IDENTIFICATION OF NEMATODES AND MORPHOLOGICAL CHARACTERIZATION OF *MELOIDOGYNE INCOGNITA* ASSOCIATED WITH *SOLANUM LYCOPERSICUM* L. IN THE ASHANTI, BRONG AHAFO AND UPPER EAST REGIONS OF GHANA

BY

HANIF LUTUF

(10442512)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF M. PHIL CROP SCIENCE DEGREE

JULY, 2015.

DECLARATION

I, Hanif Lutuf, hereby declare that with the exception of materials used and referenced which have been duly cited and acknowledged, this work submitted to the Crop Science Department of the College of Basic and Applied Sciences (CBAS), University of Ghana, for the award of a Master of Philosophy degree in Crop Science (Plant Pathology) is the result of my own research and as such has not been submitted for a degree to any other University.

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HANIF LUTUF

(STUDENT)

Date………………………

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DR. S. T. NYAKU DR.E. W. CORNELIUS

(PRINCIPAL SUPERVISOR) (CO-SUPERVISOR)

DATE……………………… DATE……...............................

ABSTRACT

Production of tomato in Ghana is threatened by plant parasitic nematodes. Identification of the types, distribution and population densities of these parasites is indispensable for the design and implementation of a proper nematode management practice. A study was conducted to: assess farmers' knowledge and perception on plant parasitic nematode occurrence and management on their farms in nine communities of Ashanti, Brong Ahafo and Upper East Regions of Ghana; Identify genera of plant parasitic nematodes associated with tomato production in the communities; determine the relative abundance of identified nematodes from tomato roots and rhizosphere soil; morphologically characterize field populations of *Meloidogyne incognita*; compare morphometric features of the parasite reported to that in literature and determine which features account for total morphological variations of the populations through principal component analysis (PCA). Semi-structured questionnaires were designed and administered to fifty four randomly selected farmers from nine communities namely Afrancho, Akumadan, Asuosu, Tono, Vea, Pwalugu, Tachimentia, Tanoso and Tuobodom. Data collected in the questionnaire were subjected to descriptive statistics. Composite rhizosphere soil and tomato roots were collected from two randomly selected farms in each of the nine communities and nematode extracted from them, identified and enumerated. Morphometric characters were measured on adult males and second stage juveniles of *Meloidogyne incognita*. Principal component analysis was performed on the measurements to determine the morphological features accounting for the total variation of measured characters. The findings indicated that farmers were ignorant of plant parasitic nematodes as pathogens of their tomato crops although they experienced symptoms of nematode infestation on their tomato fields. Most farmers (63%) continually cropped their land to tomato for 4-7 years without any form of rotation and all the farmers interviewed (100%) applied only inorganic fertilizer to their crops. Eight genera of plant parasitic nematodes were identified; (*Helicotylenchus* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchuluss* spp., *Scutellonema* spp., *Tylenchus* spp. and *Xiphinema* spp.) from rhizosphere soils and four from roots of tomato plants (*Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchulus* spp. and *Scutellonema* spp.) at varying densities. *Meloidogyne* spp. was found to be the most abundant genus (37.41%, 69.27%) in soil (200 cm^3) and roots (10 g) respectively. *Meloidogyne incognita* was the most abundant *Meloidogyne* species (59% of males and 75% of Juveniles) based on head morphology of adult males and tail shapes of second stage juveniles, Average morphometric measurements of adult males were: Body length =1131.28 µm, Greatest body width= 34.45 µm, DEGO= 3.69 µm, Gubernaculum length = 10.19 µm, Stylet length= 25.45 µm, Tail length =11.15 µm and Spicule length= 29.26 µm and second stage juveniles were: Body length = 405.54 µm, Greatest body width = 15.16 µm, Stylet length = 12.35 µm and Tail length = 46.67 µm. These measurements were generally larger than those reported in the literature for *M. incognita*. PCA indicated that total body length and greatest body width were important characters in separating adult males of *M. incognita* from four communities while tail length, body length and stylet length were important in separating J2 of *M. incognita* from the nine communities.

DEDICATION

To the Glory of the Almighty Allah, my mother (Madam Alijata), my brother (Abdul Salam) and my beloved fiancée (Faizah).

AKNOWLEDGEMENTS

I thank Almighty Allah for how far he has brought me with His mercies and directions. I am also grateful to my supervisors, Dr. S. T. Nyaku and Dr. E. W. Cornelius for their tireless efforts in making up this thesis. My appreciation goes out to the A. G. Leventis scholarship foundation for providing me with funds to undertake this research. My earnest appreciation goes to my 'squad members' Mr. Saaka Yahaya and Ms Mavis Acheampong for all their selfless contributions towards the making of this thesis. I am also grateful to the staff of Plant pathology laboratory of the Crop Science Department (Mr. Elvis Appiah, Mr. Richard Otoo, and Mrs. Matilda Okyere) and Mr. William Asante for all the assistance offered me. I am grateful to my lecturers, Prof K. A. Oduro and Prof. S. K Offei for imparting plant pathology knowledge in me. Last but not the least; I am thankful to all the AEAs and farmers in the communities surveyed for availing themselves for interviews

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CHAPTER ONE

1.0 INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables in the world, second only to potato in terms of consumption (Panthee and Chen, 2009). The crop has many uses including the preparation of soups, salads and puree (Naika *et al.*, 2005). It is a major source of lycopene, a dietary carotenoid which is an antioxidant known to combat cancer, heart diseases and premature aging (Wener, 2000). World production of this crop is estimated at 152.9 million tonnes with a value of $74.1 billion (FAOSTAT, 2009).

Tomato is regarded as a high value cash crop in Ghana and is cultivated by many households in all the agro ecological zones of the country (Diao, 2010). Its production in Ghana in 2012 stood at 321,000 tonnes from 44,750 hectares of land (FAOSTAT, 2013). The crop offers employment and income to both rural and urban inhabitants (Asare-Bediako *et al*., 2007).

Despite the high level of production, the inability to meet the local demand has resulted in the importation of both fresh and processed tomato into the country (SEND Ghana, 2008) mostly from neighboring Burkina Faso. Ghana is currently one of the largest importers of tomato paste with an average consumption rate of 25000 metric tonnes per annum, costing about $25 million annually (Aryeetey, 2006). The tomato industry in Ghana has failed to reach its full potential despite its importance (Robinson and Kolavalli, 2010). This can be attributed to several reasons of which the most important is the vulnerability of the crop to various fungal, viral, bacterial and nematode diseases (Horna *et al.,* 2006). The crop is reported to be subject to over 30 diseases in the country (Oduro, 2000; Offei *et al.*, 2008).

Nematode diseases are one of the most difficult to manage because above ground symptoms of the crop usually result from below ground infection by plant parasitic nematodes which are not easily seen by farmers. Their effects only noticed when the population is widespread and yield reduction is high (Mai, 1977).

Identification of nematodes is of fundamental importance to a wide range of scientific studies on virulence and nematode management in crops. Moreover, understanding their diversity, current distribution and population densities is very essential for implementation of nematode management strategies for sustainable tomato production. Over 60 different species from about 19 genera of plant parasitic nematodes attack tomato; however, the most destructive is the root-knot nematode (*Meloidogyne* spp.) (Sasser and Freckman, 1987) and out of the over 97 *Meloidogyne* species described, only four namely *M. incognita, M. javanica, M. arenaria* and *M. hapla* are considered as important species due to their worldwide distribution and wide host range. In Ghana, plant parasitic nematodes parasitizing tomatoes have been observed of which *M. incognita*, was reported as the most important species (Osei *et al*., 2012). Despite the importance of *M. incognita* to tomato production in the country, there is inadequate information on morphological characterization of this devastating pest. Morphological characteristics of root-knot nematodes have been studied extensively and are still valuable for species identification, Moreover, for a country like Ghana where very few molecular laboratories have been set-up, morphology based techniques become very essential for the identification of root knot-nematode.

Cultivation of tomato has been an important economic activity in Ghana, especially in the Upper East, Brong Ahafo and Ashanti regions with estimates up to 7.5 metric tonnes per hectare (Robinson and Kolavali, 2010). Therefore, the main aim of this study was to identify nematode species associated with the crop in nine communities from Ashanti, Brong Ahafo and Upper East regions of Ghana, and to characterize populations of *M. incognita* morphometrically.

The specific objectives were to:

- assess farmers' knowledge, perception and experience on plant parasitic nematode occurrence and management on their farms in Ashanti, Brong Ahafo and Upper East regions of Ghana.
- identify genera of plant parasitic nematodes associated with tomato production in nine communities and determine their frequency of occurrence and relative abundance in tomato roots and rhizosphere soil.
- morphologically characterize field populations of *M. incognita* and detect variations among populations through morphometric analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and nomenclature of tomato (*Solanum lycopersicum*)

Tomato is a perennial plant that is cultivated as an annual crop. It belongs to the solanaceae family, which also includes potato, eggplant, pepper, tobacco and petunia. Morphological descriptions placed tomato as belonging to the genus *Lycopersicum*. In 1694, Tournefort, gave cultivated tomato the name "*Lycopersicum"* which means wolf peach in Greek because in German folklore, witches used the plants to evoke werewolves. In the 18th century, Carl Linnaeus introduced a binomial system of nomenclature and chose for tomato, the name *Solanum lycopersicum* (Kriemhild and Kenneth, 2000). In 1768, Phillip Miller changed the name to *Lycopersicon esculentum* which means edible wolf peach supporting that the difference from the other Solanum species were significant and enough to justify the different nomenclature (Cuttler, 1998). A recent study based on genetic and molecular characters, has shown that it is deeply nested in the Solanum genus, forming the sister clade to potato (Peralta *et al.*, 2005). For this reason, *Lycopersicon esculentum* Mill has been renamed *Solanum lycopersicum* L.

2.2 Nutritional importance of tomato

Fresh tomatoes and canned tomato products such as concentrates, puree and paste are increasingly in demand in West Africa where they form an essential part of the diet of the inhabitants (FIAN, 2007). Tomatoes are major sources of lycopene, a dietary carotenoid (Di Mascio *et al.,* 1998), which is known to combat cancer, heart diseases and premature aging (Wener, 2000). Tomatoes are also high in vitamins A, B and C and contain good amounts of potassium, iron, and phosphorus (Wener, 2000). The crop is low in saturated fat, cholesterol and sodium and cooking or processing tomato is actually beneficial to health because it increases the bioavailability of lycopene as heating up tomato breaks down its cell walls and release more lycopene.

2.3 Tomato production in Ghana

Tomato is probably the most important vegetable grown in Ghana, and a range of areas are suitable for its production (Horna *et al.*, 2006). The crop alone makes up to 38% of vegetable expenditure in Ghana (Wolff, 1999). Cultivation of the crop has been an important economic activity in Ghana, especially in the Upper East, Brong Ahafo and Ashanti regions with estimates up to 7.5 metric tonnes per hectare (Robinson and Kolavali, 2010). Production of tomato in Ghana is seasonal and varies within agro ecological zones as a result of the variable climatic conditions experienced in the country and for that matter moisture availability for production in the various production zones (Amikuzuno and Ihle, 2010). Most of the tomato in Ghana is produced under rain fed conditions with the exception of the Upper East region where it is cultivated under irrigation (Robinson and Kolavali, 2010).

Dry season irrigation production in the Upper East region supplies markets with fresh tomatoes from late December to April or May while the Southern zone notably Ashanti and Brong Ahafo regions supply the country from June to December (Robinson and Kolavali, 2010). Varieties of the crop commonly grown in Ghana include Roma, Pectomech, Royal, Burkina and Power (Khor, 2006).

The tomato industry in Ghana has failed to reach its full potential due to several reasons. The most important among these being the susceptibility of the crop to various fungal, viral, bacterial and nematode diseases (Horna *et al*., 2006). The crop is reported to be subject to over 30 diseases in Ghana (Oduro, 2000; Offei *et al*., 2008).

2.4 Plant parasitic nematodes

Nematodes are the most numerous animals on earth. They are either free-living or parasites of plants or animals and are present in almost all possible habitats. Nematodes are aquatic organisms and their movement depends on moisture (Decraemer and Hunt, 2006). 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 exploit all parts of vascular plants but the most economically important species infect roots (Hunt *et al.* 2005). Plant parasitic nematodes exhibit three types of parasitism, i) ecto-parasitism, ii) semi-endo parasitism and iii) endo-parasitism.

The endoparasitic and semi-endo parasitic nematodes induce sophisticated trophic systems of nurse cells or sincytia in their host; the female becomes obese and lose mobility (Hunt *et al.* 2005). Plants whose root system is affected by nematodes show symptoms such as stunting, chlorosis, wilting and most importantly from an economic standpoint, reduced yields. The symptoms in the aerial part of the plant are due to the poor ability of the root system to deliver water and nutrients. These symptoms are generally confused with water or nutrient deficiencies (Hunt *et al.* 2005).

Problems associated with plant-parasitic nematodes are present in all areas where crops are grown; however, the most obvious damage occurs in the tropical or sub-tropical regions due to the high temperatures, long growing seasons and the large number of susceptible crops which will gradually increase the nematode populations year by year (Mai, 1985).

Plant-parasitic nematodes are responsible for global agricultural losses of close to $80 billion annually (Agrios, 2005). In the West African sub-region, the severity of plant parasitic nematodes has been documented. Losses in the range of 20-94% due to nematodes have been recorded in Nigeria (Olowe, 1978). Duponnois *et al*. (1995) reported *Meloidogyne* species parasitizing tomatoes in Senegal. In Ghana, Osei *et al*. (2012) have also observed populations of Plant parasitic nematodes parasitizing tomatoes of which Root knot nematode (*Meloidogyne incognita*) is the most important nematode species reported. Other nematode genera reported on tomato in Ghana include *Helicotylenchus* spp., *Pratylenchus* spp., *Tylenchus* spp., *Hoplolaimus* spp., *Scutellonema* spp. and *Xiphinema* spp. (Osei *et al*., 2012).

2.4.1 Factors influencing distribution of plant parasitic nematodes

The population density and the distribution of plant parasitic nematodes are influenced by various biotic and abiotic factors. Such factors include farming practices such as application of organic amendment in form of manure. Addition of organic matter in the soil suppresses nematodes by generating toxic compounds in the soil that are harmful to them (Wachira *et al*., 2009). Soil texture also influences the population density of nematodes (Norton, 1979). Loose soil that has high porosity, support high population of plant parasitic nematodes as a result of improved aeration and mobility of nematodes in the soil (Sultan and Ferris, 1991). Plant nematodes are able to multiply in moist soil as moisture enables mobility and infection of appropriate hosts (Sultan and Ferris, 1991). Temperature may also influence the distribution of nematodes (Norton, 1979). Most nematodes are active between 25-30° C as such higher densities of plant parasitic nematodes along the tropical region. These factors however, do not work independently to influence nematode population.

2.5 Taxonomy of plant parasitic nematodes

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

2.5.1 Order Tylenchida

This Order has the most plant parasitic nematodes, 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* spp., *Heterodera* spp. and *Globodera* spp. the female loses vermiform shape and become obese or even globose. Their body length ranges between 0.2 to 1 mm, but occasionally over 3 mm. They possess a stomatostylet, a protrusible cuticular tube generally swelling posteriorly to form a basal knob. The knobs may be rounded as in *Pratylenchus* spp. or tulip-shaped with anterior tooth-like projections as in *Hoplolaimus* spp. (Luc *et al.*, 1990, Siddiqi, 2000).

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* spp., *Scutellonema* spp. and *Hoplolaimus* spp. oesophagus overlaps intestine dorsally. In *Pratylenchus* and *Helicotylenchus* the overlap is ventral while oesophagus abuts intestine in *Tylenchorynchus* spp., *Tylenchus* spp. and *Coslenchus* spp. (Siddiqi, 2000; Hunt *et al.*, 2005). Females 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 in identification, for instance the vulva is posterior (60%-70%) in *Helicotylenchus* spp. and median in *Radopholus* spp. The shapes of the tail tip tend to vary within members of this group of nematodes. Some are dorsally convex-conoid or hemispherical as in *Helicotylenchus* spp., others are elongated as is the case with *Radopholus* spp. while others are conical in shape. For instance *Heterodera* spp. J2 has a conical tail with a hyaline portion about 50% of the tail length while

Meloidogyne J2 has a conical tail with hyaline region starting near the tail tip (Hunt *et al.*, 2005; Jabbari and Niknam, 2006). These features are significant in nematode identification.

2.5.2 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 a needle-like structure attached posteriorly to a cuticular extension- the odontophore. They include *Xiphinema* spp. and *Longidorus* spp. (Hunt *et al.*, 2005). *Xiphinema* spp. is identified by the presence of odontostylet with three prominent basal flanges and forked base. In addition, *Xiphinema* spp. stylet has a guiding ring located at the posterior of the odontostyle. These features distinguish it from *Longidorus* spp. whose amphid is poach-like, the stylet lacks flanges and the guiding ring is at the anterior half of odontostyle (Hunt *et al.*, 2005).

2.5.3 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* spp. is characterised by strong vaginal scelerotization while the vagina extend into body for about half its diameter. *Paratrichodorus* spp. has weak vaginal sclerotization that extends into body for about a third of its diameter (Hunt *et al.*, 2005).

2.6 Root knot nematode (*Meloidogyne* spp.)

Over 60 different species representing 19 genera of plant parasitic nematodes attack tomato (Berlinger, 1986), but the most destructive nematode is the root-knot nematode (Sasser and Freckman, 1987). Root-knot nematodes are obligate parasites capable of feeding inside the roots of over 3000 plant species, causing extensive crop losses worldwide (Sasser and Freckman, 1987; Roberts, 1995).

Meloidogyne species are the most extensively distributed and damaging group of nematodes responsible for the majority of losses (Sasser and Freckman, 1987). Over ninety *Meloidogyne* species have been described until now (Karssen and van Hoenselaa, 1998), which display different modes of reproduction. Few species of *Meloidogyne* are amphimictic and produce cross-fertilized eggs e.g. *M. carolinensis, M. megatyla*, and *M. pini* (Jepson, 1987). Most Root Knot nematodes however, reproduce through parthenogenesis showing variations. Some species such as *M. chitwoodi*, *M. exigua* and *M. fallax* reproduce by meiotic parthenogenesis and when males are present they also reproduce by cross fertilization. Other *Meloidogyne* species reproduce by obligatory mitotic parthenogenesis. These species e.g. *M. arenaria, M. incognita* and *M. javanica* are considered the most important species due to a worldwide distribution and their wide host range. (Triantaphyllou, 1966; Van der Beek *et al.*, 1998, Hunt and Handoo, 2009).

Meloidogyne species are obligate and sedentary endoparasites of the roots, tubers and corms and have evolved very specialized and complicated relationships with their host. Typically they reproduce and feed in a specialized cell within the plant root, inducing small or big galls or root-knots, producing malfunctioning root systems. The damage may include various levels of stunting, poor or lack of vigour and wilting under moisture stress (Hussey, 1985; Moens *et al*., 2009).

2.6.1 Biology and life cycle of root-knot nematodes

Most species of plant parasitic nematodes have a relatively simple life cycle consisting of the egg four juvenile stages and the adult male and female. The root knot nematode completes most of its life cycle within their host roots (Hunt *et al*., 2005).

A first-stage juvenile develops and molts while still in the egg to become a second stage juvenile which hatches from the egg. After hatching, root-knot nematodes move through the soil to find areas on plant roots to feed. The nematodes survive in the soil as eggs and also second stage juveniles. Mature females of root knot nematodes deposit eggs up to 1000 or more in a gelatinous matrix (egg sac or egg mass) which can be observed attached to the protruding posterior end of the females on the root surface (Hunt *et al.*, 2005). This sac protects the eggs from dehydration (Pattison, 2007). The infective second-stage juveniles hatch from the eggs and move through the soil in search of roots of suitable host plants (Davis *et al.*, 2004). The juveniles usually penetrate host roots just behind the root tip region and establish their special permanent feeding sites (giant cells) in the vascular tissues of the root (Hunt *et al*., 2005). The giant cells provide nutrients for the sedentary nematodes which continue to feed, enlarge, and molt three times. Root cells around the feeding sites are also induced to enlarge and form galls (knots) and often extensive secondary root formation and branching of the main root.

Depending upon the host and soil temperature, the entire life cycle (Fig.1) may be completed in 17 to 57 days (Hussey, 1989).

2.6.2 Methods of identification of *Meloidogyne* spp.

Exact identification of plant-parasitic nematodes is needed for more accurate research and effective control (Powers, 1992). Identification of plant-parasitic nematodes is however, not an easy task especially in the case of certain taxa or when more than one species occur in the same sample (Abrantes *et al.*, 2004). *Meloidogyne* species show wide morphological variations among and within species, making their identification difficult.

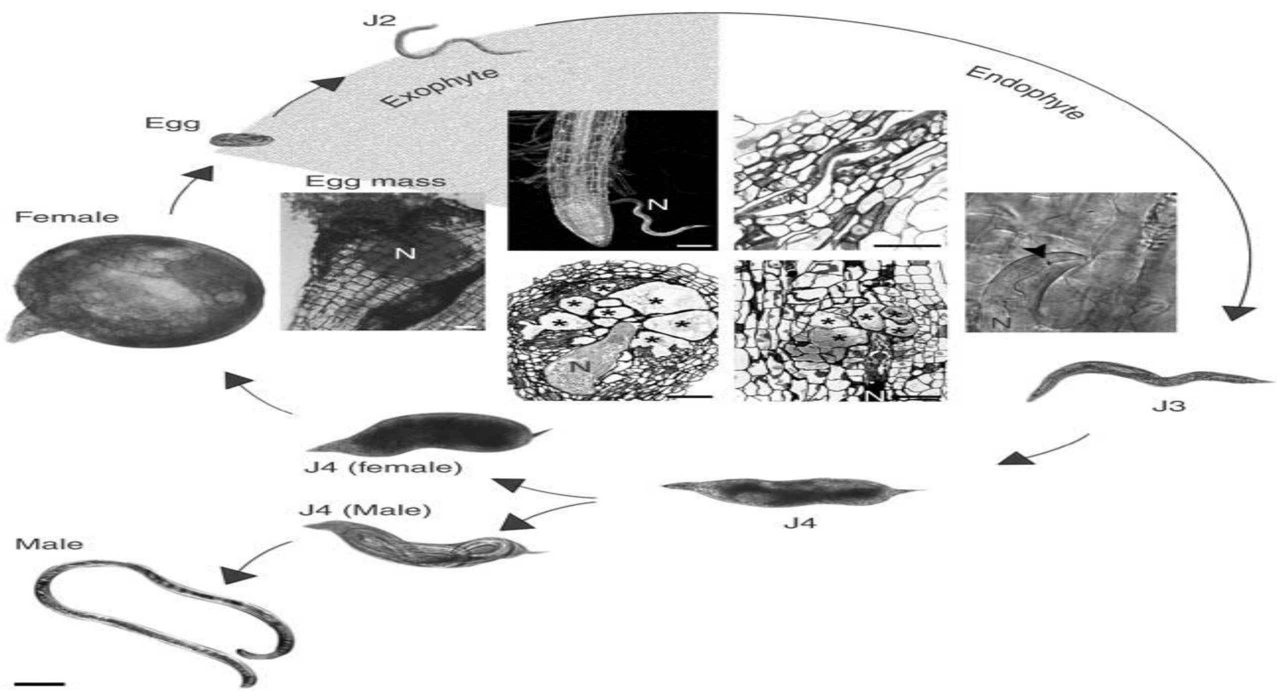

Fig. 1 Generalized life cycle of the root knot nematode. Source: Abad *et al*. (2008)

Other factors that can make identification difficult is a poor description of nematode species or high similarity between established species such as *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* (Hartman and Sasser, 1985).

The main methods of Root knot nematode identification are molecular based techniques, biochemical and traditional methods.

2.6.2.1 DNA -based methods

Molecular diagnosis of plant-parasitic nematodes has revolutionized our knowledge on the taxonomy and phylogeny of nematodes (Blok, 2005). There are several reasons why molecular data are more reliable for phylogenetic studies than morphological characters. DNA sequences are strictly heritable and the interpretation of molecular data is easier than morphological features. In addition, molecular characters are more abundant and are generated in a relatively shorter period of time as compared to morphological characteristics (Subbotin and Moens, 2006). The molecular diagnosis of plant-parasitic nematodes can be performed using bulk samples of females and second-stage juveniles (J2) or single J2 (Blok, 2005). The techniques are usually very sensitive and can detect specific species in mixed populations (Zijlstra, 2000; Subbotin *et al.*, 2001). Some methods have been developed for molecular diagnosis directly from infected roots, galls or infected soil (Blok and Powers, 2009). The polymerase chain reaction (PCR) is the key tool in the study of plant-parasitic nematodes, and can be used for phylogenetic studies, molecular breeding for resistance and molecular diagnostics (Blok, 2005). A range of DNA-based techniques is available for taxonomic or diagnostic purposes.

Ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) are among the most commonly used DNA regions for taxonomic and diagnostic purposes (Abrantes *et al.,* 2004). The rDNA repeat unit, containing 18S, 28S and 5.8S coding genes and the internal transcribed spacer (ITS), external transcribed spacer (ETS) and intergenic spacer (IGS) have been widely used for phylogenetic and diagnostic studies (Blok, 2005; Hajibabaei *et al.*, 2007). These regions have been very important for characterization of 15 species of plant-parasitic nematodes that are economically important and regulated in quarantine legislation (Blok, 2004). The 18S and 28S rDNA genes have been widely used for phylogenetic purposes (Blok, 2005). For example, the

18S rDNA of 19 populations of *Meloidogyne* species was amplified and sequenced and three main clades were identified including the most important and disseminated species, e.g. *M. incognita*, *M. javanica* and *M. arenaria* (Tigano *et al.*, 2005). Similarly, the phylogeny of 12 species of *Meloidogyne* species was deduced using the 18S rDNA sequences, indicating that this region is useful in addressing phylogeny within *Meloidogyne* species (De Ley *et al.*, 2002). Holterman *et al.* (2009) constructed a phylogeny of 116 Tylenchida taxa based on full sequence of 18S rDNA. Landa *et al.* (2008) characterized three *M. hispanica* isolates from three different origins (Brazil, Portugal and Spain) using sequences from 18S, 5.8S ITS2 and D2-D3 of 28S and found identical sequences in all three isolates. *M. hispanica* from *M. incognita* and *M. arenaria* are difficult to distinguish by morphological and biological features. The analysis of the three rDNA regions demonstrated and supported the differentiation of *M. hispanica* from *M. incognita, M. javanica* and *M. arenaria*. The first characterization of plant-parasitic nematodes was done using mitochondrial DNA, which is an interesting target for molecular diagnostics due to the high copy numbers of mtDNA in each cell and their fast rate of sequence evolution (Blouin, 2002). mtDNA is an excellent source for genetic markers for population genetics and species identification (Hu and Gasser, 2006). The mitochondrial DNA region has been used for identification of *M. mayaguensis* and to monitor the presence and distribution of this species. This region has been useful in distinguishing *M. mayaguensis* from *M. incognita* and *M. arenaria* (Blok *et al.*, 2002). A number of different kinds of molecular markers have been developed. The AFLP technique is based on the detection of genomic restriction fragments by PCR amplification, and is used to find and to assess genetic diversity (Vos *et al.*, 1995). Qualitative and quantitative analysis have been carried out to evaluate genetic variation on populations of cyst nematodes and root-knot nematodes (Xue *et al.*, 1993). Fargette *et al.* (2005)

studied the genetic diversity of *M. chitwoodi* and *M. fallax*, two major agricultural pests and found that *M. chitwoodi* displays a higher diversity than *M. fallax.* AFLP has proved useful for analysis of inter and intra-specific genetic diversity in several organisms (Han *et al.,* 2000; Tooley *et al.,* 2002).

Random Amplification of Polymorphic DNA (RAPD) has been used to discriminate to interspecific and intraspecific relationships in *Globodera* and *Meloidogyne* species (Castagnone-sereno *et al.*, 1994; Blok *et al.*, 1997). It has been used to distinguish *M. incognita*, *M. javanica* and *M. arenaria* (Cenis, 1993). RAPD markers that are different among different species can be developed into sequence characterized amplified region (SCAR) markers that can be effectively used for the diagnosis of important pests such as *Meloidogyne* species (Blok and Powers, 2009). Their specificity and sensitivity varies and depends on the number of species and isolates evaluated. Several sets of SCAR primers can be used at the same time in multiple reactions to identify many species (Zijlstra, 2000; Randig *et al.*, 2002). Zijlstra (2000) reported that SCAR markers specific to *M. chitwoodi*, *M. fallax* and *M. hapla* were amplified using DNA from eggs, juveniles and females. Tigano *et al.* (2010) detected 16 *M. enterolobii* isolates of different geographical origin using species-specific markers.

Inter simple sequence repeat (ISSR) a PCR-based molecular marker technique has proved to be highly useful for various applications including genetic diversity and population genetic structure studies of various species (Zietkiewicz *et al.*, 1994; Geleta and Bryngelsson, 2009). ISSRs uses single primer for amplification of the target regions, which is a DNA segment present at an amplifiable distance between two identical microsatellite repeat regions oriented in opposite direction (Zietkiewicz *et al.*, 1994). Carneiro *et al*. (2008) studied the diversity of *M. arenaria* using RAPD and ISSR markers and found high level of polymorphism among the isolates of this species. Microsatellites or simple sequence repeats (SSRs) have been widely used for population genetics. Analysis of the variation in microsatellites in populations can give information about the population genetic structure and differences, genetic drift and the last common ancestor (Subbotin and Moens, 2006). SSR markers have also been widely used for genetic diversity and populations studies of many species.

2.6.2.2 Biochemical and traditional methods

Analysis of proteins by polyacrylamide gel electrophoresis is an important method for species differentiation of root knot nematodes. Some enzymes, especially esterases, malate dehydrogenase and alpha-glycerolphosphate dehydrogenase exhibit distinct patterns for each species. The most used biochemical method for identification of root knot nematode is the esterase pattern (Moens *et al.*, 2009). The first use of isozyme phenotypes on the identification of root knot nematodes was done by Esbenshade and Triantaphyllou (1985).

In a survey involving 300 populations from 65 countries, Esbenshade and Triantaphyllou, (1990) reported esterase pattern for 16 *Meloidogyne* species. Carneiro *et al.* (2000) studied 111 populations of *Meloidogyne* from South America and found 18 esterase phenotypes. Zu *et al.*, (2004) found five esterase phenotypes from 46 populations of *Meloidogyne* species from China. Hernández *et al.* (2004) studied 29 isolates of *Meloidogyne* species from coffee plantations in four Central American countries and one isolate from Brazil and found six new multi-enzyme phenotypes. Isozyme phenotypes are considered attractive because of their relative stability within *Meloidogyne* species (De Waele and Elsen, 2007); nonetheless, some complications can occur due to intraspecific variation and difficulty in resolving size variants between species (e.g. *M. incognita* and *M. hapla*) and more than one enzyme is needed to confirm the species.

Traditional diagnosis of nematodes is usually based on detailed measurements and comparisons of morphological structures (Carneiro and Cofcewicz, 2008). Morphological differences between species are often based on the average of measurements from a population of individuals. The perineal pattern of egg-laying female is the most common taxonomic feature used to identify most root knot nematodes (Campos and Villain, 2005). However, identification based only on the perineal pattern may be uncertain, since some species, e.g. *M. paranaensis*, *M. konaensis, M. izalcoensis* and *M. mayaguensis* may show a perineal pattern similar to *M. incognita*, which may lead to misidentification of the species. When species identification is solely based on perineal patterns, the identification process should be done carefully, using only properly collected, prepared and mounted mature females (Carneiro and Cofcewicz, 2008). In addition, morphological characteristics of juveniles and mature males can be helpful in the identification of *Meloidogyne* species (Jepson, 1983a; Jepson, 1983b).

Although morphological and morphometric data might be inconclusive since they often vary considerably within a population (Hirschmann, 1986; Zijlstra, 2000; Carneiro and Cofcewicz, 2008), they are very useful to distinguish closely related *Meloidogyne* species especially when used as a complement to cytological, cytogenetic and molecular information (Moens *et al.*, 2009)

2.7 General nematode control options

Nematode damage to most crops, is usually always related to the initial numbers of the nematode in soil. Control strategies are, therefore, aimed at reducing these initial numbers (McSorley and Gallaher, 1991). These strategies generally can be divided into non-chemical treatments and chemical treatments. The non-chemical treatments include soil solarization and hot water treatment of planting materials, crop rotation and cover cropping, rogueing and burning diseased plants, land fallowing, flooding, application of organic amendments, use of nematode-suppressive plants, ploughing, biological control, and host plant resistance while the chemical treatments include the use of synthetic nematicides.

2.7.1 Non-chemical treatments

2.7.1.1 Soil solarization and hot water treatment of planting materials

Steam sterilization or other forms of heat treatment are often used for sterilizing soil used in greenhouses or nurseries as high temperatures will kill nematodes. Soil solarization is receiving increased attention for the management of nematodes and other soil-borne pests. It involves covering raised and moist beds with clear plastics for two-to-four months during the hottest part of the year, allowing the sun to heat the uppermost layers of soil (Elmore *et al.*, 1997).

The augmented soil temperature helps to kill many soil borne pests and pathogens including nematodes. Plant material infected with nematodes can be treated in hot water, provided that a suitable temperature range can be found which is high enough to kill nematodes but not lethal to the plant (Elmore *et al.*, 1997). Performance of this has been variable, depending on application technique and season (McSorley and Gallaher, 1991). The main drawback of this strategy is that temperature must be controlled critically and is usually just below that which injures plant tissues and most small-scale farmers in developing countries do not have enough knowledge and equipment to detect the precise temperature necessary for killing nematodes and at the same time not fatal to the plants.

2.7.1.2 Crop rotation and cover crops

Crop rotation is generally a very effective means of managing plant-parasitic nematodes. Crop rotation with a non-host crop is often adequate by itself to prevent nematode populations from reaching economically damaging levels. For example Asparagus, corn, onions and garlic are good rotation crops for reducing root knot nematode populations. *Crotalaria*, velvet bean, and grasses such as rye are usually resistant to root-knot nematodes. However, it is necessary to positively identify the species of nematode in order to know what plants are its host(s) and non-hosts (Wang *et al.*, 2004; Yepsen, 1984). Rotation crops and cover crops can be helpful in manipulating nematode populations during those times of the year when most susceptible crops cannot be successfully grown (Elmore *et al.*, 1997). Diversified crop interplantings and crop rotations can interrupt the spread, reproduction, and survival of nematodes.

Crop rotation will not eliminate infestations because most nematodes can remain in the soil as eggs for at least a year between host crops (Sherf and MacNab 1986), and most species can feed on a wide range of weeds. Due to the wide host range of root knot nematode, care must be taken in selecting alternative crops for rotation.

2.7.1.3 Land fallowing

A fallow period of two years with no susceptible plants in the field decreases nematode populations. Fallowing, in which all vegetation is kept off the infested area is a cheap and effective way to reduce nematodes numbers (Flint, 1999). This will not stop nematode eggs from hatching but, without food plant, the young nematode will die. Land scarcity in most countries has caused this control strategy to be unfeasible.

2.7.1.4 Rogueing and burning diseased plants

Rogueing and burning or discarding diseased plants in areas or farms where new outbreaks of nematode disease are discovered have been found to be effective. Rogueing will prevent or minimize the spread of nematodes from diseased plants to healthy plants along rows or between farms and nurseries (Yepsen 1984). The strategy is best for new farms or when disease is detected early. It has disadvantages of being time-consuming and when disease outbreak occurs the method is practically impossible.

2.7.1.5 Flooding

Flooding is sometimes used as a management tool to control nematodes. Nematode densities can drop significantly when soils are flooded for prolonged periods of time (Bridge 1996.). Flooding the soil for seven to nine months kills nematodes by reducing the amount of oxygen available for respiration and increasing concentrations of naturally occurring substances such as organic acids, methane, and hydrogen sulphide which are toxic to nematodes (MacGuidwin, 1993). However, it may take two years to kill all the nematode egg masses of *Meloidogyn*e spp (Yepsen, 1984). The duration of flooding for effective nematode control needs to be determined for each nematode species and it is a costly and uneconomic means of controlling nematodes. This method is practiced where water is very cheap and easily available.

2.7.1.6 Organic amendments

High soil organic matter content protects plants against nematodes by increasing soil water-holding capacity and enhancing the activity of naturally-occurring biological organisms that compete with nematodes in the soil (Akhtar and Malik, 2000; Wachira *et al.*, 2009)).

Beneficial fungi and bacteria are in high numbers in soil amended with different organic matter. Some fungi and bacteria are parasites of nematode eggs and also prey on nematodes. The parasitic nematodes do not hatch and thus population is reduced. The organic matter commonly used to control nematodes include poultry manure, saw dust and various crop residues.

2.7.1.7 Nematode-suppressive plants

Allelochemicals are plant-produced compounds that affect the behavior of other organisms in the plant's environment. For example, sudan grass and sorghum contain a chemical, dhurrin, that degrades into hydrogen cyanide, which is a powerful nematicide (Luna, 1993; Forge, *et al.*, 1995). Some cover crops have exhibited nematode-suppressive characteristics equivalent to aldicarb, a synthetic chemical pesticide.

Research has shown that incorporating sesame into rotation with cotton, peanuts, and soybeans reduced nematode population levels and yields significantly increased among those crops in fields (Anon, 1997).

Certain plants are able to kill or repel pests including nematodes, disrupt their lifecycle, or discourage them from feeding (Widmer and Abawi, 2000). Some of these plants are marigolds, castorbean, and various brassicas plant extracts, such as those from marigold (*Tagetes* spp),

Ricinus communis (L.), *Eucalyptus teretcormis* Sm. *Tridax procumbens*, *Ruta*, *Cineraria* or *Pelargonium* have also been effective in killing plant-parasitic nematodes, but results refer mainly to *in vitro* or pot experiments and practical application of these extracts is yet to be profitable (Dover *et al.*, 2003).

2.7.1.8 Ploughing

Prasad and Chawla (1991) reported that summer ploughing in parts of India allowed soil temperatures to reach 40-42^{o}C resulting in a reduction of the populations of *Heterodera avenea*, *Meloidogyne species* and *Rotylenchus reniformis* by 40%. Peacook (1957) adopted ploughing to control *Meloidogyne* species successfully at Achimota in Ghana. However, the labour needed, the difficulties of cultivating soil in the dry season, and lack of immediate and tangible benefits to the farmers normally rule out this practice for nematode control.

2.7.1.9 Biological control

Biological control is an effective alternative that can be combined with other strategies within an integrated management system (Stirling, 1991). In some soils, nematophagous fungi and bacteria have been reported to control the multiplication of nematode on susceptible crops but despite the commercialization of a few organisms, none is in widespread use.

Currently there are no effective, commercially available, biological control agents which can be successfully used to control nematodes. It has proved difficult to develop a biological control agent that is effective worldwide for any soil-borne disease. Biological control is more inconsistent, less effective and slower acting than control normally achieved with other methods (Kratochvil *et al.,* 2004). It seems likely that these limitations are intrinsic in most biological control agents and that their successful application will depend on integration with other control measures

2.7.1.10 Host plant resistance

In plant nematology, plant disease resistance is often defined as the ability of a plant to inhibit the reproduction of a nematode species relative to reproduction on a plant lacking such resistance (Friedman and Baker 2007). Resistant cultivars can produce the most dramatic increase in yields of many crops and appear to hold the solution to most nematode problems (Luc *et al.*, 2005). It is the most cost-effective and sustainable management tactic for preventing nematode damage and reducing growers' losses (Khan, 1994).

Resistance is crucial to the reliable production of food, and it provides significant reductions in agricultural use of synthetic chemicals and other inputs. Resistant crop cultivars have comparatively better crop yield than susceptible crop cultivars (Luc *et al.*, 2005).

Plant disease resistance derives both from pre-formed defenses and from infection-induced responses. Although obvious qualitative differences in disease resistance can be observed when some plants are compared after infection by the same nematode strain at similar inoculum levels, a gradation of quantitative differences in disease resistance is observed between plant lines or genotypes (Lucas, 1998). A major limiting factor affecting the effectiveness of newly introduced resistant cultivars is the selection of pathotypes or races that are able to break down the resistance (Luc *et al.*, 2005). However, strategies for utilizing resistance may be deployed to curtail breakdown of resistance.

2.7.2 Chemical control

Application of chemicals are one of the most effective methods of controlling nematodes in infected fields. Maqbool *et al.* (1985) reported that two systemic chemicals, aldicarb and carbofuran, were effective in the control of root-knot nematodes.

Phorate, a systemic chemical, was effective in reducing root knot nematode population and number of galls (Jagdale *et al*., 1985). Stephen *et al.* (1989) observed that phenamiphos 40% EC, miral 10%G and carbofuran 3%G, applied at recommended rates were effective in controlling the root knot nematode, *M. javanica* in eggplant and yield increased by 59% and 55%, respectively, compared with the untreated control.

Application of nematicides can be toxic to human and animals and they also kill the pest's natural predators, causing serious resurgence of some pests when not applied at the right time, in the right way and in the right dosage rate per hectare (McAvoy, 2000). Due to the side effects of the highly effective and broad spectrum fumigant, methyl bromide, on atmospheric ozone, the amount of the compound produced was reduced incrementally until it was totally phased out in 2005 (EPA, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Selection of site for questionnaire survey and soil and root sample collection

Tomato root and rhizosphere soil samples were obtained from nine communities in four agro-ecological zones across the Ashanti, Brong Ahafo, and Upper East Regions of Ghana. Sampling in the Upper East Region was carried out in two agro-ecological zones where tomato was being grown during the period of sampling. The agro ecological zones were Sudan savannah at Vea and Guinea savannah at Pwalugu and Tono. Sampling in the Brong Ahafo Region was carried out in the forest savanna transitional zone at Tuobodom, Tanoso and Techimantia and in the Ashanti Region carried out in the semi deciduous forest at Afrancho, Akumadan and Asuosu.

The nine communities were purposefully selected from eight districts known to produce tomatoes. The districts were Bongo-Nabdam, Kassena-Nankana and Talensi-Nabdam in Upper East Region, Tano, Tano North and Techiman North in Brong Ahafo Region and Offinso, and Offinso North districts in Ashanti Region (Table 1). Two farms were selected at random for the soil and tomato root sample collection with the help of Agricultural extension agents (AEAs) in each community and their owners interviewed with a questionnaire including four other farmers.

Table 1: Selected regions, districts, and communities, from which farmers were interviewed on plant-parasitic nematode infestations on their farms

Region	**District**	**Community**	***GPS coordinates**		**No. of respondents**
Upper East	Kassena-Nankana	Tono	10^{o} 50’N	01^{o} 5’W	6
	Bongo-Nabdam	Vea	10^{o} 45’N	10^{o} 40W	6
	Talensi-Nabdam	Pwalugu	10° 36’N	00° 51’S	6
Brong Ahafo	Tano	Tachimentia	07^{o} 10’N	02^{o} 01’W	6
	Tano North	Tanoso	07° 27’N	01^{o} 5 8’W	6
	Tachiman North	Tuobodom	07° 38’N	01° 54’W	6
Ashanti	Offinso North	Afrancho	07^{o} 23’N	01^{o} 57’W	6
		Akumadan	01° 60’W	01° 45’E	6
	Offinso	Asuosu	05^{o} 59’N	01^{o} 46’W	6
TOTAL					54

* GPS coordinates were taken from a single position on each farm

3.1.1 Questionnaire survey to assess farmers’ land use, knowledge, perception, experiences and economic impact of plant parasitic nematodes

A questionnaire survey was conducted in November, 2014 to obtain baseline data concerning farmers land use intensity, knowledge, perception, experiences on occurrence and management of plant-parasitic nematodes on their farms. Fifty- four (54) tomato farmers with varying farm sizes ranging from 0.5 acres to 10 acres were randomly selected from the study areas in the three regions. Pre-tested semi-structured questionnaires (Appendix 1) were designed and administered to participating farmers by reading these to them and their responses recorded. Pictures (Appendix 2) of nematode infested plants were also shown to the farmers to facilitate their responses.

3.1.2 Collection of rhizosphere soil and root sample of tomato

Tomato roots and rhizosphere soils were collected in November, 2014. Two farms each from the nine different communities were sampled over a period of three weeks, for the presence of nematodes. Eighteen (18) randomly chosen farms were visited and composite soil samples were taken from the rhizosphere region of tomato plants on each farm. The samples for this study were collected from farms with well-established tomato plants. The samples were taken from farms when the soil was not too wet or too dry as both extreme conditions make it difficult to collect and prepare samples for analysis. They were taken from depths of approximately 0-15 or 30 cm using a hand shovel randomly from 10 sites. The hand shovel and footwear were cleaned after sampling each farm to avoid cross-contamination of soil samples and spread of nematodes between farms. In cases where the soil was not easily penetrable, a soil core was used and samples were taken from a depth of 30 cm. The soil samples were placed in clean polyethylene bags and the date, specific location, and sample number written on adhesive paper labels stuck to bags using a permanent ink pen. The record was immediately logged into the field notebook with the sample number and GPS coordinate of the farm recorded.

Root-knot nematode infested root samples were also collected from rhizosphere of tomato plants and placed in the same bags as the soil. The samples were placed in an ice chest filled with ice to prevent dehydration of the plants.

3.2 Extraction of nematodes and assessment of root knot nematode galls

3.2.1 Extraction of nematodes from soil

Nematodes were extracted from the soil using the sieve and sucrose centrifugation method. Sucrose solution was prepared by dissolving 454 g of sugar in distilled de-ionized water and the volume brought to 1 liter. The soil sample was mixed and passed through coarse sieves to remove rocks and roots. About 200 cm^3 sub-sample of soil was poured into a beaker and weighed. Tap water was added to about twice its volume and stirred carefully and allowed to settle for three minutes. It was immediately poured through a stack of 90 µm - aperture mesh on a 36 µm - aperture mesh. Nematodes (retained on the mesh) were gently washed into centrifugation tubes for centrifugation using a water bottle.

Water was added to centrifuge tubes to equalize volumes and placed in balanced pairs. It was spinned at 1700 rpm for 5 minutes without using the brake and allowed to settle for 5 minutes. Supernatant was aspirated to about 1cm above the pellet. The tubes were filled with sucrose solution at room temperature and stirred with a spatula to break up the pellet. The sample was spinned up to 1000 rpm for 1 minute and the supernatant was poured through the 36µm - aperture mesh sieve and transferred into labeled vials up to the 10 ml mark using a fine spray water bottle.

3.2.2 Extraction of nematodes from roots

Nematodes were extracted from infested tomato roots, using modified Baermann funnel method (Whitehead, 1968). The roots were chopped with a pair of scissors and about 10 g was blended for about ten seconds and transferred to a glass funnel lined with a two ply tissue paper placed on a wire mesh. Tap water was poured gently into the funnel in which the mesh was placed until the tissue became moist. The set-up was left for 48 hrs and the water was poured separately into beakers and left overnight for the nematodes to settle. Each nematode water suspension was separately topped with tap water to 300 ml for standardization and homogenized by blowing air through with a rubber straw. The set-up for nematode extraction using Baermann funnel technique is shown in Fig 2.

Fig. 2: Set-up of Baermann funnel method used for nematode extraction from tomato roots

3.2.3 Assessment of root knot nematode galls on tomato roots

Tomato roots were washed separately and dried with tissue paper. Galling was scored using the rating chart by Bridge and Page (1980) presented below

0 = no knots on roots

1 = few small knots difficult to find

2 = small knots only but clearly visible; main roots clean

3 = some larger knots visible, but main roots clean

4 = larger knots predominate but main roots clean

5 = 50% of roots knotted; knotting on parts of main root system

6 = knotting on some of main roots

7 = majority of main roots knotted

8 = all main roots knotted; few clean roots visible

9 = all roots severely knotted; plant usually dying

10 = all roots severely knotted; no root

3.3 Identification of nematodes

Extracted nematode suspension was shaken and small batches of about 2 ml were sucked using a small pipette and transferred to a counting dish. The selected nematodes for identification were mounted on a drop of water on a microscopic slide and placed on a hot plate at 60^oC until nematode suddenly straightened out. Extracted nematodes were examined directly under a compound light microscope (Exacta – Optech Biostar B5P, Germany).

To allow for further identification and long term storage, nematodes were fixed. The Fixative, Formalin glycerol (4% Formalin with 1% glycerol) was heated to about 70°C and an excess (2-3 ml) was quickly added to the specimens to fix and kill the nematodes in one process (Seinhorst, 1966). Nematodes were then cleared with glycerol (Hooper, 1990) before being mounted on a slide and viewed under a compound light microscope. The nematodes were identified to the genus level based on their 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 mainly based on adult nematodes, but in the case of *Meloidogyne* spp., head morphology of adult males and tail shapes of second stage juveniles (J2) were used for identification to the species level.

3.3.1. Morphological observations and morphometric measurements on *Meloidogyne* spp.

Adult males and second stage juveniles (J2) were studied. Males were obtained from four communities (i.e. Afrancho, Asuosu, Tuobodom and Vea) and juveniles obtained from all nine communities selected from Ashanti, Brong Ahafo and Upper East regions of Ghana.

Picking and manipulation of the nematodes were done using a fine eyelash mounted on a steel needle (Fig. 3) unto the center of a clean glass slide. They were heat killed by adding a hot (70-75 ^{o}C) Formalin glycerol fixative on the glass slide, covered with a cover slip (Hooper *et al.*, 2005) and sealed using clear nail polish.

The specimens in prepared slides were marked on the cover slip for easier location while viewing under the compound light microscope. All slides were labeled with the sample number, number of specimen and the date of preparation using a fine tip permanent marker. The prepared slides were viewed under a binocular compound light microscope connected to a computer with Scope-Image-Professional Imaging software (Version 9.0) for processing and storing the images (Fig. 3).

The Scope-Image-Professional Imaging software was used to snap digital pictures from the slides of the nematode specimen at 100×, 200×, 400× and 1000× magnification to assist in *Meloidogyne* spp. identification and morphometric measurements of *M. incognita*.

The measurements obtained from the juveniles included the total body length, greatest body width, stylet length, tail length and the hyaline tail length. Male body length, greatest body width, DEGO, gubernaculum length, tail length and spicule length were also measured to assist with the species characterization.

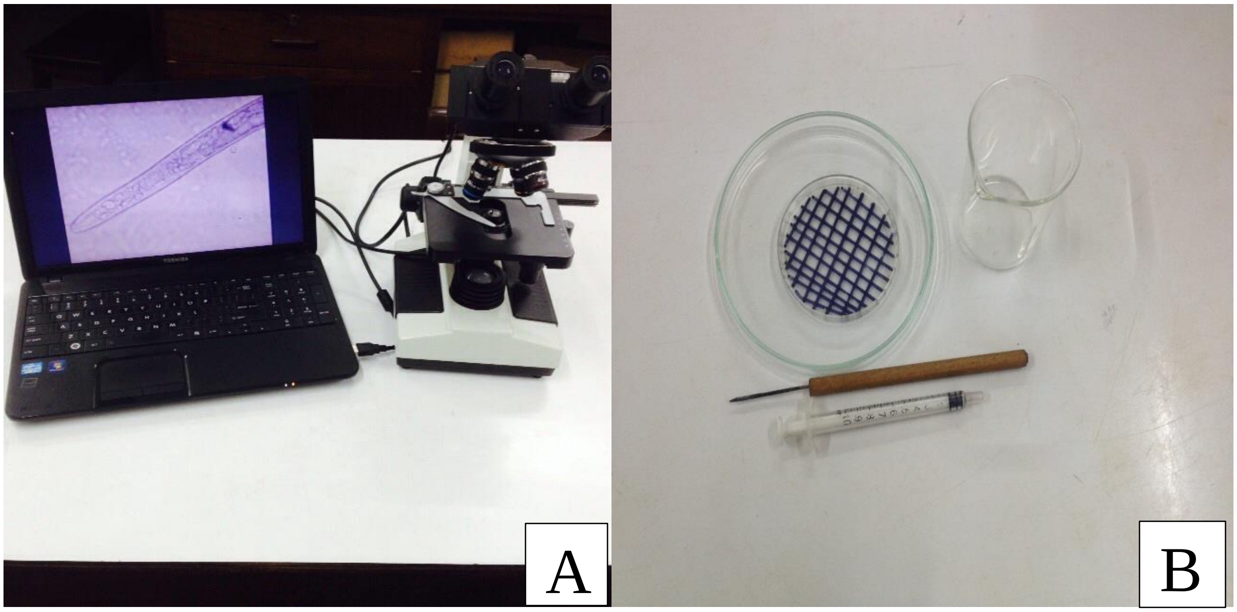

Fig. 3: Binocular compound light microscope connected to a computer with Scope-Image Professional Imaging software (A) and Tools used to handle nematodes (counting dish, syringe and picking bristles (B))

De Man's indices, a = (total body length /greatest body width) and c = (total body length /tail length) were calculated for both stages of development of *M. incognita*.

All measurements were recorded for each community, the data was summarized by calculating the averages, range and standard deviation. The morphometric measurements were compared with the species descriptions by Singh (2009) for *M. incognita*.

3.4 Data analysis

Data collected from the questionnaire survey were coded and subjected to descriptive statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0.

Regression analysis was performed on population densities of nematodes recovered from tomato roots and rhizosphere soils from the nine communities using General statistics (Genstat) version 12.0.

Principal component analysis of morphometric data of adult males and second stage juveniles of *M. incognita* was done using Excel Statistics (Xlstat) version 2015.

CHAPTER FOUR

4.0 RESULTS

4.1 Farmers' knowledge, perception and experience of plant parasitic nematodes on their tomato farms

4.1.1 General background of tomato farmers

Majority of the farmers (77.8%) from the three regions interviewed were males with females making up only 22.2% of respondents. Varied educational levels were observed among the respondents with most of them (51.9%), attaining primary education, some (44.4%) attaining secondary education and only (3.7%) attaining tertiary education (Table 2).

Table 2: Educational levels of tomato farmers in Ashanti, Brong Ahafo and Upper East Regions of Ghana

Educational level	**Percentage %**
Primary	51.9
Secondary (JHS/ SHS)	44.4
Tertiary	3.7
Total	**100**

4.1.2 Farmers land use intensity and crop production systems

Most of the farmers (63%) continually cropped their tomato fields for 4-6 years whiles others (22.2%), cultivated tomato on the same piece of land for 7-10 years. A few farmers (13%) cropped their land for 1-3 years and only 1.9% of them cropped theirs for more than 11 years (Fig. 4).

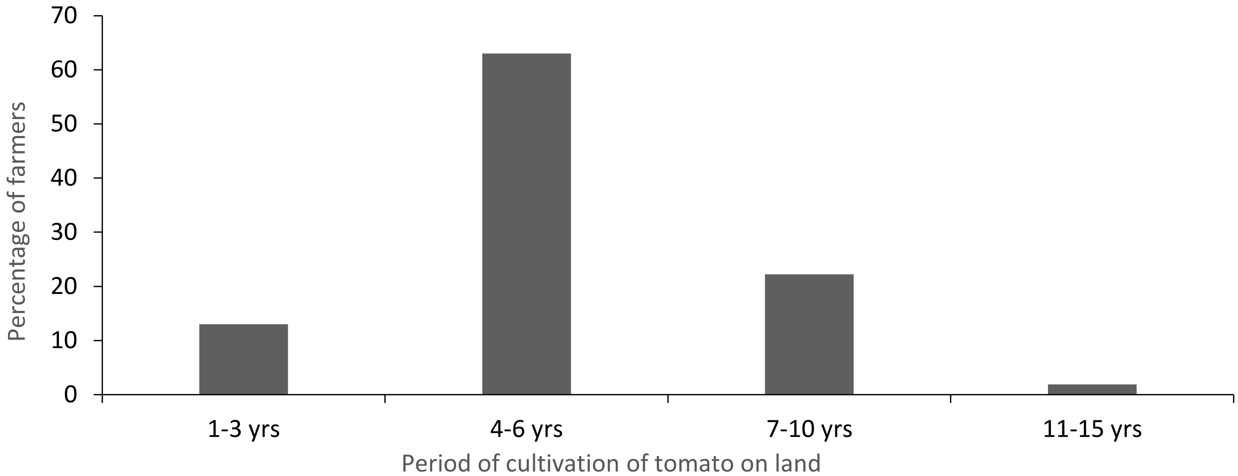

Fig. 4: Intensity of farmers' land use for tomato production in Ashanti, Brong Ahafo and Upper East regions of Ghana.

From the survey, it was also realized that 63% of the farmers in the study areas practiced crop rotation whiles the remaining 37% never rotated their tomato with any crop. 50% of those that practiced crop rotation, rotated with pepper, okra, maize and cassava while 35.3% rotated their tomato with only pepper.

Some of the farmers (45.7% and 40%) practiced crop rotation for less than a year and 1 year respectively and a few (14.3%) practiced it for 2 years. All the farmers interviewed (100%) applied inorganic fertilizers on their fields in the course of the growing season.

4.1.3 Farmers' source of planting material

Majority of the farmers (55.6%) across the three regions sourced their tomato seeds from certified agro dealers. Some (42.5%) used their own seeds from previous harvest whiles only 1.9% got theirs from friends and neighbours (Table 3).

Table 3: Main sources of tomato seeds used by farmers in Ashanti, Brong Ahafo and Upper East Regions of Ghana

Source of seeds	**Percentage (%)**
Agro dealers	55.6
Farmer extracted seeds	42.5
Friends / Neighbours	1.9
Total	**100**

4.1.4 Farmers' knowledge and perception on the occurrence and symptoms of nematode diseases

Most of the respondents (79.6%) did not know what plant-parasitic nematodes were and the rest (20.4%), however, had some knowledge about them.

Majority (72.6%) of those with knowledge about nematodes thought that they were below ground parasites, and 18.2% believed they were both below and above ground parasites. A few (9.1%) of them, however, thought they were only above ground parasites (Fig. 5).

Majority (88.7%) of the respondents could not differentiate among nematode infestation, nutrient deficiency, and moisture stress, others (11.3%), however, could differentiate among the various symptoms. About 84.1% of respondents indicated they observed galls, lesions on the tomato roots and stems (14.5), wilting (12.3) and chlorosis of leaves (9.5) (Table 4).

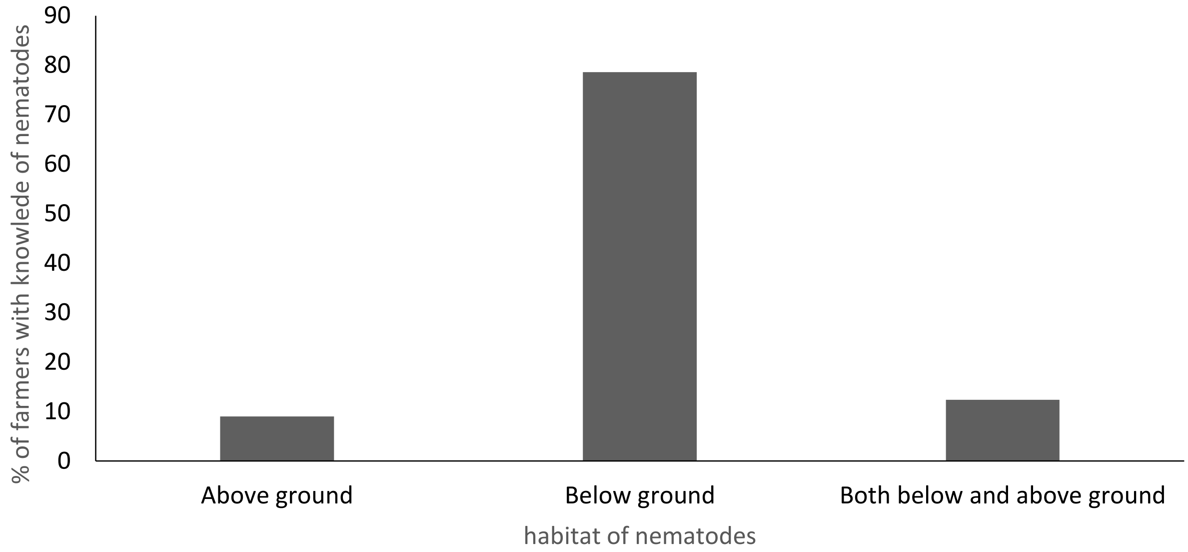

Fig. 5: Farmer's perception of where nematodes reside

Table 4: Symptoms of nematode infestation observed by tomato farmers on their farms in the Ashanti, Brong Ahafo and Upper East Regions of Ghana

Disease symptoms	Percentage of respondents (%)
Galls on the roots	84.1
Lesions on stem and roots	14.5
Wilting of leaves	12.3
Chlorosis	9.5
Poor fruit set	2.3
Small fruit size	2.3

Varieties of tomato mostly cultivated across the areas surveyed were 'Pectomech', 'Wososwoso' 'Powerano', 'local' and an unnamed cultivar. All the farmers (100%) indicated that all the varieties were susceptible to nematode infestation at varying levels. They however, could not tell which variety was more susceptible compared to the other.

Generally, most farmers (64.2%) indicated that nematode infestation occurred in periods where rainfall was high. Others (33.9%) linked nematode infestation to periods in the dry season. A few (1.9%) linked it to both dry and rainy periods within the growing season (Fig. 6).

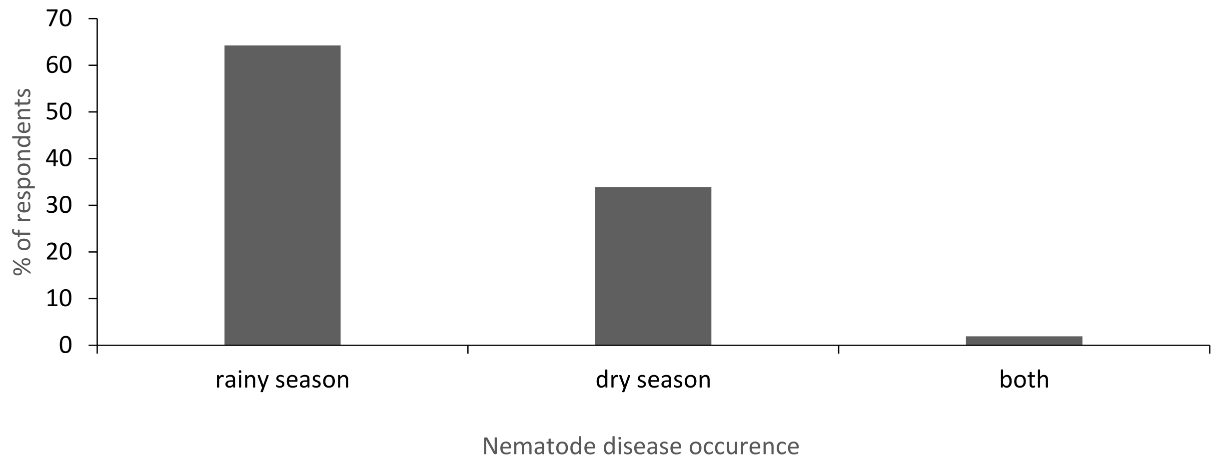

Fig. 6: Farmers perception of period of pronounced infestation of their farms by nematodes

Furthermore, majority of farmers (73.6%) perceived that these symptoms were most pronounced in the vegetative and fruiting stages, while others (11.3%) thought them to be most pronounced in the vegetative stage (Fig 7).

4.1.5 Farmers' knowledge and perception on the spread and control of nematodes of tomato

Tomato farmers in the three regions had different perceptions on the mode of spread of nematodes within and among farms. A high proportion of the farmers (45.3%) had no idea as to how they were spread within and among farms, while 21.6% attributed their spread to rain water. A few (20.6%) thought the spread of nematodes was associated with the planting of infected seeds, whiles 12.5% perceived nematode spread to be soil borne (Fig. 8).

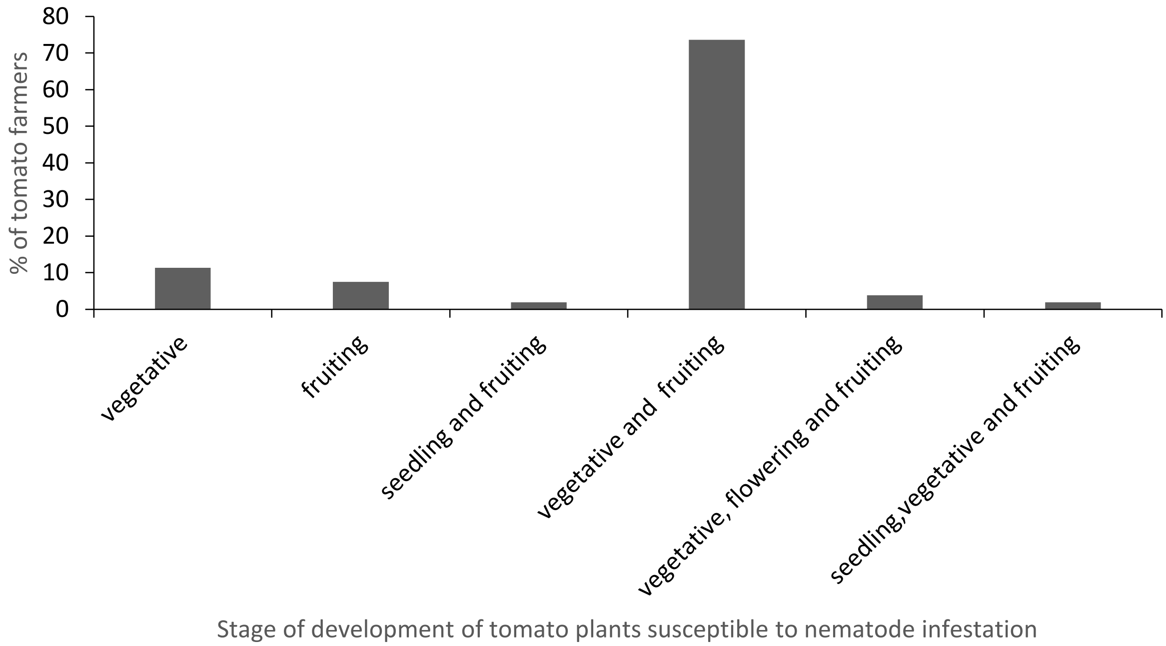

Fig. 7: Farmers perception of stage of development of tomato where symptoms of nematode infestation pronounced

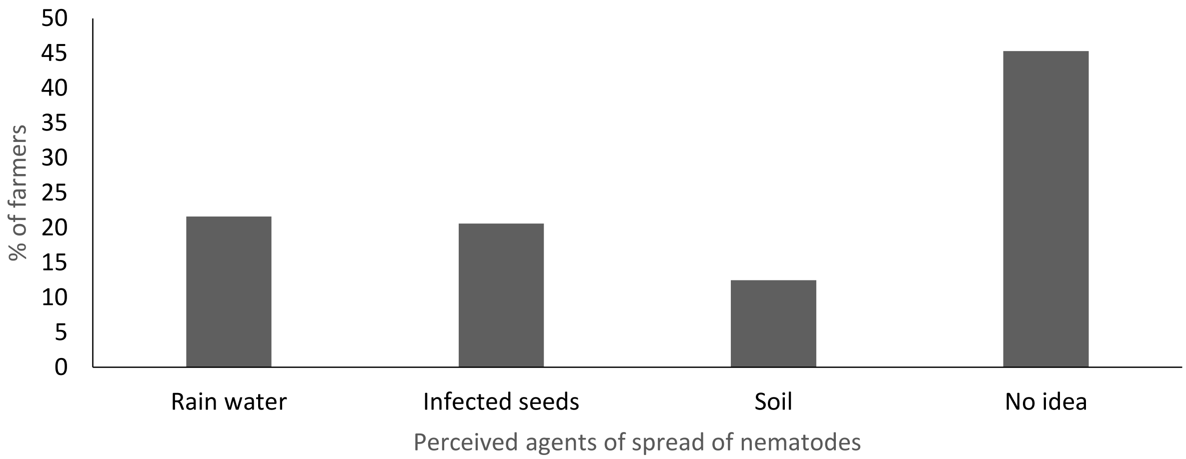

Fig. 8: Perception of farmers on agents of spread of nematodes in tomato fields

Majority (66%) of farmers in the eight districts indicated that nematodes could not be controlled while a few (34%) of them believed that they were controllable. Out of those that thought they were controllable, 74.5% indicated that application of appropriate chemicals could control them About 9.8% of the respondents had no idea of any control measure, although they believed the disease could be controlled, 8.9% perceived use of healthy seeds as the most effective nematode control measure, and 6.8% believed land fallowing would control nematodes (Fig. 9).

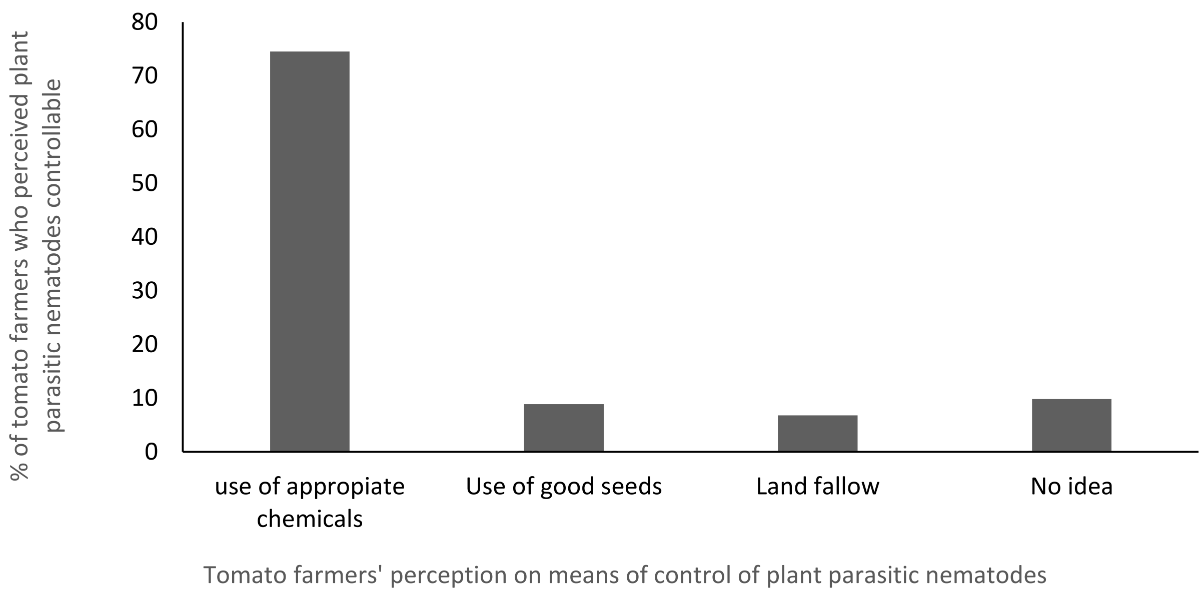

Fig. 9: Farmer’s perception of means of nematode control in tomato fields in Ashanti, Brong Ahafo and Upper East Regions of Ghana.

4.1.6 Knowledge and experience of farmers on yield and economic impact of nematodes on livelihoods of farmers

All the tomato farmers (100%) in the three regions interviewed engaged in tomato cultivation as an income generating venture. Most of the farmers (32.7%) estimated to harvest tomato ranging from 61-80 crates* per hectare per season whiles only 3.7% estimated to harvest between 30-40 crates per hectare per season (Table 5).

Table 5: Farmers estimated yield of tomato per hectare in Ashanti, Brong Ahafo and Upper East regions of Ghana

Expected yield (crates /ha/ annum)	**Percentage of farmers**
30-40	3.7
41-50	13.9
51-60	6.8
61-80	32.7
81-100	20.7
101-150	17.1
151-200	2.8
201-300	2.3
Total	**100**

*1 crate = 120kg (Robinson and Kolavalli, 2010).

Most of the farmers (34.5%) however, realized yields of 41-60 crates per hectare per season (Table 6). The highest yield (151- 200) was realized by only 3.4% of the farmers and the lowest (less than 20 crates) realized by 17.5% of farmers

Majority (63.7%) of the respondents attributed their yield losses to high incidence of tomato diseases. The rest ascribed theirs to excessive drought (15.8%), excessive rainfall (6.2%), poor market (2.7%) and 0.9% assigned their yield losses to poor land preparation (Table 7).

Table 6: Actual yields of tomato realized by farmers in Ashanti, Brong Ahafo and Upper East Regions of Ghana

Actual yields (crates/ ha/ annum)	**Percentage of farmers**
less than 20	17.5
21-40	9.2
41-60	34.5
61-80	5.9
81-100	20.4
101-150	9.1
151-200	3.4
Total	**100**

Table 7: Farmers reasons for not achieving expected yields of tomato in the study areas

Reasons	**Percentage of farmers**
Tomato diseases	68.5
Excessive drought	12.4
Excessive rainfall	6.9
Poor market	8.7
Poor land preparation	3.5
Total	**100**

4.2 Plant parasitic nematodes associated with tomato in Ashanti, Brong Ahafo and Upper East Regions of Ghana

4.2.1 Genera of plant parasitic nematodes in nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana

Eight (8) genera belonging to five families of order Tylenchida and Dorylaimida were consistently extracted and identified from tomato roots and rhizosphere soil of farmers' fields in nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana. The genera were: *Helicotylenchulus* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchulus* spp., *Scutellonema* spp., *Tylenchus* spp. and *Xiphinema* spp.

Their identification were based on the following:

Helicotylenchus spp.

Small to medium sized (0.4-1.2mm), vermiform, usually spiral, rarely arcuate when killed with gentle heat. Lip region continuous to slightly offset, rounded, or anteriorly flattened (truncate) with or without annulation, longitudinal striations on annules absent. Lateral field with four incisures, areolated usually in anterior part of body only. Labial framework moderately to strongly sclerotized. Stylet well developed, with rounded or anteriorly indented knobs. Orifice of dorsal esophageal gland one fourth or more of stylet length behind stylet base (Fig. 10)

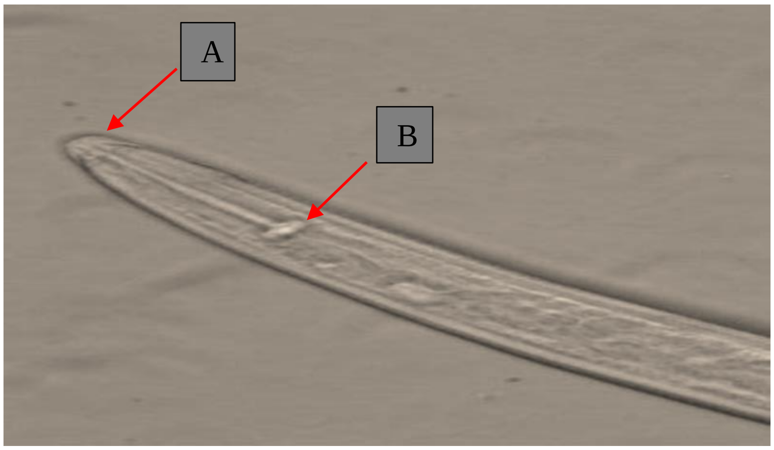

Fig. 10: Micrograph of the head region of *Helicotylenchus* spp. showing a continuous to slightly offset and rounded lip region (A) with moderately indented stylet knobs (B)

Hoplolaimus spp.

Lip region high offset, with prominent transverse and longitudinal striae cephalic framework massive, basal divided squares. Stylet is also massive (31-61 µm), with compact tulip- shaped basal knobs bearing anterior tooth- like projections (Fig. 11)

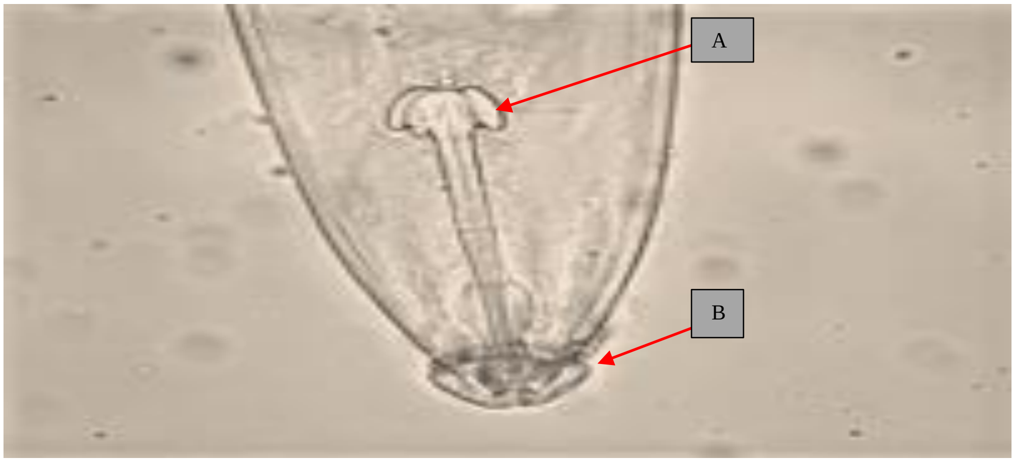

Fig. 11: Micrograph of the head region of *Hoplolaimus* spp. showing a compact tulip- shaped basal knobs (A) and a high offset lip region (B)

Pratylenchus spp.

Small nematodes (<1 mm long) slightly curved ventrally on application of gentle heat when fixing. Labial region strongly sclerotized, low, flattened, usually appearing as a dark, flat cap under the stereomicroscope (Fig. 12).

Rotylenchulus spp.

Juveniles, males and young females vermiform, arcuate to spiral upon relaxation. Stylet in juveniles and female two to three times cephalic region width long. Orifice of dorsal oesophageal gland usually about one stylet length behind stylet base. Juvenile tail more rounded terminally and with shorter hyaline terminal portion than that of a female.

Fig. 12: Micrograph of *Pratylenchus* spp. with strongly sclerotized lip region appearing as a dark, flat cap (red arrow)

Scutellonema spp.

Small to medium-sized nematodes (0.3–1.5 mm) usually dying in a C-shape or open spiral. Labial region with moderate sclerotization. Stylet of medium development with rounded knobs. Oesophagus with dorsal overlap (Fig. 13)

Tylenchus spp.

Tails of both sexes elongate, conoid to filiform.

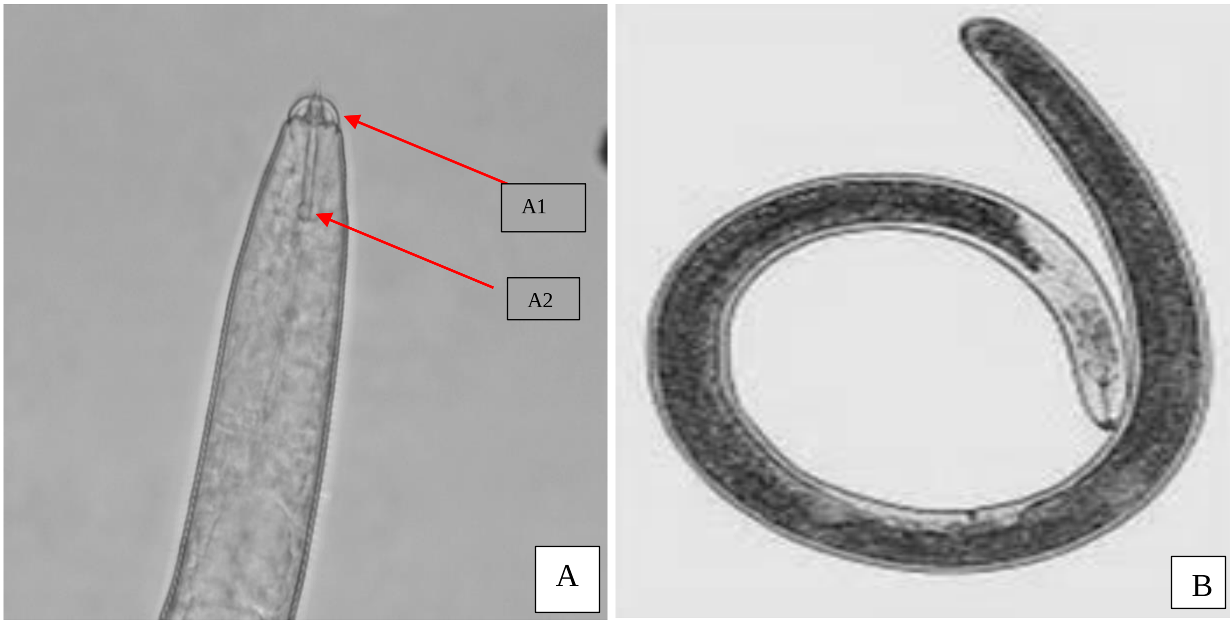

Fig. 13: Micrographs of *Scutellonema* spp. with a labial region of moderate sclerotization, (A1) and rounded stylet knobs (A2) (A) and spiral shape of a fixed nematode (B)

Xiphinema spp.

Body length medium to long (1.2- 3.0 mm); Lip region rarely continuous, usually demarcated by a shallow depression or well offset. Tail is short, conoid, rounded to slightly digitate and rarely broadly rounded. Tail terminus generally pointed or rounded (Fig. 14)

Fig. 14: Micrographs of *Xiphinema* spp. with a short, conoid pointed tail (red arrow) (A) and a head region with a shallow depressed lip region (red arrow) (B)

Meloidogyne spp.

Adult males and second stage juveniles of *Meloidogyne* spp. were identified to the species level based on the following features:

Meloidogyne incognita

Adult males are characterized by a large, round labial disc, raised above of the median lips. The labial disc is concave to flat, and the high head cap is nearly as wide as the head region in lateral view. The head region is usually marked by 2-5 annulations but may be completely smooth and is not offset from the rest of the body (Fig. 15). Tails of second stage juveniles have a hyaline terminus which is broadly rounded (Fig. 16)

Fig. 15: Micrograph of the head region of an adult male *M. incognita* showing a labial disc, raised above of the median lips (red arrow)

Fig. 16: Micrograph of second stage juvenile *M. incognita* showing a broadly rounded tail (red arrow)

Meloidogyne javanica

Adult males are characterized by a high, rounded head cap, distinctly set off from the head region. The labial disc and medial lips are fused and form one smooth, continuous structure which is almost as wide as the head region in lateral view. The head region may be smooth or marked by 2-3 incomplete head annulations. The head region is not distinctly set off from the rest of the body. Tails of second stage juveniles have a narrow tapering tail terminus ending in finely rounded tip; there are often cuticular constrictions mid-way along the hyaline terminus (Fig. 17)

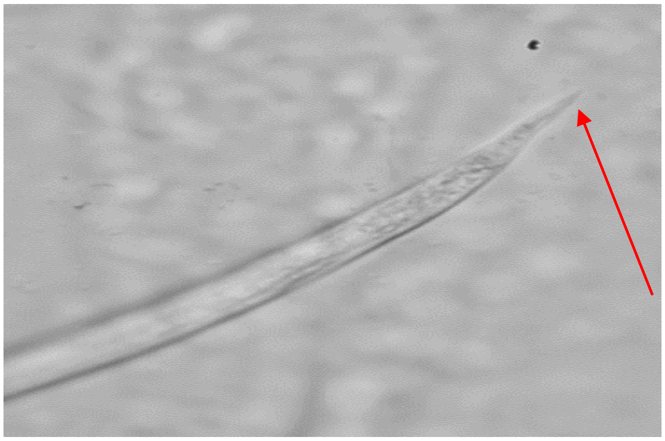

Fig. 17: Micrograph of tail region of second stage juvenile of *M. javanica* which had a finely rounded tip

Meloidogyne arenaria or M. hapla

Adult males are characterized by a high and narrow head cap and set-off head region. The labial disc and median lips are fused and form one smooth, continuous structure that is much narrower than the head region. The head region is smooth and distinctly set off from the body annulations. The body annules increase in width and diameter posteriorly (Fig. 18)

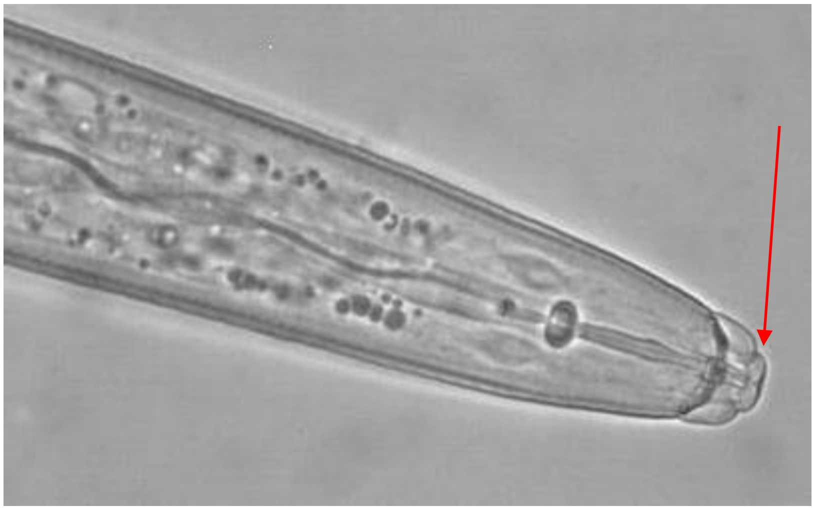

Fig. 18: Micrograph of adult male *M. arenaria* or *M. hapla* with a much narrower head cap (red arrow)

Seventy eight percent (87.5%) of the nematode genera identified belonged to the Order Tylenchida, while 12.5% belonged to the Order Dolylaimida. Family Hoplolaimidae had the highest representative with four genera out of the eight encountered (Table 8).

Meloidogyne spp, *Pratylenchus* spp, *Rotylenchulus* spp *and Scutellonema* spp were present in both tomato roots and rhizosphere soils

4.2.2 Diversity and density of plant parasitic nematodes

4.2.2.1 Soil

580 nematodes were identified from soil samples collected from the nine communities in the Ashanti, Brong Ahafo and Upper East Regions of Ghana. Upper East and Brong Ahafo Regions had all eight genera of nematodes identified; however, Upper East Region recorded the least total nematode density (91) while Ashanti Region had the greatest nematode density (258)

Table 8: Genera of plant parasitic nematodes extracted from soils and roots of tomato in the Ashanti, Brong Ahafo and Upper East Regions of Ghana and their percentage abundance

Order	**% of occurrence**	**Suborder**	**Family**	**Genus**
Tylenchida	87.5	Tylenchina	Pratylenchidae	*Pratylenchus* spp.
			Hoplolaimidae	*Hoplolaimus* spp.
				Helicotylenchus spp.
				Rotylenchulus spp.
				Scutellonema spp.
			Meloidogynidae	*Meloidogyne* spp.
			Tylenchulidae	*Tylenchus* spp.
Dorylaimida	12.5	Dorylaimina	Longidoridae	*Xiphinema* spp.

Seven genera were encountered in Ashanti Region with the exception of *Hoplolaimus* spp. Population densities of *Helicotylenchus spp.*, *Meloidogyne* spp., *Pratylenchus* spp., and *Rotylenchulus* spp. from Upper East Region were significantly lower than population densities of these genera in soils from Ashanti and Brong Ahafo Regions (Fig 19)

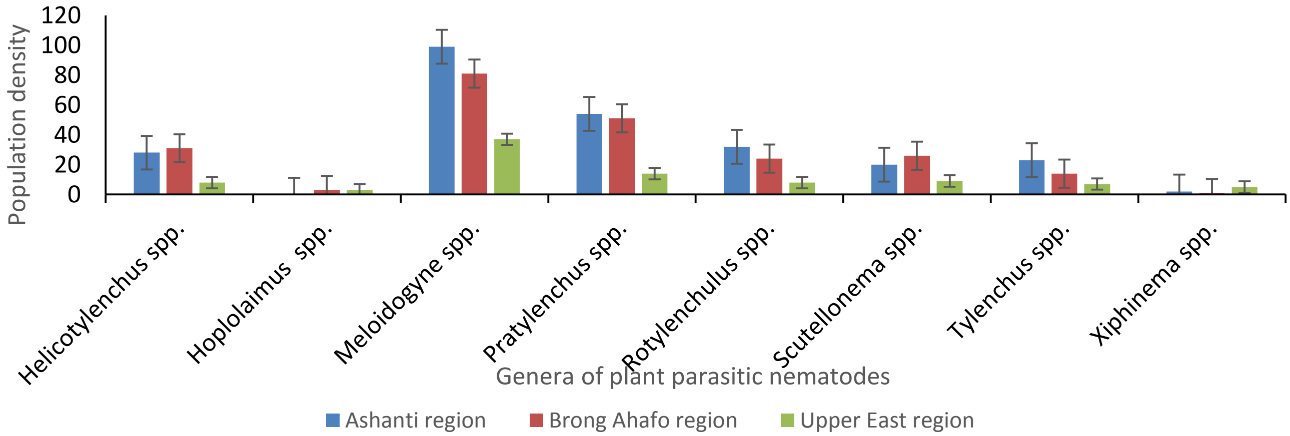

Fig. 19: Population densities of genera of plant parasitic nematode extracted in 200 cm^3 soil from Ashanti, Brong Ahafo and Upper East Regions of Ghana

Afrancho had the highest densities of *Helicotylenchus* spp. (20), *Rotylenchulus reniformis* (16) and *Scutellonema* spp. (11) and *Tylenchus* spp. (19) while Akumadan had the highest density of *Meloidogyne* spp. (45) (Table 9).

4.2.2.2 Roots

A total of 205 nematodes were identified from root samples collected from the nine communities in the Ashanti, Brong Ahafo and Upper East Regions of Ghana. Ashanti and Brong Ahafo Regions had all four genera of nematodes identified. Upper East Region recorded the least total nematode density (41) while Brong Ahafo Region had the greatest (94) Population density of *Meloidogyne* spp. recovered in 10 g of tomato roots from farmers' fields in Brong Ahafo Region was significantly higher than densities of the genus in roots from Ashanti and Upper East Regions (Fig. 20)

Table 9: Diversity and density of genera of plant parasitic nematodes (200cm^3 tomato soil) from nine communities in Ashanti, Brong Ahafo and Upper East Region of Ghana

Community	*Meloidogyne* spp.	*Pratylenchus* spp.	*Helicotylenchus* spp.	*Rotylenchulus* spp.	*Scutellonema* spp.	*Tylenchus* spp.	*Xiphinema* spp.
Afrancho	39	16	20	16	11	19	2
Akumadan	45	11	5	11	3	3	0
Asuosu	15	6	3	3	6	1	0
Tuobodom	40	22	10	10	7	6	0
Techimantia	16	9	8	4	8	2	1
Tanoso	25	22	13	10	7	6	0
Vea	10	3	3	2	4	3	2
Pwalugu	12	2	4	4	2	3	1
Tono	15	9	1	2	3	1	2

Figures are counts of nematode genera from corresponding community

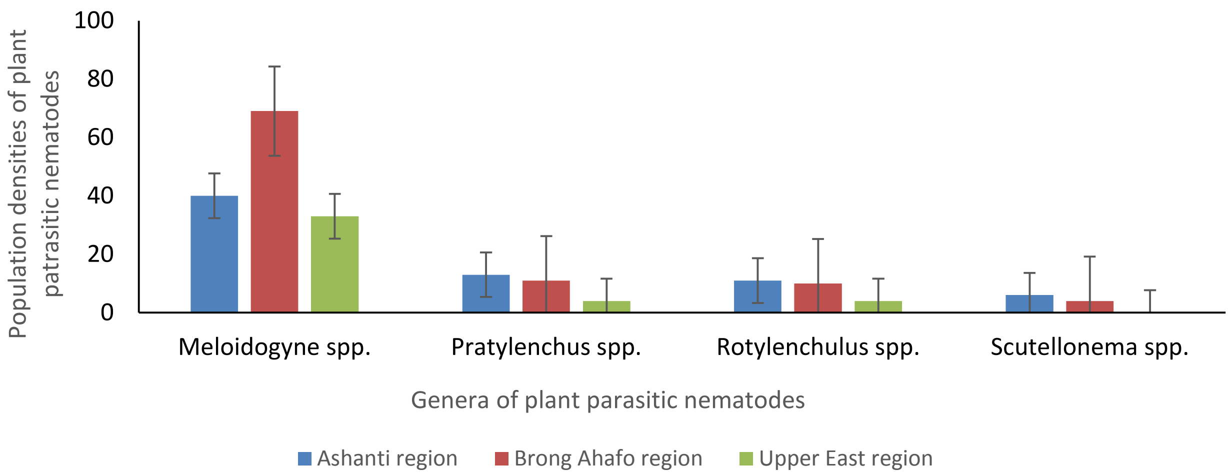

Fig. 20: Population densities of genera of plant parasitic nematode extracted in 10 g tomato roots from Ashanti, Brong Ahafo and Upper East Regions of Ghana

Afrancho had the highest nematode densities of *Pratylenchus* spp. (7), *Rotylenchulus* spp. (6) and *Scutellonema* spp. (4) while Tuobodom and Tachimantia had the highest density of *Meloidogyne* spp. (28). Only *Meloidogyne* spp. (10) were retrieved from tomato roots from Vea (Table 10)..

Table 10: Diversity and density of plant parasitic nematodes (10 g tomato roots) from nine communities in Ashanti, Brong Ahafo and Upper East Region of Ghana

Community	*Meloidogyne* spp.	*Pratylenchus* spp.	*Rotylenchulus* spp.	*Scutellonema* spp.
Afrancho	17	7	6	4
Akumadan	15	3	3	1
Asuosu	8	3	2	1
Tuobodom	28	5	6	3
Techimantia	28	3	0	0
Tanoso	13	3	4	1
Vea	10	0	0	0
Pwalugu	12	1	3	0
Tono	11	2	1	0

Figures are counts of nematode genera from corresponding community

4.2.3 Frequency of occurrence and relative abundance of plant parasitic nematodes

4.2.3.1 Soil

Plant parasitic nematodes were detected in nearly every sample but at varying frequencies of occurrence. *Helicotylenchulus* spp, *Melodogyne* spp. and *Rotylenchulus* spp. were present in the soils of all the farms sampled. *Xiphinema* spp. was present in the soils of only five of the eighteen farms sampled. *Meloidogyne* spp. was the most abundant nematode genus (relative abundance of 37.4%), *Pratylenchus* spp. had a relative abundance of 20.5%. *Scutellonema* spp. and *Tylenchus* spp. had lower relative abundance although they were found in the soils of all communities (Table 11).

Table 11: Frequency of occurrence of plant parasitic nematodes (18 farms) and their relative abundance (200 cm^3 soil) from nine communities in Ashanti Brong Ahafo and Upper East Regions of Ghana

Nematode genus	Frequency of occurrence	Relative abundance (%)
Helicotylenchus spp.	18[a]	11.55[b]
Hoplolaimus spp.	8	1.03
Meloidogyne spp.	18	37.41
Pratylenchus spp.	16	20.52
Rotylenchulus spp.	18	11.03
Scutellonema spp.	14	9.48
Tylenchus spp.	10	7.59
Xiphinema spp.	5	1.38

[a] Figures represent number of farms where corresponding genera of nematodes were recovered.
[b] Figures represent relative abundance (%) of each nematode genus given as ($\frac{n}{N} x\, 100$). n= the number of individuals of nematode genus, N= the total number of nematodes identified and counted from soil.

4.2.3.2 Roots

Melodogyne spp. was present in tomato roots from all the farms sampled while *Scutellonema* spp. was present in tomato roots of only five of the eighteen farms sampled. *Meloidogyne* spp.

was the most abundant nematode genus (69.3) encountered while *Scutellonema* spp. had the lowest relative abundance (4.9%) (Table 12).

Table 12: Frequency of occurrence of plant parasitic nematodes (18 farms) and their relative abundance recovered from 10 g tomato roots from nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana

Nematode genus	Frequency of occurrence	Relative abundance (%)
Meloidogyne spp.	18[a]	69.27[b]
Pratylenchus spp.	14	13.66
Rotylenchulus spp.	12	12.2
Scutellonema spp.	5	4.88

[a] Figures represent number of farms where corresponding genera of nematodes were recovered.
[b] Figures represent relative abundance (%) of each nematode genus given as ($\frac{n}{N} x\ 100$). n= the number of individuals of nematode genus, N= the total number of nematodes identified and counted from soil.

4.2.4 Regression analysis on population density of plant-parasitic nematodes in soil and tomato root samples

There was a significant ($p<0.05$) relationship between the population of plant parasitic nematode in the soil and those obtained from the roots with $R^2 = 0.55$. The analysis revealed a positive relationship (y = 2.53x + 6. 17) between them. The mean change in the population density of plant parasitic nematodes in 200 cm^3 of rhizosphere soil for a unit change in their population density in 10 g of tomato roots was about 3 nematodes (Fig.21).

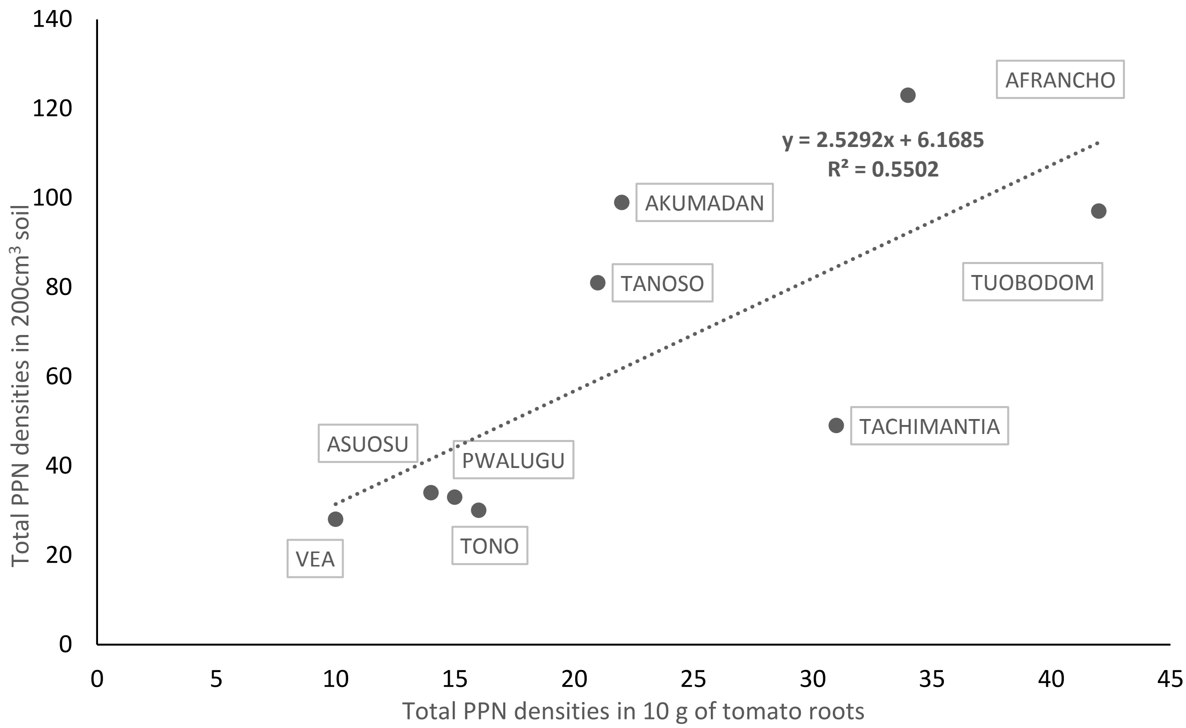

Fig. 21: Relationship between plant parasitic nematodes recovered from 200 cm^3 of soil from root rhizosphere and 10 g of tomato roots from nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana

4.2.5 Root gall index (RGI) tomato roots

Tomato roots from Tuobodom and Vea were the most and least galled respectively (Fig. 22; Table13)

Fig. 22: Diseased tomato roots from Tuobodom showing galls (red arrows) (A) and healthy tomato roots from Vea (B).

Table 13: Tomato root galling indices of the mean of three tomato roots each from nine communities in Ashanti, Brong Ahafo and UpperEast Regions of Ghana

Community	**Root Knot galling index**
Afrancho	6.0
Akumadan	6.3
Asuosu	5.0
Tuobodom	7.0
Techimantia	5.3
Tanoso	5.6
Vea	2.0
Pwalugu	6.3
Tono	2.3

4.3 Percentage occurrence (%) and morphometric measurements of *Meloidogyne* species

4.3.1 Percentage occurrence (%) of *Meloidogyne* species.

The results of morphological examinations carried out on populations of male *Meloidogyne* spp. from four communities showed that *M. incognita* (59 %), *M. javanica* (27%) and *M. hapla* or *M. arenaria*.(8%) were all identified (Fig. 23). Additionally, some 6% of the populations showed features which were not specific to any species.

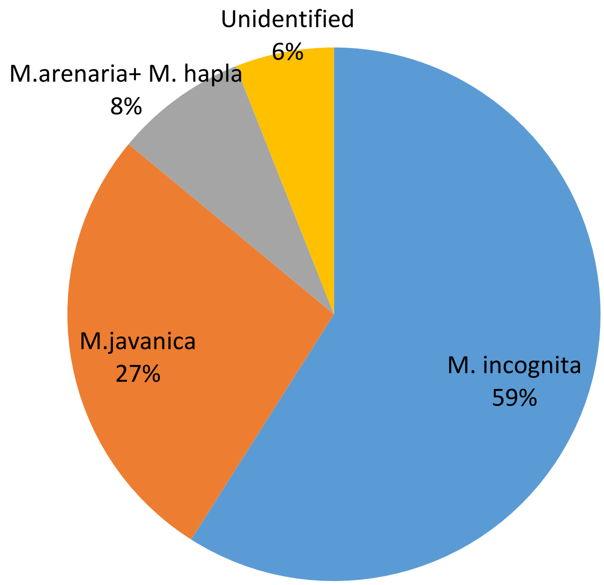

Fig. 23: Composition of adult males of *Meloidogyne* spp. from four communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana

Morphological examinations carried out on populations of second stage juveniles (J2) of *Meloidogyne* spp. from nine communities showed that *M. incognita* (75%) and *M. javanica* (20%) were identified. 5% of the populations showing characteristics not specific to any species (Fig. 24).

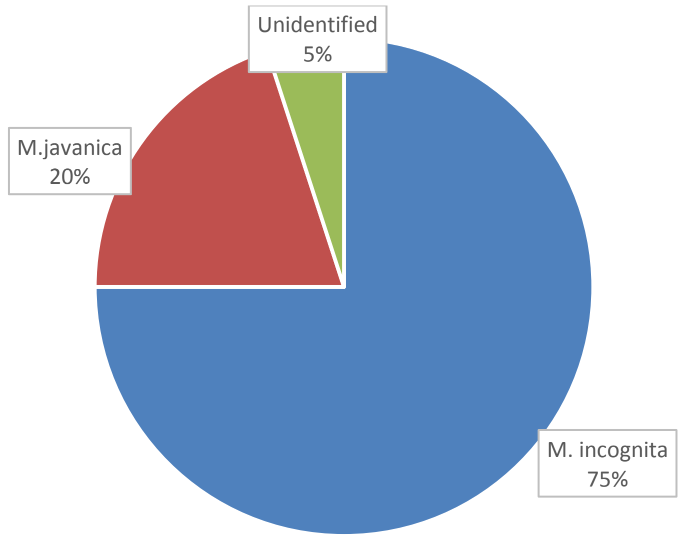

Fig. 24: Composition of second stage juveniles of *Meloidogyne* spp. from nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana

4.3.2 Morphometric characteristics of *Meloidogyne incognita*

4.3.2.1 Adult males

Results obtained when morphometric measurements of adult males of *Meloidogyne incognita* from four communities in Ashanti, Brong Ahafo and Upper East Regions were undertaken and the best differentiating characters (i.e. body length, greatest body width, DEGO, gubernaculum length, stylet length, tail length, and spicule length) were compared to morphometric measurements of adult males of *Meloidogyne incognita* reported by Singh (2009) are represented in Table 14.

Table 14: Morphometric measurements of male *Meloidogyne incognita* (n=4) from tomato fields of four communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana compared to those reported by Singh (2009)

Body characters	**Vea**	**Asuosu**	**Afrancho**	**Tuobodom**	**Mean**	**Singh, 2009**
Body length	1115.47 ± 3.02 (1113.52-1120.31)	1136.42 ± 3.92 (1132.28- 1141.45)	1157.63 ±1.26 (1156.85- 1196.86)	1115.60 ± 0.60 (1114.83- 1116.92)	1131.28	1749.5
Greatest body width	32.3± 0.92 (32.15-32.42)	36.44±1.23 (34.58-37.28)	36.19±1.12 (34.57-36.85)	32.88± 0.99 (21.37-33.78)	34.45	24.1
DEGO	3.7±0.21 (3.43-3.92)	3.75±0.12 (3.69-3.82)	3.74±0.18 (3.68-3.86)	3.58±0.21 (3.68-3.84)	3.96	2.9
Gubernaculum length	9.91±0.04 (9.71-10.04)	9.85±0.37 (9.68-9.99)	10.84±0.04 (9.86-11.02)	10.19±0.05 (9.88-11.25)	10.20	9.3
Stylet length	25.58±0.54 (23.54-26.75)	25.05±0.56 (24.48-26.54)	26.05±0.48 (25.77-27.53)	25.15±0.53 (24.43-26.82)	25.46	24.1
Tail length	11.19±0.11 (10.86-11.86)	11.18±0.12 (10.5-11.53)	11.16±0.14 (10.58-11.88)	11.09±0.12 (10.64-11.92)	11.16	-
Spicule length	28.65±0.55 (27.11-28.93)	28.42±0.43 (27.43-28.78)	30.21±0.65 (28.15-31.24)	29.76±0.54 (28.72-30.27)	29.26	28.5
‘a’	34.53±0.17 (33.72-35.75)	31.18±0.18 (30.99-32.07)	31.98±0.37 (30.59-33.27)	33.92±0.52 (31.27-34.42)	32.93	
‘c’	99.68±1.09 (97.82-100.12)	101.64±1.27 (99.83-102.45)	103.76±1.31 (101.28-104.75)	100.59±1.11 (99.73-104.45)	101.54	

Values are in µm in the form mean ± standard deviation (range)

4.3.2.2 Second stage juveniles

Results obtained when morphometric measurements of second stage juveniles of Meloidogyne incognita from nine communities in Ashanti, Brong Ahafo and Upper East Regions were undertaken and the best differentiating characters (i.e. body length, greatest body width, stylet length, tail length, and length of hyaline portion of tail) were compared to morphometric measurements of second stage juveniles of *Meloidogyne incognita* reported by Singh (2009) are presented in Table 15.

Table 15: Morphometric measurements of second stage juveniles of *Meloidogyne incognita* (n=10) from tomato fields of nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana compared to those reported by Singh (2009)

Body characters	**Vea**	**Pwalugu**	**Tono**	**Akumadan**	**Afrancho**
Body length	379.87±38.24 (320.01-426.43)	402.16±24.24 (334.27-425.73)	407.05± 15.67 (382.75-432.27	405.91±14.27 (392.42-410.48)	396.33±15.57 (347-423.75)
Stylet length	11.64±1.06 (11.53-11.79	12.32±1.16 (10.32-12.48)	11.75±2.6 (10.58-12.63)	11.83± 1.82 (10.64-12.83)	12.28±1.93 (11.01-13.02)
Tail length	47.10±1.65 (39.58-49.32)	46.86±1.54 (38.58-49.42)	46.71±1.63 (38.98-49.42)	46.39±1.45 (39.68-48.32)	45.73±1.34 (37.58-49.72)
Hyaline tail length	11.65±0.96 (10.32-12.98)	12.61±0.95 (10.94-13.88)	12.22±0.91 (10.44-13.48)	12.27± 0.93 (10.54-13.98)	12.50±0.96 (11.34-13.98)
Greatest body width	15.33±0.12 (14.82- 15.95)	15.02±0.56 (14.82-16.73	15.02±0.46 (14.92-16.73)	15.12± 0.12 (14.82- 15.95)	14.94±0.53 (14.02-15.73)
‘a’	24.79±1.06 (24.58-25.04)	26.77±2.32 (25.93-26.99)	27.11±2.58 (26.27-27.86)	26.84±2.32 (25.93-27.19)	26.54±2.58 (26.47-27.96)
‘c’	8.06±0.95 (7.98-8.99)	8.58±0.89 (7.71-8.95)	8.71±0.97 (8.12-9.18)	8.75±0.87 (8.21-8.95)	8.67±0.89 (8.01-9.05)

Table 15 cont: Morphometric measurements of second stage juveniles of *Meloidogyne incognita* (n=10) from tomato fields of nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana compared to those reported by Singh (2009)

Body characters	**Asuosu**	**Tachimantia**	**Tuobodom**	**Tanoso**	**Mean**	**Singh, 2009**
Body length	414.08±16.84 (375.48-430.95)	415.58±15.34 (390.47-440.27)	417.26± 16.75 (399.57-420.18)	411.65±28.17 (326.17-434.78)	405.54	359.3
Stylet length	12.46±1.33 (11.01-13.01)	12.91 ±1.45 (10.27-13.05)	13.23±1.32 (12.06-13.37)	12.77± 0.79 (11.23-13.10	12.36	10.6
Tail length	46.79±1.65 (39.78-47.32)	46.99±1.48 (38.55-49.42)	46.79±1.65 (37.58-49.62)	46.70±1.49 (38.58-48.32)	46.67	48.8
Hyaline tail length	12.88±0.96 (11.54-13.98)	13.11± 0.97 (11.64-14.98)	13.34±0.92 (10.34-14.99)	12.49±1.06 (11.34-12.78)	12.56	10.7
Greatest body width	15.21±0.46 (14.82-16.69	15.30± 0.42 (13.82- 15.95)	15.33±0.58 (14.72-16.63)	15.27±0.15 (14.62- 16.15)	15.17	-
‘a’	27.22±2.68 (26.27-27.88)	27.17±2.08 (26.77-28.56)	27.23±2.32 (25.93-28.79)	26.97±2.42 (25.93-26.99)		
‘c’	8.85±0.95 (7.98-9.09)	8.84±1.02 (8.01-9.21)	8.92±0.98 (7.98-9.11)	8.81±0.87 (8.21-8.95)		

Values are in µm in the form mean ± standard deviation (range)

Adult males of *M. incognita* from Afrancho population had the largest values of body length, gubernaculum length, stylet length, tail length and spicule length. Asuosu populations had the highest values of greatest body width and DEGO while recording the lowest values in gubernaculum length, stylet length and spicule length.

The mean body length of male *M. incognita* reported by Singh (2009) was larger than the mean body length of male *M. incognita* of all four populations. The mean greatest body width, DEGO, gubernaculum length, stylet length and spicule length of male *M. incognita* of all four populations were however larger than those reported by Singh (2009). Tail length, 'a' and 'c' were not reported by Singh (2009) and therefore not compared to those from the four communities.

4.3.3 Principal component analysis (PCA) of *Meloidogyne incognita*

4.3.3.1 Adult males

The first two principal components accounted for 61.09% of the total variance for male *M. incognita* in the various populations (Table 16). Seven eigenvalues were noted for male *Meloidogyne incognita* populations. High variability was observed for Factors (F1 and F2) compared to the other factors. The cumulative variability for F1 and F2 were 40.0% and 61% respectively F1 is dominated by high positive eigenvectors (> 0.40) for total body length, greatest body width and gubernaculum length., small positive eigenvectors (between 0 and 0.40) in stylet length DEGO, spicule length and 'c' and a negative eigenvectors (< 0) for tail length and 'a'

Table 16: Eigenvectors and eigenvalues of principal component analysis derived from morphometric characters of male *Meloidogyne incognita* recovered from four communities in Ashanti, Brong Ahafo and Upper East Region of Ghana

Body characters	**F1**	**F2**
Body length	0.414	0.334
Greatest body width	0.416	0.404
DEGO	0.018	0.201
Gubernaculum length	0.407	-0.215
Stylet length	0.259	-0.223
Tail length	-0.240	0.511
Spicule length	0.257	-0.194
‘a’	-0.377	-0.400
‘c’	0.396	-0.359
Eigenvalue	3.60	1.89
Variability (%)	40.00	21.09
Cumulative %	40.00	61.09

F2 is dominated by negative eigenvectors for stylet length, spicule length, gubernaculum length, ‘a’ and ‘c’, small positive eigenvectors for total body length and DEGO and a high positive eigenvectors (> 0.40) for tail length and greatest body width.

There was a strong positive correlation between body length and greatest body width (r = 0.79), gubernaculum length and ‘c’ (r = 0.57), spicule length and gubernaculum length (r = 0.55), and body length and gubernaculum length (r = 0.54) in decreasing order of strength of correlation There was also a strong negative correlation between greatest body width and ‘a’ (r = 0.98), tail length and ‘c’ (r = -0.92) and body length and ‘c’ (-0.66) also in decreasing order of strength (Table 17).

Biplots (F1 and F2) for male *M. incognita* populations revealed specific groupings (Fig. 25). *Meloidogyne incognita* males from Vea grouped to the lower left corner, and the upper right corner was dominated by *M. incognita* males from Asuosu and Afrancho. The greatest body width, body length, and DEGO contributed to the clustering of the Asuosu and Afrancho populations. The ratio ‘a’ separated the Vea population from the others.

From the PCA, the variables which contributed most to the total variation of morphometric characters of adult males of *M. incognita* from the four communities were greatest body width (16.81 %), tail length (15.9%) and the body length (14.15%).

Table 17: Correlation matrix of morphometric variables of male *Meloidogyne incognita* from four communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana.

Variables of morphometric characters	**Body length**	**Greatest body width**	**DEGO**	**Gubernaculum length**	**Stylet length**	**Tail length**	**Spicule length**	**'a'**	**'c'**
Body length									
Greatest body width	0.790								
DEGO	0.247	0.058							
Gubernaculum length	0.541	0.365	-0.129						
Stylet length	0.259	0.136	0.189	0.468					
Tail length	0.062	-0.069	0.133	-0.368	-0.220				
Spicule length	0.350	0.130	-0.077	0.549	0.417	-0.085			
'a'	-0.658	-0.981	0.022	-0.277	-0.076	0.086	-0.046		
'c'	0.338	0.377	-0.043	0.577	0.324	-0.917	0.258	-0.340	

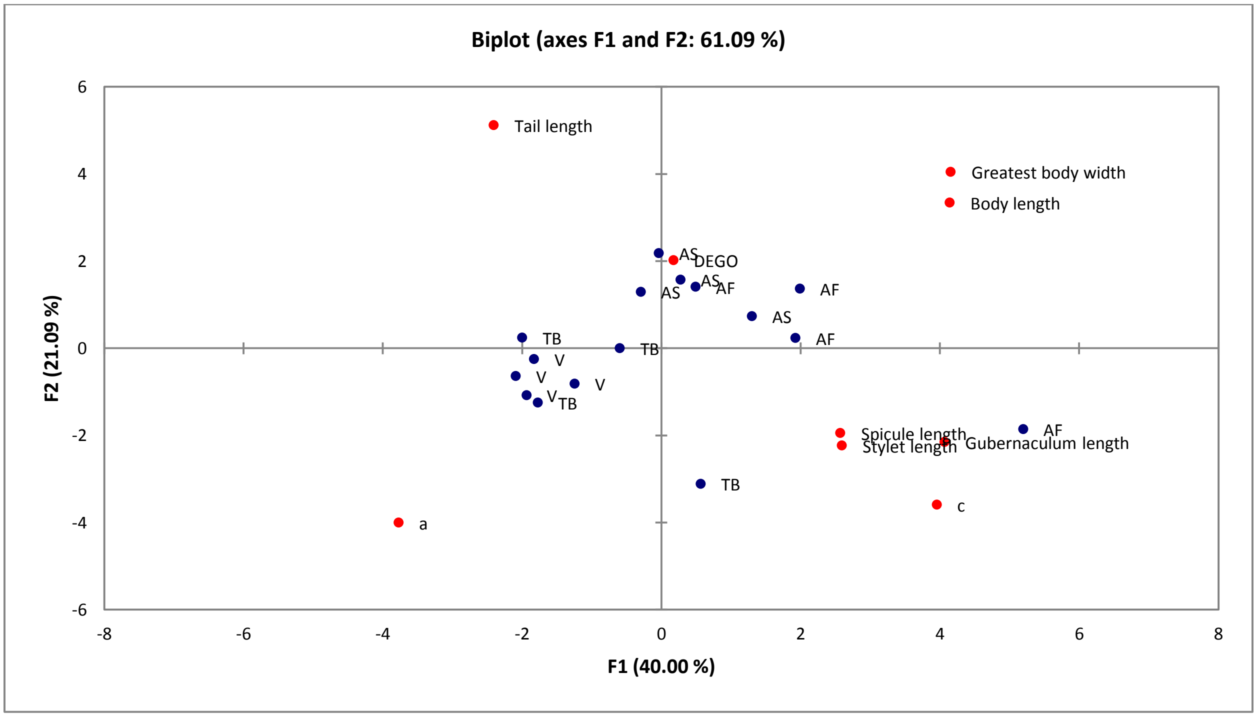

Fig. 25: Biplot of Morphometric variables and their measurements of *Meloidogyne incognita* males from four communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana.

Average tail length in all the populations of *Meloidogyne incognita* juveniles was 46.67µm. The population with the smallest mean tail length (45.73 µm) was Afrancho whiles the longest tails came from the Vea population (47.10 µm). Average hyaline tail terminus length for all the populations of *Meloidogyne incognita* juveniles was 12.56 µm. The Vea population had the shortest hyaline tail terminus (11.65µm) whiles the longest hyaline tail terminus came from the Techimantia population (13.34 µm).

The mean body length (359.3 µm), stylet length (10.6 µm) and hyaline tail length (10.7 µm) of second stage juveniles of *M. incognita* reported by Singh (2009) was smaller than those recorded from all nine populations of second stage juveniles of *M. incognita*. The mean tail length of second stage juveniles of *M. incognita* of all nine populations were however smaller than that reported by Singh (2009). Tail length, 'a' and 'c' were not reported by Singh (2009) and hence could not be compared to those from the nine communities.

4.3.3.2 Second stage juveniles

The first two principal components accounted for about 59.14% of the total variance for second stage juveniles of *M. incognita* in the various populations (Table 18). Seven eigenvalues were noted for second stage juveniles of *Meloidogyne incognita* populations. High variability was observed for Factors (F1 and F2) compared to the other factors. The cumulative variability for F1 and F2 were 39.47% and 59.14% respectively

Table 18: Eigenvectors and eigenvalues of principal component analysis derived from morphometric characters of second stage juveniles of *Meloidogyne incognita* recovered from nine communities in Ashanti, Brong Ahafo and Upper East Region of Ghana

Body characters	**F1**	**F2**
Body length	0.554	0.258
Stylet length	0.21	-0.425
Tail length	-0.078	0.737
Hyaline tail length	0.185	-0.238
Greatest body width	-0.088	-0.116
‘a’	0.555	0.294
‘c’	0.542	-0.231
Eigenvalue	2.76	1.38
Variability (%)	39.47	19.67
Cumulative variability (%)	39.46	59.14

F1 is characterized by high positive eigenvectors (> 0.50) for total body length, ‘a’ and ‘c’ and length negative eigenvectors for tail length and greatest body width.

F2 is characterized by negative eigenvectors for stylet length, hyaline tail length, greatest body width and ‘c’ and positive eigenvectors for total body length, ‘a’ and tail length.

There was a strong positive correlation between body length and ratio a (r = 0.91), body length and ratio c (0.78), ‘a’ and ‘c’ (0.72) and stylet length and ratio c (0.30) in decreasing order of strength of correlation. There was also a strong negative correlation between greatest body width and ratio c (-0.35) and tail length and ratio c (-0.48). (Table 19)

Table 19: Correlation matrix of morphometric variables of second stage juveniles of *Meloidogyne incognita* from nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana.

Variables	Body length	Stylet length	Tail length	Hyaline tail length	Greatest body width	'a'	'c'
Body length							
Stylet length	0.176						
Tail length	0.176	-0.178					
Hyaline tail length	0.160	0.229	-0.061				
Greatest body width	0.065	0.040	0.074	-0.033			
'a'	0.912	0.148	0.140	0.161	-0.350		
'c'	0.780	0.255	-0.475	0.172	0.013	0.724	

Biplots (F1 and F2) for second stage juveniles of *M. incognita* populations revealed no specific groupings (Fig. 26). Second stage juveniles of *M. incognita* of all the nine populations grouped around the middle.

The variables which contributed most to the total variation of morphometric characters of second stage juveniles of *M. incognita* from the nine communities were tail length (54.9 %) and body length (37.2%).

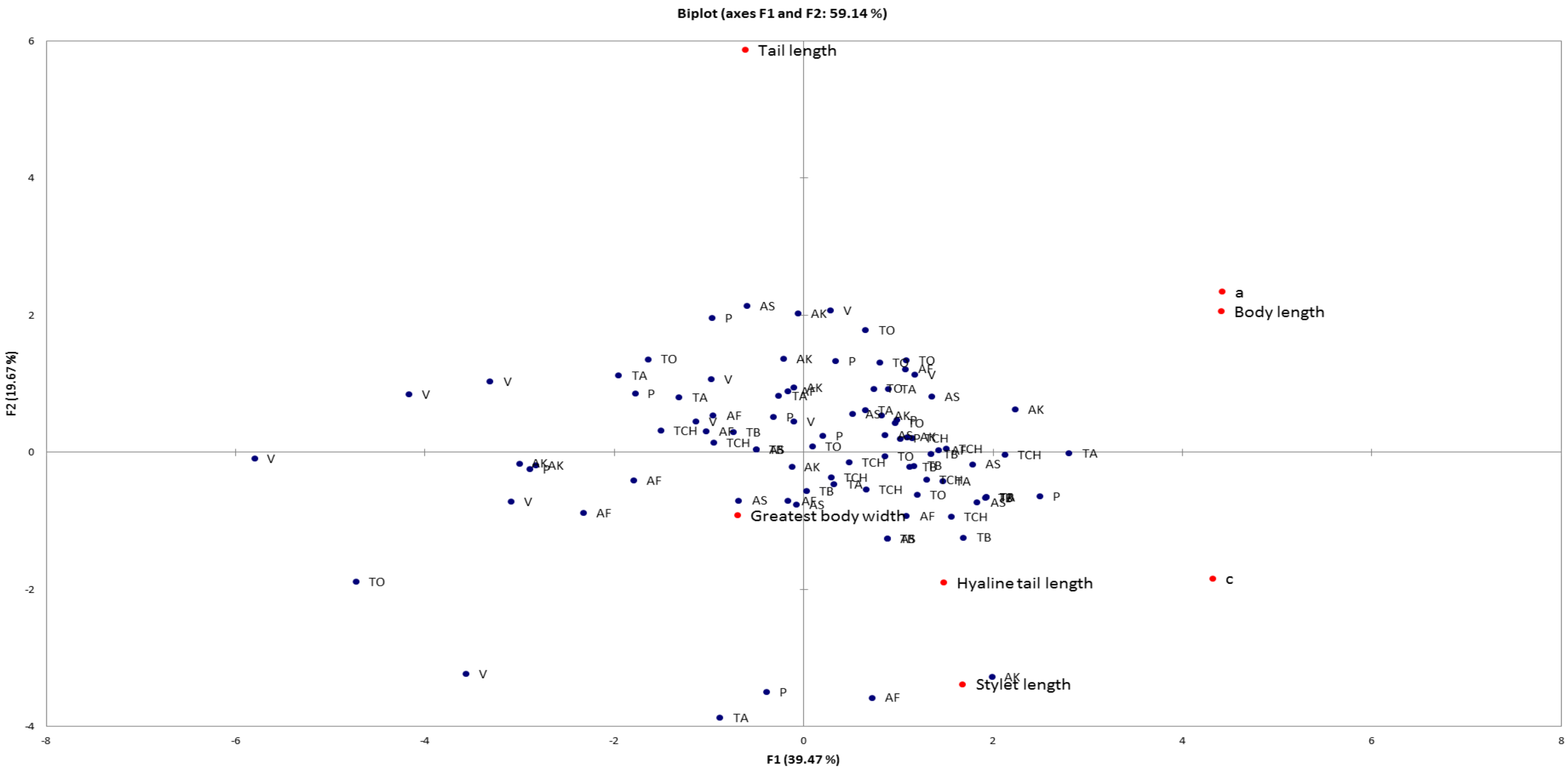

Fig. 26: Biplot of Morphometric variables and their measurements of second stage juveniles of *Meloidogyne incognita* from nine communities in Ashanti, Brong Ahafo and Upper East Regions of Ghana.

CHAPTER 5

5.0 DISCUSSION

Most of the farmers had little or no formal education (51%). Perhaps, most people with higher education sought for jobs in the cities and thus, have left farming activities in the hands of those who had little or no formal education. A study by Sesay *et al.* (2013) revealed that, farming in Sierra Leone was left in the hands of individuals with little or no formal education, while the highly educated sought for jobs in the cities. The analysis also revealed that majority of the tomato farmers were males (78%). This was perhaps due to the general assumption that medium to large scale farming of tomato was exclusively reserved for men in most of the localities surveyed. Their lack of knowledge about nematodes may have contributed to the high prevalence of these nematode diseases resulting from improper cultural practices, e.g., non-removal of weeds during the off-season which may serve as alternative hosts for these pests.

Many farmers continually cropped their tomato fields without any form of crop rotation, while others rotated their tomato crops with other solanaceous crops such as pepper (*Capsicum* spp.) and garden eggs (*Solanum melongena*). These practices could have resulted in the persistence of the nematodes in most farmers' fields, because nematodes were capable of surviving on alternative host plants especially those in the solanaceae family (Luc *et al.*, 2005; Sikora and Fernandez, 2005).

Another observation relates to farmers in some communities not applying manure or organic fertilizers to their fields during the growing season which had a negative impact on plant-parasitic nematodes. Addition of organic carbon to the soils in the form of manure leads to an increase in the number of free-living and predatory nematodes, and a decrease in plant-parasitic nematodes (Dackman *et al.* (1987); Wachira *et al*. (2009)). High organic matter in the soil reduces plant parasitic nematodes 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 nematodes in organically grown tomatoes.

Majority of farmers did not have knowledge about nematode parasitism, and therefore could not differentiate among symptoms induced through nematode infestation, nutrient deficiency, and moisture stress. Furthermore, farmers' ignorance about the spread of nematodes through movements within and between farms, and the use of nematode-infested implements contribute to their spread. Nematodes were capable of being spread on soil adhering to footwear and clothes (Hunt *et al.*, 2005).

This study revealed that, tomato was a host to a diverse group of plant- parasitic nematodes in all the agro-ecological zones surveyed. Production of tomato in West Africa has been reported to be adversely affected by plant-parasitic nematodes. In Ghana for example, tomato yield losses (73-100%) have been reported in the Guinea savannah zone (Hemeng, 1981).

Higher numbers of nematodes were recovered from the tomato rhizosphere soils and roots of plants cultivated around the Ashanti and Brong-Ahafo Regions, compared to the Upper East Region of the country. The Ashanti and Brong-Ahafo Regions received relatively high amounts of rainfall, thus the soils had high moisture content most part of the year creating a conducive environment for nematodes to thrive and to move and infect tomato and other crops. Additionally, mixed-cropping practiced by most of the farmers where tomatoes are sometimes intercropped with other crops such as pepper, okra and maize might also explain the relatively rich nematode genera. On the other hand, the extremely high temperatures and long drought spells in the Upper East Region probably accounted for the comparatively lower nematode densities in those areas.

The high reproductive rates of plant parasitic nematodes under favourable soil conditions (Ananhirunsalee *et al.,* 1995) together with their short life cycle of 20-30 days (Crow and Dunn, 2005) might result in a rapid build-up of the nematode population during the growing season and therefore cause economic damage to crops. Furthermore, interaction between plant-parasitic nematodes and other plant pathogenic soil organisms, particularly fungi and bacteria, in the development of some disease complexes makes nematodes very important even at very low densities.

Meloidogyne spp. was the most important plant-parasitic nematode isolated from both soil and root samples. *Meloidogyne* spp. has been reported to be the most important species affecting tomato worldwide (Sasser and Freckman, 1987). *Rotylenchulus reniformis* and *Pratylenchus* spp. which were also identified in this study have been reported as parasites of tomato roots (Robinson *et al*., 1997; Sikora and Fernandez, 2005), therefore if they are not managed, they could also serve as constraints in tomato production.

Helicotylenchus spp., *Hoplolaimus* spp., *Tylenchus* spp. and *Xiphinema* spp. were known to attack vegetables and other crops, however, their economic importance has not yet been evaluated. The three endoparasitic nematode genera referred to above have a wide host-range (Robinson *et al.,* 1997; Castillo and Vovlas, 2007; Rich *et al.,* 2009) and consequently, they have the potential to damage other crops rotated with tomato in Ghana.

Rapid and reliable identification of nematode species is required for a number of reasons including utilizing appropriate crop rotations, managing diseases and pest resistance effectively, developing bio-control strategies, and quarantine purposes and for studying virulence and plant-nematode interactions (Cenis, 1993; Zijlstra *et al.,* 2000; Baicheva, 2002; Zijlstra and Van Hoof, 2006). There has been an increased pressure on nematologists to accurately identify nematodes up to the species level particularly after restrictions on the use of some broad spectrum chemical nematicides such as methyl bromide (Manzanillalopez *et al.,* 2004; EPA, 2015). Morphological characteristics of root-knot nematodes have been studied extensively and are still valuable for species identification as they can be used to distinguish between species. Morphological descriptions are also required when describing new species.

The study revealed the presence of *M. incognita, M. arenaria* and *M. javanica*. These species have been identified by other investigators in tropical regions (Moens *et al.*, 2009). In this study, *M. incognita* populations were high in both male (59%) and juvenile (75%) isolations. These observations are in agreement with Eisenback *et al.*, (1981) who reported that *M. incognita* was the most prevalent among all *Meloidogyne* species in the studied areas in the international *Meloidogyne* project. *Meloidogyne incognita* was identified as the most abundant nematode species during a survey of tomato fields in Ghana (Osei *et al.*, 2012). However, in Tanzania *M. javanica* was the most abundant root-knot nematodes species (89%) in infected tomato samples (Nono-womdin *et al.*, 2002).

The undescribed species in the samples was expected with the present movement of plant-derived materials around the world, climate change, continuing growth of human population and consequent changes in land use and agricultural practices (Oliveira *et al.*, 2011).

Most of the communities selected for sampling are located in agro-ecological zones with a relatively high annual rainfall (SRID, 2011). This satisfies the reason as to why *M. javanica* and *M. arenaria* occurred together in the same field populations. In regions where rainfall is evenly distributed over the year, *M. javanica*, *M. incognita*, and occasionally *M. arenaria* occur together in field populations (Eisenback *et al.,* 1981). This is very important for management of root knot nematodes especially during the rainy season.

A combination of characters of males, females and second stage juveniles provides reliable identification. Males' head shape, the tail size and shape of second stage juveniles, and perineal patterns of females have been shown to be the most reliable and stable species-specific characters (Rammah and Hirschmann, 1990). In this study, the same characters were important except that the female perineal pattern was not studied due to the unavailability of females.

Morphometrics of second-stage juveniles and males were generally larger than those reported in literature for *M .incognita*. However, one could not make a conclusion from the results as was also indicated by Waeyenberge and Moens (2001). It's only after a combination of different methods used for species identification that assertion may be accurately made as assertion based on quantitative features could be wrong.

PCA (principal component analysis) of the morphometric characters of adult male indicated that, total body and greatest body width were important characters in separating *Meloidogyne incognita* populations. For second stage juveniles, body length, tail length and stylet length were important characters to separate. This is supported by Jepson (1987) report which emphasized their use in combination with other characters from other stages for proper identification as well.

Generally, during the study, it was found that morphological and morphometrical identification was time-consuming because sometimes it was not easy to view some characters under a light microscope in great detail. Many studies have shown the usefulness of molecular based analysis as the quickest and most reliable method to identify *Meloidogyne* spp. (Esbenshade and Triantaphyllou, 1985; Carneiro, 2000). However, the cost of setting up and maintaining molecular laboratories is high and nematode systematics is a relatively poorly funded discipline in Ghana. Therefore, morphology based techniques are very essential because offer a relatively inexpensive method of identification of root knot nematodes.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

- The study revealed that most tomato farmers in Ashanti, Brong Ahafo and Upper East Regions of Ghana (63%) continually cropped their land to tomato for 4-7 years without any form of rotation. Most farmers (73%) were ignorant of plant parasitic nematodes as plant pathogens although infestation was widespread on their fields. Many farmers could not distinguish among nematode infestation, nutrient deficiency and moisture stress. All the farmers (100%) applied only inorganic fertilizer to their crops and high yield losses were attributed to diseases and pests.

- *Helicotylenchulus* spp, *Hoplolaimus* spp, *Tylenchus* , *Xiphinema* spp. were found only in soil whiles *Pratylenchus* spp., *Meloidogyne* spp., *Rotylenchulus* spp., *Scutellonema* spp were found in both soil and roots of tomato plants. It has also shown that tomato is a host to *Helicotylenchulus* spp. (11.5% in soil), *Hoplolaimus* spp. (1.0 % in soil), *Meloidogyne* spp. (37.4% in soil and 69.3% in roots), *Pratylenchus* spp. (20.6% in soil and 13.7% in roots), *Rotylenchulus* spp. (11.0% in soil and 12.2% in roots), *Scutellonema* spp. (9.5% in soil and 4.9% in roots), *Tylenchus* spp.(7.6% in soil) and *Xiphinema* spp. (1.4% in soil) across the nine communities surveyed. Ashanti Region recorded the highest population density of nematodes (258 in 200 cm^2 soil, 70 in 10 g tomato roots) and Upper East Region recorded the least population density of nematodes (91 in 200 cm^2 soil, 41 in 10 g tomato roots).

- Average morphometric features of *M. incognita* males from four populations are: Body length =1131.28µm, Greatest body width= 34.45 µm, DEGO= 3.69 µm, Gubernaculum length = 10.19 µm, Stylet length= 25.45 µm, Tail length =11.15 µm and Spicule length= 29.26 µm. Average morphometric features of *M. incognita* second stage juveniles (J2) from nine are: Body length = 405.54 µm, Greatest body width = 15.16 µm, Stylet length = 12.35 µm and Tail length = 46.67 µm. These measurements were generally larger than those reported in literature for *M .incognita*. Morphometric measurements of second stage juveniles from all the nine communities appeared uniform whiles adult males from Asuosu and Afrancho were similar in morphometric measurements when subjected to principal component analysis. Body length and greatest body width of males and tail length, body length and stylet length of second stage juveniles were important characters in separating populations of *M. incognita.*

It is therefore recommended that:

- Farmers in these communities should be thought on symptoms of plant parasitic nematode infestation in tomato and their management since most farmers were ignorant of plant parasitic nematodes as plant pathogens.
- There is also the need to establish the economic importance of *Meloidogyne* spp, *Rotylenchulus* spp and *Pratylenchus* spp since these nematode genera were found in both soils and roots of tomato.

- Since there were often overlaps of morphological characters, molecular characterization of the *M. incognita* found is recommended to detect variations in populations if present.

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APPENDICES

APPENDIX 1**: Questionnaire survey on the types of nematodes associated with tomato production in Upper East, Brong Ahafo and Ashanti Regions of Ghana**

Documentation of farmers' knowledge, perceptions, and experiences concerning occurrence and management of nematodes

QUESTIONAIRE NO ……….

A. Background

1. Name of farmer ………………………………………….
2. Sex a [Male] b [Female]
3. Level of education a [Primary] b. [JHS] c [SHS] d. [Tertiary] e. [Other]
4. Name of district ……………………………
5. Name of locality …………………………..
6. Size of farm, year of cultivation and source of planting material

Year of cultivation	Size of farm	Varieties	Source of planting materials
2009			
2010			
2011			
2012			

B. Land use intensity

7. How long have you cultivated this land a.[1-3 years] b. [4-6 years] c. [7-10 years] d. [11- 15years] e. [more than 15 years]
8. What type of crops have you planted on this piece of land in the past? .

a. Tomato

b. Pepper

c. Garden eggs

d. Okra

e. Others, (please specify) ………………………………….

9. How long have you planted tomato on this piece of land a. [1-3 years] b. [4-6 years]

c. [7-10 years] d. [11- 15years] e. [more than 15 years]

10. Have you rotated your tomato crops before a. [yes] b. [no]

11. If yes, with what crop(s) ……………………….

12. What was the period of rotation a. [Less than a year] b.[1 year] c.[2 years] d,[3 years]

e. [more than 3 years]

13. Have you practiced land fallow in the past? a. [Yes] b. [No]

14. If yes, how long was the fallow period? a. [Less than a year] b. [1 year] c. [2 years] d,[3 years]

e.[more than 3 years]

15. Do you apply fertilizer to your crops in the course of the growing season? a. [Yes] b. [No]

16. If yes, which type of fertilizer? a. [Organic] b. [Inorganic]

17. What varieties of tomato do you grow ? ……………………………………………

C. Farmers knowledge, perception and experiences concerning occurrence of nematodes on farm

18. Have you experienced the following symptoms on your crops in the past?

19. Do you know what nematodes are? a. [Yes] b. [No]

20. If yes, are they a. [Above ground] b. [Below ground] c. [Both below and above-ground]

21. Can you differentiate between nematode attack, nutrient deficiency and moisture stress?

a. [Yes] b. [No]

22. What symptoms and signs do you rely on to identify the presence of nematodes on your tomato crops?

a. Galls on the roots [ ]

b. Lesions on roots and stem [ ]

c. Sand encrusted roots [ ]

d. Stunted plants [ ]

e. Wilting [ ]

f. Yellowing of leaves [ ]

g. lost leaves [ ]

h. poor fruit set [ ]

i. small fruit size [ ]

j. Poor stand of crops [ ]

Others, please specify …………………………………..

23. Do all your tomato varieties express these symptoms?

a. [Yes] b.[No]

24.If yes, which is the most susceptible?

…………………………………………

25.If no, which does not express any of these symptoms?

………………………………………..

26. What time of the year do you experience symptoms of nematode damage?

a. [Rainy season] b.[Dry season] c. Others, (please specify)

…………………………..

27. What are the conditions of the soil on your with a high incidence of nematodes (these symptoms)

a. Lowland (Waterlogged) [ ]

b. Well drained soil [ ]

c. Low fertility [ ]

d. Sandy soil [ ]

e. Loamy soil [ ]

f. Clayey soil [ ]

28. At what stage in the development of your crops are these symptoms pronounced

a. [Seedling] b. [Vegetative] c.[Flowering] d.[Fruiting] e.[Others], please specify

…………………………………………………………………..

D. Farmers knowledge, perception and experiences concerning spread and control/ management of nematodes.

29. How do these symptoms spread within the farm?

……………………………………………………………………

30. How do these symptoms spread to other farms?

…………………………………………………………………….

31. Can nematode damage be controlled or managed? a. [Yes] b. [No]

32. If no to Q24, give reasons. A. [no reason] b. [I do not know how to control them] c. Other, (please specify)…………………………………..

33. If yes, what do you think can be done to control the damage by nematodes

What can be done	Who should do it	Why it should be done

34. Do you practice any management/ control measures? a. [Yes] b.[No]

35. If yes, what control measures do you practice? ……………………………………….

E. Farmers knowledge, perception and experiences concerning economic importance of nematodes.

36. What are your reasons for farming tomatoes?

……………………………………………………

37. What are your expected and actual yields obtained over the years.

Year	Farm size	Expected yield	Volume harvested	Reasons for difference (if any)
2009				
2010				
2011				
2012				

APPENDIX 2 : Pictures used to facilitate farmers responses during questionnaire administration