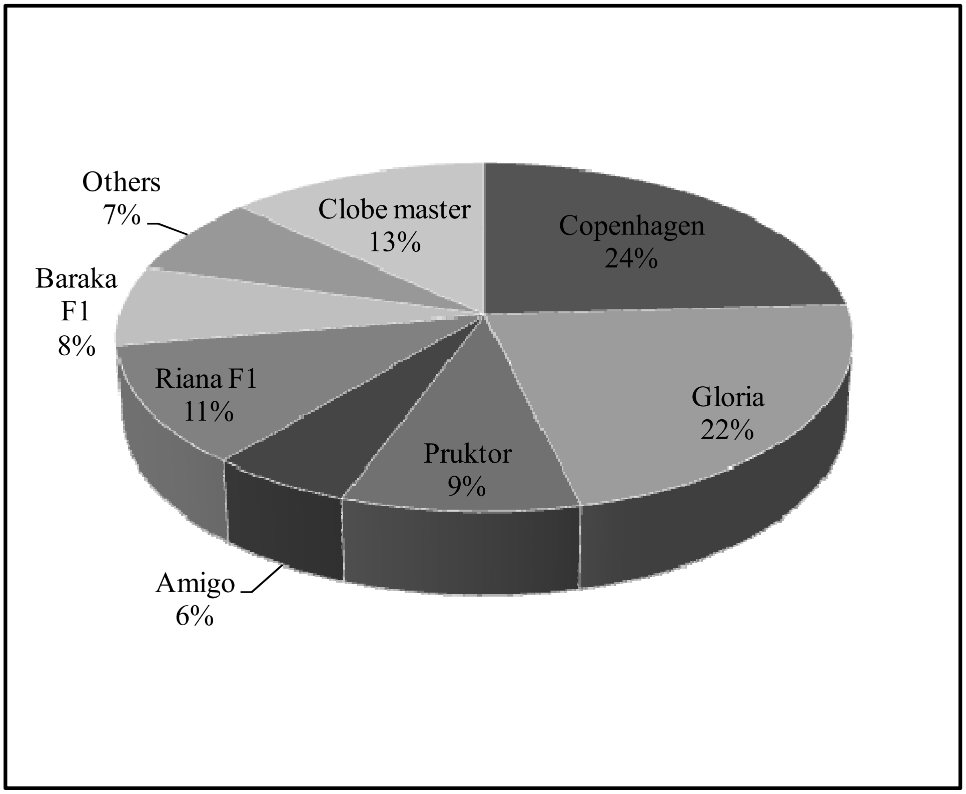


Figure 4.11 Cabbage varieties commonly grown in Nyandarua and Embu AEZs

Five out of the seven commonly grown varieties were only found in upper highland zones while two; Riana F1 and Amigo were exclusively cultivated at upper midland zone. Analysis revealed a highly significant ($P<0.05$) differences between population densities of soil PPNs in different cabbage varieties. However, there was no significant ($P>0.05$) difference in the density of PPNs from cabbage root in various cabbage varieties (Fig 4.12). The Amigo and Riana F1, which were exclusively grown in UM1, recorded the lowest nematode population which was significantly ($P<0.05$) different from all the other varieties.

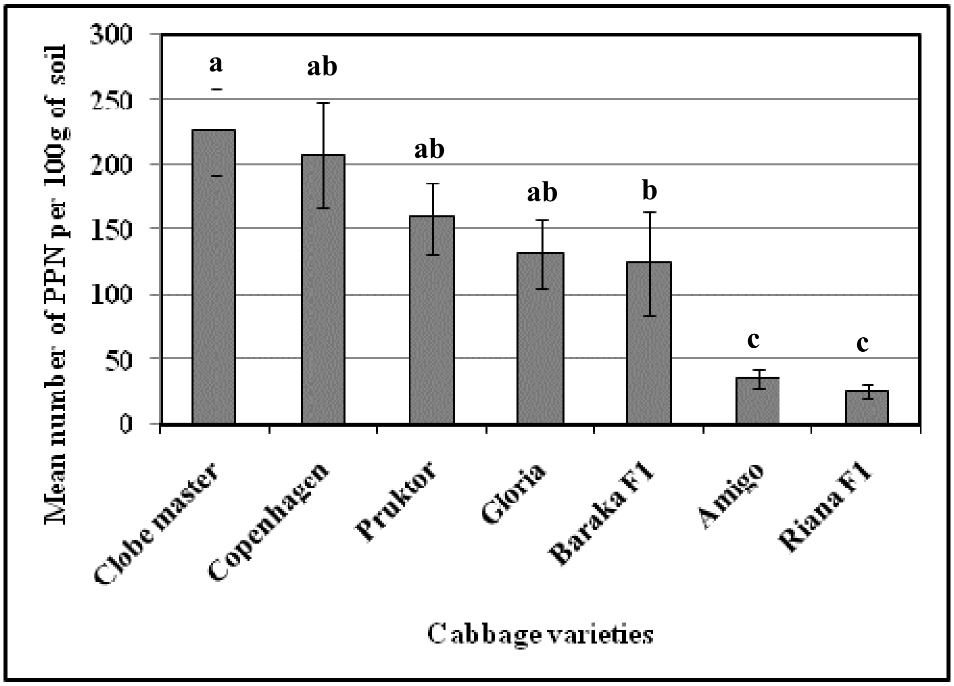


Figure 4.12 Population of plant PPNs from rhizosphere soil of various cabbage varieties in Nyandarua and Embu AEZs. †Bars showing the same letters are not significantly different

Clobe master had the highest density of PPNs with a mean of 226 individual per 100 g of soil. This figure was significantly ($P<0.05$) different from Baraka F1, Riana F1 and Amigo but not different from Copenhagen, Pruktor and Gloria. Amigo and Riana F1 recorded lowest number (36 and 26 respectively) of plant parasitic nematodes in all sampled zones and their means were significantly different from all other cabbage varieties (Fig 4.12).

4.4.2 Common farming practices applied by cabbage farmers in Nyandarua and Embu AEZs

Cabbage farmers were found applying various types of manure amendment in their farms (Table 4.13). Cow manure was the most preferred by 43.33% of the cabbage farmers. This was followed by sheep manure (15%), compost (6.67%), and chicken (5%), only 1.67% of farmers applied green manure. On the other hand, 17% of cabbage farmers did not apply manure in their farm.

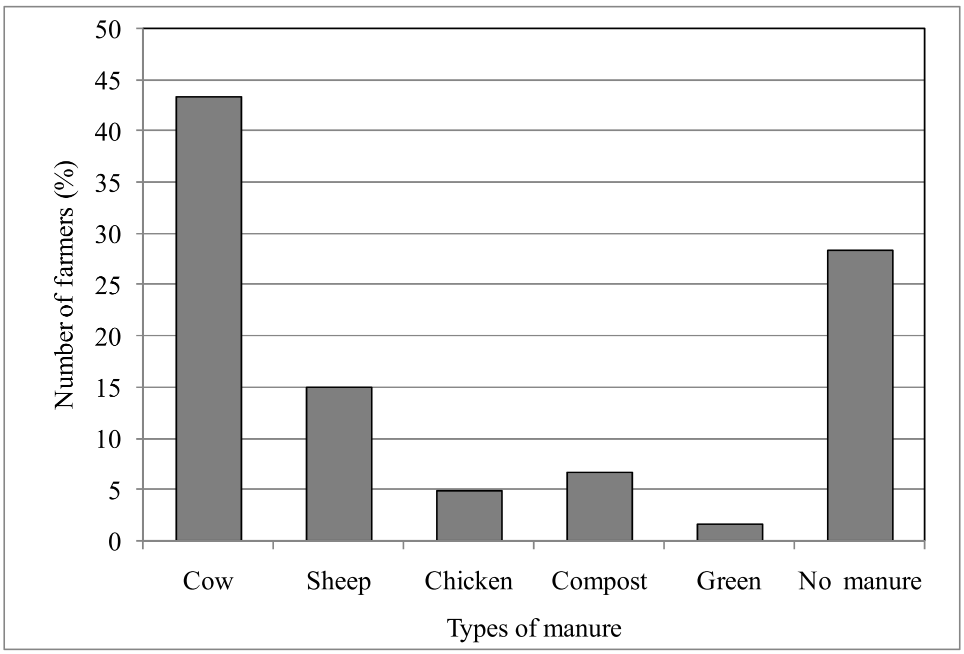


Table 4.13 Proportion of manure use by cabbage farmers in Nyandarua and Embu AEZs

Ninety percent of the interviewed farmers practiced crop rotation. Potato was the most preferred rotational crop by 37% of the farmers. Maize was preferred by 15% of farmers, while carrots and beans were preferred 10% each. Other crops included in the rotational are; oat, pea, and kale among others (Fig 4.13).

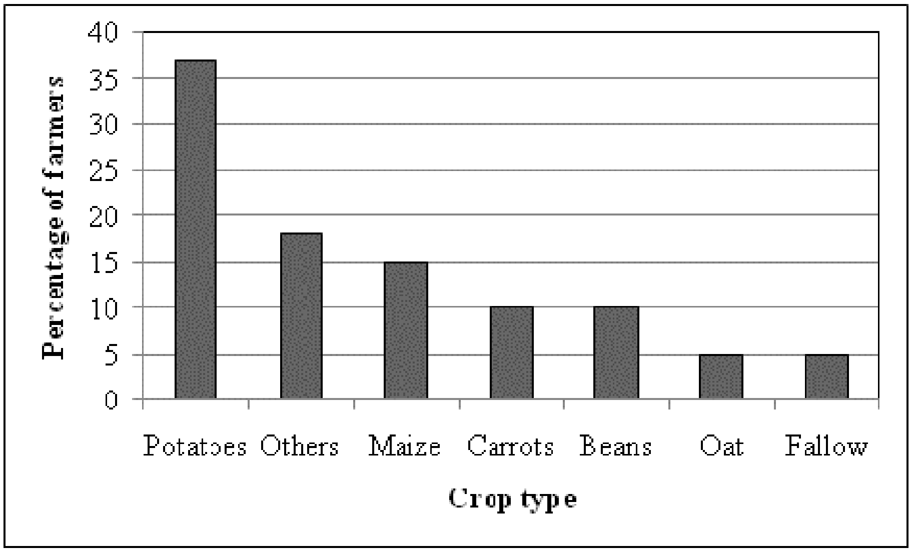


Figure 4.13 Preferred rotational crops by cabbage farmers in Nyandarua and Embu AEZs

4.4.3 Effects of some soil elements on soil PPN population in Nyandarua and Embu AEZs

There were differences in soil properties across AEZs as indicated in tables 3.1-3.3. The mean population of PPN was found to correlate with some soil elements. There was a significant negative correlation ($P<0.05$, $r= -0.56$) between PPN from cabbage rhizosphere and organic carbon in the soil (Fig 4.14). Similarly a significant negative correlation ($P<0.05$, $r= -0.56$) between PPN in cabbage rhizosphere and nitrogen in the soil was noted (Fig 4.14). However, no significant correlation ($P>0.05$) was found between PPN in cabbage roots and organic carbon and nitrogen. A significant positive correlation ($P<0.05$, r=0. 36) between the PPN in cabbage roots and phosphorus in the soil was noted (Fig 4.15). However, there was no significant correlation between PPN density in cabbage rhizosphere and phosphorus and potassium levels in the soil.

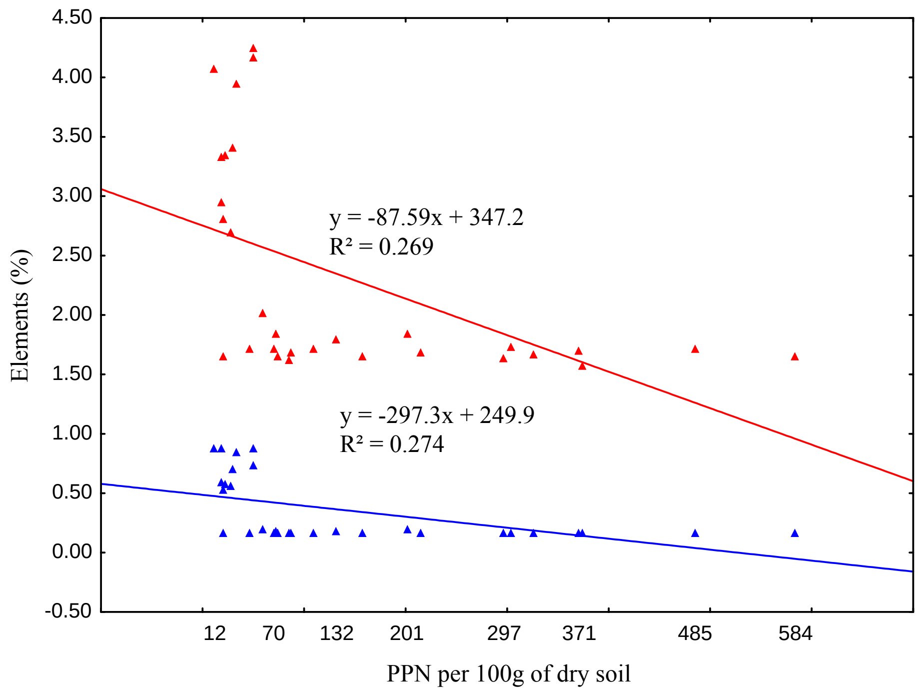


Figure 4.14 Relationship between organic carbon and nitrogen and the plant parasitic nematodes in cabbage rhizosphere in Nyandarua and Embu AEZs

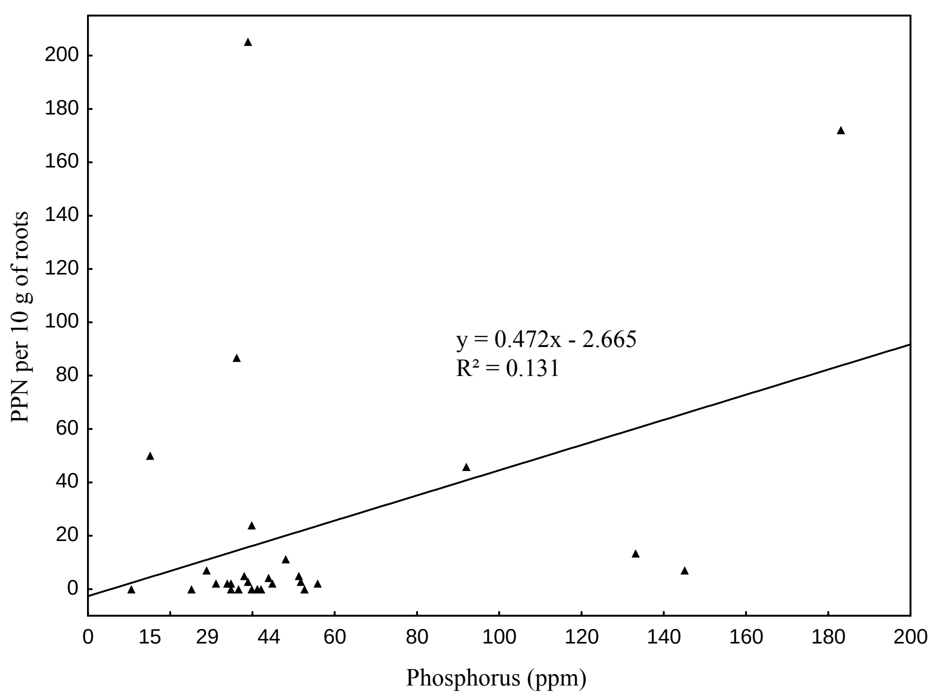


Figure 4.15 Relationship between PPN in cabbage rhizosphere and amount of Phosphorus in the soil in Nyandarua and Embu AEZs

CHAPTER FIVE

DISCUSSION

5.1 Plant parasitic nematodes associating with cabbages in Nyandarua and Embu AEZs

This study has revealed that cabbage is a host to a diversity of plant-parasitic nematodes in Nyandarua and Embu agro-ecological zones. A large number of PPNs were recovered in the cabbage rhizosphere and roots. These nematodes were found across all AEZs at varying population densities. The study areas are located in potential agricultural zones that receive good amount of precipitation while soil is able to retain moisture most part of the year (Jaetzold and Schmidt, 1983), thus creating a conducive environment for nematodes to thrive. Plant parasitic nematodes have been reported in other parts of Kenya (Waceke, 2007) and other parts of the world (Potter and Olthof, 1993; Mennan and Handoo, 2006).

Presence of high soil moisture and/or watering of cabbages during drought allow nematodes to move and infect cabbage and other crops easily. On the other hand, soil structure in these AEZs (Clay-Loam) might have contributed to the high number of PPN present. This type of soil is known to have high porosity and aeration that favour nematode mobility (Olabiyi *et al.*, 2009 and Chirchir *et al.*, 2008). In addition, intensive and mixed cropping might explain the relatively rich nematode genera where cabbages are sometimes intercropped with among other crops such as maize and potatoes.

5.2 Populations of PPN associating with cabbages in various agro-ecological zones of Nyandarua and Embu

Free-living nematodes are known for their high sensitivity to soil perturbations. Low population of free-living nematodes in the cultivated plots often indicates the degradation of soil properties and a loss of fertility (Bongers and Bongers 1998). This could have been the case in UH1a and UH4 AEZs where the level of PPNs was found to be relatively higher than free-living. In such conditions, PPNs rapidly become a problem, especially when the same crops are continuously grown for several seasons (Hillocks and Waller, 1997). On the other hand, high level of free-living nematodes in UH2, UH3 and UM1 could be attributed to high soil organic matter or less soil perturbation (Bongers and Bongers, 1998). Cabbage farmers in these areas were found to apply various types of manure amendment in their farms which increased soil organic matter.

As reported by Dackman *et al.*, (1987) and Wachira *et al.*, (2009), addition of organic carbon to the soils in form of manure leads to an increase in the number of free-living and predatory nematodes and a decrease in PPNs. High organic matter in the soil reduces PPNs in agricultural land by generating toxic compounds in the soil which suppress nematodes or by changing the soil's microfauna and microflora and increasing the populations and activities of microorganisms that are antagonistic to nematodes (Oka, 2010). Nahar *et al.*, (2006) observed a negative correlation between plant parasitic nematodes and free-living in organically grown tomatoes.

High numbers of plant nematodes were found associating with cabbage roots in UH1a. This could be attributed to among other factors intercropping cabbage with other susceptible crops such as maize and continuous growth of vegetable in the same plot of land as observed during survey. Most vegetables e.g. spinach, are known to host a variety of PPNs. This could have lead to accumulation of nematodes in the soil thus providing initial inoculums upon planting. It has been found that continuous growing of a highly susceptible host plant as a mono-crop greatly increases populations of PPN pests and cause damage to the crop; conversely, growing a poor host will significantly suppress the nematode populations (Bridge, 1996). On the contrary, low number of nematodes from roots identified in other AEZs may have been as a result of good farmer practices such as use of organic amendment, soil physical-chemical properties, variations in moisture content or influence due to cabbage varieties grown. This may have been the case in UM1 which recorded low number of nematodes in roots and soils. In this area the variety of cabbage grown were different from those grown in other areas.

The population of PPN was found to be slightly higher in upper AEZs (UH1a and UH1b) as opposed to lower agro-ecological zones (UM1) which recorded the lowest number of nematodes. This may be attributed to among other factors increase in soil moisture. The upper AEZs receives relatively high amount of rainfall, coupled with low temperature and hence the soil is able to retain moisture for a longer duration (Jaetzold and Schmidt, 1983) as opposed to the lower AEZs. However, UH4 although it is slightly lower than UH1a, UH1b, UH2 and UH3, recorded a higher population of nematodes. This may be attributed to irrigation, which provided much needed soil moisture for nematode mobility to infest crop rhizosphere. Norton (1979) reported moisture as the most important abiotic parameter governing nematode populations, directly or indirectly, an argument that might explain presence of more nematodes in upper AEZs and watered farms.

The population and distribution of PPNs in AEZs may also have been influenced by soil physical-chemical characteristics. These characteristics are important relative to occurrence and population dynamics of nematodes (Wallance, 1963). However, the interrelations of nematodes, soil physical-chemical characteristics and the plant hosts are complex, and many soil factors do not work independently to influence nematode population (Norton *et al.*, 1971). High number of nematodes is associated with loose soil that has high porosity and aeration that favour nematode mobility (Norton, 1979); most soils in Nyandarua and Embu are clay-loam (CL) which is capable of supporting significant nematode population.

Low PPN in UM1 may be attributed to high level of carbon and nitrogen in the area, in contrast, UH1a, UH1b and UH3 AEZs which recorded high numbers of root and soil nematode and had low levels of carbon and nitrogen. A negative correlation between PPNs and soil organic carbon is consistent since organic carbon increases the number of antagonistic organisms in the soil resulting in reduction in the PPN population. Linford *et al.*, (1938) found that incorporation of organic amendment in the soil reduces population of *Meloidogyne* spp. in the soil probably by increasing the population of antagonistic micro-organism. Jaffee *et al.*, (1998) found that the number of nematode-trapping fungi was higher in organic than in conventional soils, and that population densities of some fungi were also higher in organic soils. Increase in antagonism is likely to exhibit a positive relationship with the nematode suppression in the soil (Oka, 2010) and this may explain the negative correlation of nematode genera with soil carbon reported from this study.

These findings are also in agreement with those of Talwana *et al.,* (2008) in Uganda where they found that nitrogen level in the soil was associated with a decrease in nematode population densities. In addition, Rodrỉguez-kåbana *et al.,* (1987) noted a negative linear relationship between nitrogen content in organic amendment applied to soil and infestation of plants by RKN. This emphasizes the importance of nitrogen addition in the soil to manage PPN and support the findings by Talwana *et al.*, (2008) that presence of a negative correlation of nematode populations with soil nitrogen is a relevant indication that use of fertilizers may mitigate nematode infestation. Nitrogen is thought to act indirectly on nematodes by increasing the incidence of fungi which attack nematodes (Cooke, 1962). Organic amendments have been shown to reduce soil bulk density and increase soil nitrogen and carbon supply (Doran, 1995).

The levels of potassium in soil were relatively high in five out of the six AEZs; UM1 is the only AEZ that had low potassium levels. This may also explain the low population density of PPN in this zone. Increase in the level of potassium in the soil has been shown to increase PPNs (Kincaid *et al*., 1970; Badra and Yousif, 1979) especially *Pratylenchus* spp. since availability of potassium in the host plant increases the rate of nematode reproduction (Agu, 2008).

The Victoria, Riana, Pruktor, Rinda, Gloria, Golden Acre, and Amigo have been listed as the most common varieties grown by farmers in Kenya (Ministry of Agriculture, 2007). However, two cabbage varieties widely cultivated in UM1 (Amigo and Riana F1) recorded the lowest nematode population as compared to the varieties grown in upper highland zones. The difference in plant nematodes population recorded in different cabbage varieties may be attributed to the ability of the host genes to resist or prevent nematode multiplication in host species (resistance) (Trudgill, 1986) or the ability of the host genotype to withstand or recover from the damaging effects of nematode attack and yield well (tolerance) (Cook and Evans, 1997). Various crop varieties are said to possess varying degrees of resistance and tolerance to disease and pests (Trudgell, 1991). Additionally some plants are known to produce allelochemical compounds that deter pests and disease causing agents from infecting a plant. Some brassica plants have been known to possess such allelopathic potential (Halbrendt, 1996).

5.3 The difference in population densities of various PPN genera associated with cabbage, within Nyandarua and Embu AEZs.

This research has demonstrated that different genera of PPNs are widespread in cabbage growing fields in various AEZs of Nyandarua and Embu regions. *Pratylenchus* spp. was the most common PPN in cabbage roots and soils. It was found in high population density and frequency of occurrence in samples from all AEZs. High presence of *Pratylenchus* spp. in nearly all the studied AEZs may be attributed to the farming practices common among farmers.

It emerged that most farmers used crops that are host to lesion nematodes in their crop rotation cycles. Thirty seven percent (37%) of farmers intercropped cabbage with potatoes (*Solanum tuberosum*) while *Pratylenchus* spp. has been consistently associated with *S. tuberosum* (Brown *et al*., 1980). Therefore, intercropping and/or crop rotation involving potato and cabbage could have resulted in a high frequency of the lesion nematodes. Others (15%) intercropped cabbage with maize which is a good host of lesion nematodes. Some seedbeds were prepared in maize plantation as noted during survey. This might not only have led to infestation of cabbage by maize lesion nematode but exposed the seedlings to lesion nematodes at a very tender stage before they are transplanted into the field.

The *Pratylenchus* spp. is among the most commonly encountered nematode pests in agronomic settings and is responsible for significant yield losses worldwide. In Kenya for example, it is the most important nematode parasite of maize (Kimenju *et al*., 1998) and it has been identified in cabbage (Waceke, 2007). *Pratylenchus penetrans* has been shown to cause wilting and death of cabbage (Acedo and Rohde, 1968) although Oltholf and Potter (1973) had reported cabbage to be relatively tolerant to *P. penetrans*. In a green house host range experiment with onion, broccoli and cabbage, Rich *et al*., (1977) found cabbage to be a good host to *P. scribneri*.

The Root-knot nematode (RKN) was reported in low number in root system. Low galling and egg mass indices were noted. Among the species identified was *Meloidogyne javanica*. *Meloidogyne* spp. J2 and galls were mostly detected in UM1 and UH2 probably due to inclusion of diversity of vegetable crops in cabbage production system, which are known to be good hosts of RKN.

The findings of RKN associating with cabbage in Nyandarua and Embu agree with other research work done. Waceke (2007) reported 26% frequency of occurrence in cabbage roots while Mennan and Handoo (2006) recorded a low frequency of 2% in Turkey. Bafokuzara (1996) considered *Meloidogyne* spp. as a potential important pest of cabbage among other vegetables in Uganda as a result of its high occurrence (28% in cabbage).

The findings also corresponds with Mcsorley and Frederick (1995) work, where commonly grown crucifers were found to host all four major *Meloidogyne* species and races. Liébanas and Castillo (2004) differently arrived to a similar conclusion. They found various crucifer species being infected by *M. arenaria* race 2, *M. incognita* race 1 and *M. javanica*, indicating them to be potential hosts for *Meloidogyne* spp. *Meloidogyne javanica* is known to parasitizes over 770 plant hosts, *Brassica oleracea* and *Brassica rapa*, being some of them (Potter and Oltholf, 1993).

However, cabbages infected by RKN were found to posses very small egg masses and tiny, inconspicuous galls. Presence of RKN at low population in cabbage roots support reports by other researchers, indicating cabbage to be a poor host to RKN (Waceke, 2007). Presence of RKN at a relatively lower density could be due to suppressive ability of cabbage as reported by Bafokuzara (1983). Research carried out in Brazil showed that kale, a member of Brassica group was found to be resistant to *M. javanica*, *M. incognita* race 3 and *M. arenaria* but not to *M. hapla*. Cabbage was found to be moderately susceptible to *M. javanica, M. incognita* and *M. hapla* but highly susceptible to *M. arenaria* (Carneiro *et al*., 2000).

The *Helicotylenchus* spp. was second in frequency of occurrence (more than 80% of soil samples) in five of the study sites. Since this nematode has a wide host range its high presence was probably influenced by cropping history such as mixed cropping thus predisposing cabbage to higher levels of attack. *Helicotylenchus* spp. has been recorded in cabbage in Kenya (Waceke, 2007). Bafokuzara (1996) found this genus in all vegetables grown in six districts of Uganda at a frequency of 50%. This nematode tend to burrow into the roots of their host-plants and feed within the root cortex leading to brown necrotic areas on attacked roots. This was clearly visible from the root samples.

The *Heterodera* spp. was present in all AEZs of Nyandarua but entirely absent in UM1 samples. This could be attributed to potato production in Nyandarua; *Heterodera* is an important potato nematode. Intercropping of cabbage with potatoes by 37% of farmers may have led to high incidences of this cyst nematode. *Heterodera* spp. has been reported in cabbage in various parts of the world. Mennan and Handoo (2006) reported *H. cruciferae* in soil samples from cabbage rhizosphere occurring at a frequency of 20%. This nematode genus has been known to cause significant yield loss in cabbage (Abawi and Mai, 1980; Ltholf *et al.,* 1974) its high presence in cabbage leads to a reduced root system that results in a smaller and less compact head (Abawi and Mai, 1980). This also correlates with a research done by Abawi and Mai (1980) which indicated the damaging level of *Heterodera* spp. in field micro plots inoculated with six eggs/g of soil. This could be the first report of cyst nematode associating with cabbages in Kenya and further investigation is necessary.

The stubby root nematodes (*Trichodorus* and *Paratrichodorus)* which attack crop roots leading to thickened 'stubby' roots, with branched lateral roots resulting in poor top growth and subsequent reduction in yield, were present in all the AEZs. They have been reported in Kenya (Waceke, 2007) and in various parts of the world associated with vegetable crops (Netscher and Sikora, 1990). They are important parasites of vegetables and they are known in potatoes, not so much for their direct damage, but for the tobacco rattle virus which they transmit causing corky ring-spot which is a serious disease of potatoes (Hafez *et al*., 1990).

The *Tylenchorhynchus* spp. was present in relatively high frequency but at low population density. Although it is an important nematode of citrus, a species of this genus has been identified in vegetable. *Tylenchorhychus brassicae* is a serious problem on most cruciferous crops and it impact negatively in them if present in high numbers (Khan, 1969). *Tylenchorhynchus* spp. has been reported in various parts of the world, Brown (1962) in his survey of PPN in Karachi, reported widespread occurrence of stunt nematode on cabbage among other crops. It was reported in Kiambu and Kajiado Kenya by Waceke (2007) as one of the most destructive nematode pest of vegetables.

Other PPNs identified include *Xiphinema* spp. and *Longidorus* spp. These Longidoridae are known potential pests of vegetables. They cause damage especially in sandy soil, often overlooked where RKN predominate (Netscher and Sikora, 1990). In this study, they occurred at low frequency (< 30%) in both soil and roots. They are common plant nematodes having been reported in vegetable and cabbage crop (Waceke, 2007; Bridge *et al*., 1996).

The *Scutellonema* spp. is commonly found in vegetable crops. However, its economic importance in the field has not been established (Netscher and Sikora, 1990). It was present in soils at a lower frequency. *Peltamigratus* spp.

was only present in UH4 AEZ. In addition, this is the first report of *Peltamigratus* spp. associating with cabbage in Kenya. However, it has been associated with vegetables production in other parts of the world, (Bridge *et al*., 1996).

The *Quinisulcius* spp. has been reported in Kenya (Waceke, 2007); Uganda (Bafokuzara, 1996), and is suggested to be a damaging pest known to cause yield loss of cabbage in Central America, where unusual high density of this nematode was found around cabbage roots and this indicated its ability to attack and damage cabbage (Bridge *et al*., 1996). For this study, *Quinisulcius* occurred in very low frequency which cannot cause much alarm.

On the other hand, ectoparasites like *Tylenchu*s, *Filenchus* and *Psilenchus* are either considered weak root hair feeders (Yeates *et al*., 1993) or fungal feeders (Okada *et al*., 2002). Moreover *Tylenchus* and *Filenchus* were among the dominant genera identified but they are presumed to be algal feeders and weak PPN and have not been demonstrated to be pathogenic to economically important crops (Hafez *et al*., 1992; Yeates *et al*., 1993).

Some nematodes were often encountered at low densities. Their low densities may have been due to their poor performances in presence of other root nematodes or lesion forming nematodes (Blake, 1969) and low population density may have resulted from inter-specific competition. Presence of some other nematodes could have resulted from weeds in the cabbage fields. Some weeds were seen to possess some nematode signs such as galls. These weeds may have been serving as an alternate host to the nematodes (Umar and Chubado, 2008).

Crop cultivars, cropping systems and farm management probably affected abundance pattern of individual groups of PPNs (Wallance, 1963). Nematode diversity has been shown to decrease with intensity of land cultivation or soil disturbance (Kimenju, *et al*., 2009), this could have been the case in upper agro-ecological zones where PPN diversity was slightly lower than UM1. According to Yeates and Bongers, (1999) the decrease in diversity of the nematode fauna with increasing intensity of cultivation can be attributed to physical disturbance, change in quantity and quality of organic matter being returned to the soil and increase in numbers of specific plant feeding nematodes that are favoured by the crops selected.

The genus richness is said to be influenced by agrochemical inputs and tillage, whose increase is said to increase total nematode biomass which is dominated by plant parasitic nematodes (Bouwman and Zwart, 1994). Such soils are rated as the most disturbed ecosystem. High inputs of agrochemicals particularly pesticides in addition to monocultures can be the main contributing factors to increased loss in nematode diversity which tend to favour PPNs than free living nematodes (Kimenju, *et al*., 2009).

Agro-ecological zones which practiced intensive agriculture characterized with cultivation of different crops was found to support high nematode levels. This is in agreement with work done (Kimenju, *et al*., 2009) which revealed that land use has an effect on the nematode community structure and that increase in soil disturbance results in increase of plant parasitic nematodes. This condition is attributed to the increased feeding sites for the nematodes from the many plant roots produced by a crop due to fertilizer application.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has clearly shown that cabbage is a host to economically important plant parasitic nematodes in Nyandarua and Embu counties. Plant nematodes were found associated with cabbage across the agro-ecological zones at varied population densities. However nematode population was slightly higher in upper AEZs (UH1a and UH1b) as opposed to lower AEZs (UM1). The findings challenge the consistent use of cabbage as a rotation crop in management of plant parasitic nematodes especially in vegetable in vegetable production.

The *Pratylenchus* spp. is probably the most important nematode found associating with cabbages in Kenya. This genus was found in high population in all agro-ecological zones. As such, *Pratylenchus* could be a major limiting factor in vegetable production. Besides, the results discussed here have indentified key nematodes that are of economic importance in cabbage vegetable production. They include *Helicotylenchus* spp., *Paratrichodorus* spp., *Tylenchorynchus* spp., and *Paratylenchus* spp. Presence of *Meloidogyne* spp. in low population density confirm the findings that cabbage is a poor host to RKN.

Various farming practices in the two counties may have significantly influenced the presence and distribution of nematode populations. These include practices such as intercropping, mono-cropping, use of organic amendments in form of manure and choice of cabbage varieties. Additionally, some soil physical-chemical factors such as the level of organic carbon and nitrogen in the soil may have influenced the nematode distribution.

6.2 Recommendations

- There is need for tests to establish the economic importance of the reported suspected nematode pest in cabbage with an aim of designing a proper nematode management strategy. This survey provides important background information for planning and administering nematode management strategies in Kenya.

- There is need for Pathogenicity tests for various PPN reported herein in order to quantify damages caused by individual nematode genera and other nematode pests reported in other vegetable crops.

- Various cabbage varieties reported in this study should be screened for resistance and tolerance to various plant parasitic nematodes with an aim of designing a proper nematode management strategy. The suitability of cabbage varieties/hybrids grown in the different AEZs as hosts to PPN genera need to be tested since the host genotypes play an important role in the level of root infection and soil infection by the nematode pests.

- Farmers in the two counties should be educated on management of plant parasitic nematodes with an aim of boosting agricultural production in these AEZs.

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APPENDICES

Appendix 1: Field work questionnaire

Date______________ Questionnaire no: ______________GPS readings________

Particulars of the study area:

Division_____________location________________sublocation_______________

Village ____________Agro-ecological zone ___________soil type ____________

Name (optional) ____________________cell phone (optional) ______________

Questions:

<u>Tick where appropriate</u>

1. a) Do you grow cabbages? Yes ☐ No ☐

   b) If yes above, what proportion of your farm is under cabbage cultivation?

   Less than 0.5acre ☐ 0.5-1acre ☐ 1acres ☐ 2acres ☐ more than 3acres ☐

2. What do you grow cabbage for?

   a) Home consumption ☐

   b) For sale ☐

   c) Both ☐

   d) Others (specify) ____

3. When did you plant these cabbages?

   Before April ☐ April ☐ May ☐ June ☐ July ☐ August ☐

4. What variety of cabbage are they?

   Sugar loaf ☐ Drum head ☐ Copenhagen market ☐ Gloria ☐ Golden acre ☐

   Others (specify) ____

5. Where did you obtain your seedlings?

   a) Own nursery bed Yes ☐ No ☐

   b) From other nurseries yes ☐ No ☐

C) If from other nurseries specify____

6. Did you use any fertilizer during planting?

a) Yes ☐ No ☐

b) If yes in 6 (a) above, which one?

D.A.P ☐ C.A.N ☐ T.S.P ☐ S.S.P ☐ DSP ☐ Others ☐

7. a) Do you use manure on cabbages? Yes ☐ No ☐

b) If yes in 7 (a) above which type of manure did you use?

Compost manure ☐ Green manure ☐ Cow ☐ Goat ☐ Chicken manure ☐

8. (i) Did you top dress? Yes ☐ No ☐

(ii) If yes what did you top dress with?

____

9. a) How do you water your cabbages?

ii) Irrigation Yes ☐ No ☐ ii) rain Yes ☐ No ☐

b) If you irrigate where you do get water from?

____

10. a) Which of the following plant disease causing organisms do you know?

(i) Nematodes (ii) fungi (iii) bacteria (iv) viruses

b) If yes in 10a (i) above do nematodes attack your cabbages? Yes ☐ No ☐

c) How do you control them?

____

11. a) Do you use any chemical in your farm? Yes ☐ No ☐

b) If yes in (a) above, against what is the chemical used?

Nematodes ☐ fungi ☐ bacteria ☐ virus ☐ insect ☐ others ☐

12. a) Do you carry out any soil treatment? Yes ☐ No ☐

b) If yes in 12 (a) against what? _________________ using what? ______________________

13.i) Do you carry out crop rotation? Yes No

ii) If yes in i) above, which crop(s) do you use in your rotation? ____

14. Which crops were planted where cabbages are during the previous season? ____

15. For how long does your field remain fallow between harvesting and planting?

Four months Three months Two months One month Less than a month

16. In your opinion, is cabbage cultivation profitable? Yes No

18. Would you like to make any other comment about cabbage production? ____

Thank you for taking time to respond to our questions and for allowing us to pick sample from your farm.