UNIVERSITY OF CAPE COAST

AN EVALUATION OF THE NEMATICIDAL POTENTIAL OF FIVE BOTANICALS IN THE MANAGEMENT OF *MELOIDOGYNE* SPP ON TOMATO (*SOLANUM LYCOPERSICUM*)

BY

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research work and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Name: Vester Jobah Saydee

Signature: …… Date: ……

Supervisor's Declaration

I hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Supervisor's Name: Mr. Yaw Opoku-Asiama

Signature:…… Date……

ABSTRACT

In Ghana, information on plants antagonistic to nematodes is scanty compelling farmers to rely on synthetic chemicals which are effective but pose an environmental threat. This work aimed to evaluate the effect of aqueous leaf extracts of *Khaya senegalensis*, *Tectona grandis*, *Azadirachta indica*, *Vernonia amygdalina* and *Bryophyllum pinnatum* plants for the control of *Meloidogyne* spp. In evaluating the botanicals, a laboratory, field and pot experiments were conducted. The first experiment evaluated the effect of extracts from fresh leaves of *K. senegalensis*, *T. grandis*, *A. indica*, *V. amygdalina* and *B. pinnatum* on eggs hatchability and nematicidal activity against second stage juveniles of *Meloidogyne* spp in the laboratory. Results show that lower egg hatch and higher juvenile mortality occurred in the extracts and was concentration dependent. *A. indica* at 25% gave the lowest mean egg hatch of 2.33 and highest juvenile mortality of 65.16. The second experiment was carried out to evaluate the effect of aqueous leaf extracts of *K. senegalensis*, *T. grandis, A. indica, V. amygdalina* and *B. pinnatum* applied to soil as drench on tomato plants. Although the botanicals were effective in the laboratory and pot experiments, they were moderately effective in the field.. The third experiment was carried out to evaluate the application of leaf extracts of *K. senegalensis*, *T. grandis, A. indica, V. amygdalina* and *B. pinnatum* as mulch and soil admixture on growth and severity of nematode infection on tomato plants. The results indicated that *K. senegalensis, T. grandis, A. indica, V. amygdalina* and *B. pinnatum* applied as soil-admixtures were more effective in controlling *Meloidogyne* than applied as mulch.

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DEDICATION

To my mother, Madam Nancy Q. Hinneh (Late), family and my dearest son David Chelsea Cheeks (Mr.), who stood by me in making this dream a reality.

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CHAPTER ONE

INTRODUCTION

Tomato (*Solanum lycopersicum L.*) is one of the most widely accepted fruits in the world because it is mostly consumed worldwide. It is one of the most important nutritious vegetable crops grown around the world. Ali *et al.,* (2012) have reported that, in terms of area cultivated, tomato ranks next to potato whereas, as a processing crop it ranks first in the world. It belongs to the family of Solanaceae, including crops such as eggplant, pepper, tobacco and potato.

Tomato is believed to have originated from tropical central and South America (Cobley & Steele, 1974). Its cultivars in Africa are believed to have descended from varieties later introduced from Europe (Villareal, 1980). Several researchers have shown that it was introduced in Ghana in the 16^{th} and 17^{th} centuries by the Portuguese and has become the most popular vegetable crop (Norman 1992 & Nkansah *et al.,* 2003). Amuti, (1971) stated that most of the local varieties produced in Ghana today evolved from varieties that were introduced by the Portuguese in the 16^{th} and 17^{th} centuries.

Several researchers have reported that not all varieties are successful for cultivation in the country. This observation was made by some researchers who noted that in the coastal savannah areas of Ghana, varieties which proved successful for cultivation are the local types, Fireball, Wosowoso, OK1, OK5, OK7-2, OK7-3, ImprovedZuraungu, Marglobe, Victor and Pusa Early Dwarf (Sinnadurai, 1967; Amuti, 1971 & Blay, 1978).

Tomato has a good adaptation to a wide range of climatic conditions, and so is found throughout tropical Africa (De Lannoy, 2001). According to FAO

(2005), Ghana has wide range of areas that are suitable for tomato production. Production of the crop in Ghana is done by small-scale farmers who grow it basically for its fresh use. However, with the introduction of irrigation projects, large scale monoculture has become wide spread, especially in the Northern and Upper Regions, and around southern Volta region. Tomato production is also vibrant in Akumadan and the Wenchi Districts. Tomato is also grown commercially at Derma, Techimantia and Tanoso in the Brong-Ahafo region. Cooperative farming according to Norman (1992) is concentrated around Mankessim, Swedru, Nsawam, Amasaman, Sege and Dodowa. Farming methods applied for tomato cultivation are often based on availability of water. The sources of water such as rainfall, irrigation, wells and riverbeds determine both the season of farming and the number of times farming is undertaken within the year. In addition, post harvest losses are very high in Ghana especially during the peak harvesting period when there is a glut. Norman (1992) reported that production and yield of tomato in Ghana is affected by several factors. Pests and diseases have been found to be a major constraint to production, and these affect the quality and quantity of the produce. Major pests that attack tomato include plant parasitic nematodes (Berlinger, 1986).

Tomato is an important crop in Ghana (Norman, 1992). It can be grown under varied conditions; from green house to the field. It contains important chemical compounds of medicinal importance (Sahlin, 2004). The ripe tomato fruit is rich in vitamins and a good sourec of A, B, C and minerals which areimportant in the human diet (Willcox Catignani & Lazarus, 2003). About

125 million tonnes of fresh tomatoes were produced in the world in 2008 (FAO, 2010).

In Ghana, about 12,000 hectares of land are under tomato cultivation and it is estimated that more than 60,000 farmers grow tomato; Policy Planning and Monitoring and Evaluation Departmentof the Ministry of Food and Agriculture(PPMED, 1993). In 1987, tomato contributed about 130,000 metric tons to the total agricultural productions and about 13 billion cedis in revenue to the Ghanaian economy (PPMED, 1991). Tomato is one of the major sources of income to farmers and traders in Ghana. It's usefulness in fresh or processed form has played a major role in its rapid and widespread adoption as an important food commodity in the country (Norman, 1992, Horna, Melinda & Jose., 2006 and Asare-Bediako, Showeminio and Buah& Ushawu., 2007). The crop is the largest contributor to Gross Domestic Product (GDP) in the countryGhana Statistical Service (1999). In 2004, tomato alone contributed 607 metric tons to the total agricultural growth and about US$ 56,000 in revenue (SRID, 2005).

F A O in 2005 reported that tomato contributed $437,000 to the Ghanaian economy from export of 4,368 metric tonnes.

Despite the intensive cultivation and potential of tomato in the tropics, yield in Ghana is very low compared to other tropical countries. GIPC (2005) and Danquah and Fulton (2007) reported that yield of tomato in the country is still low (about 7.5 tonnes/hactare) compare to other tropical counties like Nigeria at 1,860,600metric tones (FAO, 2010). The global production area for tomato in 2010 was estimated at 151,699,505 tonnes (FAO, 2010). Production in the United States of America in 2010 was 12,858,700 tonnes (FAO, 2010), Ghana

produced just three tonnes per hactare (SRID, 2010). The low production of tomato in the tropic and sub-tropical regions has been attributed to the persistent pest problems and inadequate pest management practices (COPR, 1983). Some of these problems include limited availability of improved planting material, high cost of labour for land preparation, staking, weeding, harvesting, storability, and nematode diseases among others. Root-knot nematodes (*Meloidogyne spp*) according to De Lannoy (2001) are the major pest of tomato.

Meloidogyne arenaria, *Meloidogyne javanica* and *Meloidogyne incognita* are reported as the main species which attack tomato Clerk (1974). Apart from tomato which is highly susceptible other members of the Solanaceae family like the garden egg, pepper and tobacco when grown on even lightly infested land, fail to produce any remarkable fruit and on severely affected soil they are killed totally while still young Clerk (1974).

Hemeng (1981) observed 73 – 100% yield loss in tomato in the Guinea Savannah Zone of Northern region of Ghana to be caused by root-knot nematodes.

Even though Farmers use a high percentage of their income to fight nematodes problems every year, their effort is still not enough to reduce the losses.

Mostfarmers would not use nematicide; rather nematicides are recommended like carbofuran as control measure against root knot disease, but expensive and these chemicals are toxic to animals and man and therefore pose great danger to peasant farmers most of whom are illiterates (MoFA, 1995).

Current efforts and campaign being made by Governments of various countries including Ghana to improve the standard of living through the

production and utilization of vegetable (like tomato), could only be successful if solutions to the problems of pests including plant parasitic nematodes are found. MoFA (1995) reported that the use of synthetic nematicides is considered the most effective practical means of combating the menace of plant parasitic nematodes in tomato but has some serious constraints. The assault on the environment through the indiserminate use of synthetic agro-chemicals (Bell, 2000) and unreliable results from crop rotation systems (Sikora & Fernandez, 2005) has necessitated the search for sustainable, effective and environmentally acceptable nematode management options.

The World Health Organization (WHO) reported that 20% of pesticide use in the world poses danger to human health as well as the environment (Hurtig et *al*., 2003). Higher level of pesticide has been found in people residing closer to agricultural fields (Quandt *etal*., 2004). It has also been reported that in late 2010, 15 farmers died from suspected pesticide poisoning in Upper East region of Ghana (NPAS, 2012).

Currently the Ghanaian public and government have come to realize that the use of chemical pesticides by vegetable farmers to control pests and diseases in the country is increasing and if agricultural production is to be sustainable and safe to humans and the environment, then intensive farming systems should become less dependent on chemical pesticides (Okorley, Zinnah & Bampoe., 2002).

With concern about the adverse long-term effects of pesticides on the environment and human health adequate measures are therefore required to promote the appropriate management of pests and pesticides. Proper management will ensure that increased and sustainable agricultural production

and farm incomes are achieved; that diseases and insect pest are managed in a sustainable manner, and that the risks to human health and the environment associated with pesticide use are kept to an acceptable level.

The use of plant extracts and antagonistic microorganisms as a component of integrated nematode management is gaining wide acceptance worldwide. Their environmental safety in an environmentally conscious world also holds promise for their acceptability and use by resource-poor African farmers Egunjobi and Onayemi, 1981 Zurren and Khan, 1984; and Adegbite, 2003.

The potential for nematicidal activity of indigenous plants and their products as an alternative for traditional nematicides has been studied by several works Prot and Kornprobst, (1983); Haseeb, Siiddiqui and Alam, (1984), Pracer, Tarjan and Hodgson,(1987); Osmam and Viglierchio, (1988); Adegbite and Adesiyan (2005); and Hasabo and Noweer (2005).

The use of botanical extracts for controlling pest is appealing because of the growing problem of environmental pollution caused by synthetic nematicides. Plant extracts have been found to be effective for the control of plant parasitic nematodes Siddiqui and Allam (1987), (Hussain, Kumar, Kahn & Tito, 1984); they are easily degraded, leave no harmful residues, are cheaper, nontoxic to host plant and humansand availability in many tropical countriesAmadioha, (2003). Extracts of many plants with anti-helminthic and antimicrobial properties have been proven effective in controlling plant parasitic nematodes (Ferris & Zheng, 1998). Such plant species produce different allele-chemicals which have tremendous nematicidal potential (Sukul, 1992). Compounds occurring in the plants with nematicidal activity comprise a wide variety of phytochemicals e.g. polythienyls, acetylenes, alkaloids, fatty acids and

derivatives, phenolics, terpenoids alkaloids, fatty acids and derivatives, phenolics, terpenoids (Chitwood, 1992).He further stated that use of phytochemicals in crop production could offer sustainable management option.

Nematicidal phytochemicals are generally safe for the environment and these compounds include repellents, attractants, hatching stimulants or inhibitors and nematotoxicants, either constitutive or formed in response to nematode presence (Chitwood, 2002). Information on plants antagonistic to nematodes is scanty compelling farmers to rely on synthetic chemicals which are effective but pose an environmental threat (Osei, Fening, Gowen & Jama, 2010) this lead to increased interest in developing pesticides of natural origins during recent years.

The objective of this study was, therefore to evaluate the use of aqueous leaf extract of some plants in Ghana for the management of root-knot nematode infection on tomato.

The specific objectives were to evaluate:

i. the effect of*Azadirachta indica*, *Bryophyllum pinnatum, Khaya senegalensis*, *Tectona grandis* and *Vernonia amygdalina* on egg hatchability and nematicidal activity against juveniles of *Meloidogyne* spp.
ii. the effect of the aqueous leaf extract of the five medicinal plants on growth and yield of tomato in the field.
iii. the application of the five plants *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis*, *Tectona grandis* and *Vernonia*

amygdalina extracts as mulch and soil admixture for the control of root-knot disease and growth of tomato.

CHAPTER TWO

LITERATURE REVIEW

Nematodes

Nematodes are small worm-like aquatic animals that are very common in most habitats. They have a wide host range, and cause problems in many crops. The majority of nematodes species live freely in fresh or salt water or in the soil and feed on microscopic plants and animals (Endo, 1975). Some nematodes are ecto-parasites and so remain outside the plant feeding on surfaces tissues inserting their stylet into the host tissue (Decraemer and Hunt 2006). They can also be endo-parasites and these enter plant tissues completely or with large portions of their bodies. These may be migratory in roots, stems, buds and leaves (Crowling, 1979). Many of the plant parasitic nematodes are found within the tissues of the host plants where there is high moisture content (Jenkins & Taylor, 1967).

Symptoms of nematode infection are indistinguishable and can often be mistaken for numerous other pathogens as well as abiotic factors (Windham & Edwards, 1999; Castillo & Vovlas, 2007), making accurate diagnosis a more involved process. Above ground plant parts may show symptoms like stunting growth, chlorosis, lodging and wilting (Norton & Hinz, 1976; Norton, 1983; Duncan & Moens, 2006; Castillo & Vovlas, 2007).

Affected root systems can exhibit heavily branched root tips, stunted root growth, lack of root hairs, and dark redbrown lesions (Windham & Edwards, 1999; Agrios, 2008). Injury to root cells caused by nematode renders the root less productive in absorbing water and nutrients from the soil. As feeding continues, root tissue begins to breakdown (Windham & Edwards, 1999) and

as the number of nematodes feeding on the root increases the rate of tissue decay intensifies. Studies have shown that root damage on crop as early as three week old, can reduce yield (Kiesselbach, 1999). Nematodes are also usually found in mixed populations, so determining the actual species that caused yield loss may be difficult to assess (Windham & Edwards, 1999). The only way to correctly identify a nematode population is by analysis of a sample collected from the infested soil or root material (Windham & Edwards, 1999).

Agricultural crops have different kinds of pathogens that reduce yield potentials every season. The understanding or knowledge of some of these like nematodes, by farmers is very little. Almost 4,100 species of plant parasitic nematodes have been described worldwide (Decraemer & Hunt, 2006). The three genera having the greatest economic impact are in order of importance: cyst nematode (*Heterodera* spp), root-knotnematode (*Meloidogyne* spp), and root-lesionnematode (*Pratylenchus* spp) (Sasser & Freckman, 1987). Pathogens that cause high infection include the ecto-parasites to which *Meloidogyne* spp belong. Hussey and Williamson (1998) stated that the entry and internal migration of endo-parasitic nematodes can cause significant physical damage to the root system, therefore reducing yield potentials very early in the infection process. Openings in the root tissue made by nematodes allow secondary pathogens access to the damaged tissue, further decreasing the plant's ability for growth, production, and in some cases, survival (Krall, 1978; Windham & Edwards 1999; Duncan & Moens, 2006). Such nematode-microbe interactions constitute disease complexes. Duncan and Moens (2006) also reported nematode-microbe interactions with various

fungal and bacterial pathogens including*Fusarium moniliforme, F. oxysporum, Gibberella zeae, Helminthosporium pedicellatum, H. sativum, Rhizoctonia fragariae, R. solani, and Verticillium dahliae* (Duncan & Moens, 2006). Plant parasitic nematodes have common morphological characteristics that can be use as common features like microscopic, transparent, worm-like animals with un-segmented, bilaterally symmetrical bodies can be a key in identifying them(Decraemer and Hunt, 2006). They further stated that the bodies of Plant parasitic can be described as a tube within a tube; the outer tube being the body wall, or cuticle, and the inner tube containing the reproductive system (Decraemer and Hunt, 2006).

The life cycles of most nematodes have six stages, beginning with an embryo followed by four juvenile stages, then to an adult (Decraemer & Hunt, 2006). Eggs can be laid in soil or root tissue. A newly laid egg contains a nematode in the first juvenile stage, also known as the J1 stage (Khan, 2008). While inside the egg, most nematode will molt into a second juvenile stage, the J2 stage. The J2 hatches from the egg using its stylet to pierce the shell. At this growth stage, most nematode can begin feeding on a suitable host. Juveniles will go through three additional molts, finally reaching adulthood.On average, life cycles range from 2 to 6 weeks depending on species and environmental factors (Windham & Edwards, 1999; Castillo & Vovlas, 2007; Agrios, 2008; Khan, 2008). Sexual identities are established during the last molt into adulthood. Most genera are dioecious, having separate male and female nematodes (Ferris & Ferris, 1998; Decraemer & Hunt, 2006). Reproduction occurs either between mating partners or parthenogenetically, where females bear only female offspring without need of fertilization (Windham &

Edwards, 1999). Parthenogenesis reproduction in nematode is specific by species within genera (Agrios, 2008).

Nematodes search for a suitable host when they are ready to feed. Nematodes can find host tissues by random movement within soil or through chemotaxis and chemokinesis (Khan, 2008). Nematode use sensory organs, such as phasmids or amphids, to detect changes in temperature, moisture, carbon dioxide, oxygen, and chemical substrates (Khan, 2008; Robinson and Perry, 2006). This sensory information guides the nematode through the soil profile; the data leads them either to potential hosts or away from harmful environments.

The nematode is attracted to root exudates of host plants (Tsai and Van Gundy, 1990). Depending on species migratory tendencies, nematodes may settle on one root or move between several roots for feeding (Huang and Ole Becker, 1997; Todd and Oakley, 1996). Once a host is found, the nematode then searches for a suitable feeding site by touching various areas on the root surface (Khan, 2008; Zunke, 1990). Finding a feeding site, the nematode begins feeding by insertion of its stylet into the root tissue. The stylet is used by plant parasitic nematodes primarily for feeding and is generally a hollow sclerotized tooth-like structure (Agrios, 2008; Ferris and Ferris, 1998). Feeding depth within the root varies by genus (Robinson and Perry, 2006). Most nematodes secrete chemicals to aid in the breakdown of root tissues (Zunke, 1990). An organ within the digestive tract, known as the median bulb, expands and contracts, acting as a pump to aid the nematode in ingesting plant cytoplasm (Khan, 2008). The host plant is commonly fed upon as long as it provides sufficient nutrients to the nematodes. Once the root tissue has ceased

activity or the plant has perished, the nematodes may either move on to a new plant specimen or wait in quiescence for another favorable host or environment (Duncan and Moens, 2006).

Most plant parasitic nematodes are obligate parasites, needing live plant material to feed upon (Khan, 2008; Windham and Edwards, 1999); they also cannot reproduce well, or sometimes survive, on non-host plants. Although a host is needed for adequate sustenance, most nematodes have key behavioral and physiological strategies to endure lack of host or unfavorable environmental conditions, such as quiescence (Agrios, 2008; Castillo and Vovlas, 2007; Ferris and Ferris, 1998; Norton and Niblack, 1991). During quiescence, the nematode is in a reduced metabolic state induced by levels of water, salt concentration, temperature, or oxygen. Survival while in dormancy depends upon a number of factors including, but not limited to, duration, predators, and host availability (Ferris and Ferris, 1998; Norton and Niblack, 1991). Nathan A. Cobb once discussed the prevalence of all nematode communities stating, "In short, if all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes" (Cobb, 1915). Plant parasitic nematodes are no exception as they can be found on every continent in every ecosystem in the world (Castillo and Vovlas, 2007). Since they are obligate parasites, they are concentrated in areas containing suitable host species.

Nematode communities are affected by several biotic and abiotic factors. Soil organisms, parasites, and predators often influence nematode survivability and

reproduction (Bilgrami, A. L., C. Brey, and R. Gaugler, 2008; Sikora, 1992). Soil texture, aeration, temperature, moisture, pH and aeration, and other edaphic factors may also influence nematode life processes (Agrios, 2008; Castillo and Vovlas, 2007; Decraemer and Hunt, 2006; Khan, 2008; Norton and Niblack, 1991).

Nematode spatial distribution is highly aggregated in soils and can be irregularly distributed within fields (Norton and Niblack, 1991). Vertical distribution of nematodes can be temporal and affected by several factors. Soil texture, soil type, moisture, temperature, root distribution and host cultivar/variety can influence the presence and reproduction of phytoparasitic nematodes (Castillo and Vovlas, 2007; Forge, T. A., R. DeYoung, and T. C. Vrain, 1998; Norton and Niblack, 1991; Nyczepir and Lewis, 1979; Rebois and Huettel, 1986; Taylor and Evans, 1998). Nematodes can be classified as migratory or sedentary. Migratory plant parasites move frequently, feeding on several areas of the root system. Sedentary nematodes find a suitable feeding source and remain in that location for the rest of their lifetime or the host's lifetime. Nematodes require a film of water for movement through pore spaces between soil particles (Decraemer and Hunt, 2006). Movement in a season can range from 0.3 to 2 meters in a year (Agrios, 2008; Castillo and Vovlas, 2007; Khan, 2008; Windham and Edwards, 1999). Movement requires the alternate contraction of muscles within the nematode body, creating undulations in the dorso-ventral plane (Decraemer and Hunt, 2006; Norton and Niblack, 1991). During dry periods, the nematode movement and survival is limited (Agrios, 2008). Optimum temperature for nematode activity is from 16-32 °C (Windham and Edwards, 1999). Ideal temperatures vary by species,

environment, and stage of development (Robinson and Perry, 2006; Windham and Edwards, 1999). Since nematodes have little range of dispersal on their own, their long distance transportation is dependent on other means. Nematodes can be carried by water or wind-blown soil particles and plant tissue, but mechanical transfer of infested material is the primary mode of nematode dispersal (Castillo and Vovlas, 2007; Duncan and Moens, 2006; Morgan, G. D., A. E. MacGuidwin, J. Zhu, and L. K. Binning, 2002; Windham and Edwards, 1999). This dissemination can occur locally, within a single field, or globally. The highly inconsistent population distribution within a field causes very high statistical variability when conducting research on nematodes. This leads to challenges for producers to manage the populations accurately, economically and efficiently. Having a precise assessment of nematode populations is imperative for proper management strategies to be implemented (Decraemer and Hunt, 2006).

Plant parasitic nematodes are widely distributed and heavy infestations have been reported from all over Ghana, (Peacock, 1957 and Chona, 1965). Addoh, 1970 identified the presence of *Meloidogyne arenaria, M. incognita* and *M. javanica* in Ghana. The distribution of many species has been influenced by human activities (Kerry, 1981). Edward (1953) during his researches on root nematode on weeds and cultivated plants listed 76 host plants in Ghana. The most commonly cultivated among the host plants are banana, onion, pepper, watermelon and tomato. Thompson andWebster, (1973) and Clerk (1974), observed that *Meloidogyne* spp is responsible for deleterious effect on tomato

which is most susceptible to the attack of the disease; growth resulting in low yield and poor quality.

Meloidogyne Species

Many different types of crops, fruits, leaves and bulbs are grown world-wide have a range of plant parasitic nematodes associated with them. *Meloidogyne* species are of particular importance (Bridge & Starr, 2007) and affect plants in general and especially vegetables (Noling, 2005).

The *Meloidogyne* spp are obligate parasites of the roots of thousands of plant species. Major pests of vegetable crop of the most economically damaging species of the sixty known species are *M. javanica, M. arenaria, M. incognita* and *M. halpa.* They are known worldwide as pests of major importance in plants ranging from field crops through pasture and grasses to horticultural, ornamental and vegetable crops (Stirling, Stanton & Marshall., 1992).

Meloidogyne infected plants may appear chlorotic, stunted, necrotic, and/or wilted, especially during periods of moisture stress and high temperature (Pattison, 2007). However, diagnostic symptoms appear on roots of infected plants in the form of galls or knots. These galls vary from 1 to 10 mm or larger in diameter, depending on the nematode species involved location of galls in the root system and the susceptibility of the host plant (Mai & Abawi, 1987). Severely galled root systems become malformed, with shortened and thickened individual roots. Such roots may appear as a mass of galls. Growth rate of roots and root branching are frequently suppressed by infection with root-knot nematodes.

The altered root growth results in reduced root volume and surface area. Thus, the root has a reduced capacity for water and mineral uptake as well as the synthesis of cytokinins, gibberellins, and other growth determining metabolites. Intensive galling seriously reduces root efficiency and often results in permanent wilting, premature defoliation, and eventually plant death (Mai andAbawi, 1987).

Meloidogyne spp may interact with other soil-inhabiting plant pathogens to form disease complexes in which case the resulting disease is much more severe than components of the complex would cause alone. *Meloidogyne* species are known to interact with both Verticillium and Fusarium, which cause wilt diseases of pepper, tomatoes, potatoes, and other plants Mai and Abawi (1987).

Management Options for Nematode Disease

Plant parasitic nematodes are a major constraint to agricultural production worldwide (Luc, Sikora & Bridge, 2005). General symptoms of nematode infection include chlorosis, wilting, galling of roots and tubers, stunted growth, root lesion and yield loss. Effective management of plant parasitic nematodes is essentialfor sustainable food production.

Control of a plant disease can be achieved by a single procedure but satisfactorily control of most diseases requires the application of multiple control measures and usually involves an integrated programme of manipulation of environmental, biological and chemical factors (Singh, 2001). There are several potential methods for managing root knot nematodes. These

methods are grouped into two main categories namely, non-chemical methods and the use of nematicides.

A number of cultural, chemical, and biological techniques for nematode management have been utilized over decades. Many cultural practices have been shown to aid in reducing nematode population densities. Rotation and cover crops using non-host plants have proven very beneficial in reducing plant parasitic nematodes (Ball-Coelho, B, A. J. Bruin, R. C. Roy, and E. Riga, 2003; Duncan, 1991; Jackson, T.A., G.S. Smith, and T.L. Niblack., 2005; Johnson, A. W., C. C. Dowler, and E. W. Hauser, 1975; Koenning, Koenning, S. R., D. P. Schmitt, and K. R. Barker, 1985; Kratochvil, R. J., S. Sardanelli, K. Everts, and E. Gallagher., 2004; LaMondia, 2006; McSorley and Gallaher, 1993). Some nematode species behave differently within a genus and they have a broad host range; therefore, rotation is not a viable option (Barker and Olthof, 1976; Bélair, G., N. Dauphinais, D. L. Benoit, and Y. Fournier, 2007; Jordaan and De Waele, 1988). Delayed planting dates can influence nematode infection (Koenning, S. R., D. P. Schmitt, and K. R. Barker, 1985). Leaving land fallow for a growing season is also an effective cultural practice for control of nematodes, however, can be very costly to the producer (Koenning Koenning, S. R., D. P. Schmitt, and K. R. Barker, 1985; Kratochvil, R. J., S. Sardanelli, K. Everts, and E. Gallagher, 2004; Windham, 1998). With no host crop, nematodes do not have a food source and the soil becomes very warm and dry. Tillage practices have demonstrated beneficial effects, but results vary by nematode species, soil type, host plant and location (McSorely and Gallaher, 1993; Thomas, 1978; Windham and Edwards, 1999). Soil amendments have also caused reductions in plant parasite populations

(Hassan, *et. al*., 2009; Kratochvil, R. J., S. Sardanelli, K. Everts, and E. Gallagher, 2004). Normal cultural practices, such as adding nitrogen to soil, can also have nematicidal activity. When cultural techniques leave farmers with few desirable options, they turn to more potent chemical alternatives. Synthetic chemical these products are quite effective, there are negative environmental aspects that far out-weigh the benefits. These chemicals have been shown to kill beneficial soil microorganisms (Nyczepir and Lewis, 1979). Nematodes are classified as animals so the chemicals used for their control (such as carbamates and organophosphates) are harmful to humans, too. Contact with these chemicals through mixing, application, cleaning, and storage can be very dangerous to the producers, and thus a shift has appeared in the nematicide market. The government, due to groundwater contamination, has restricted their use (Duncan, 1991). Chemicals can be expensive and, depending on the nematode species and population density, control may not be warranted (Duncan and Moens, 2006; Windham and Edwards, 1999). Several other methods have been studied for nematode control. The choice of one, or a combination of several, is heavily dependent upon cost, efficacy, and potential for economic return.

Integrated pest management (IPM) is an important part of many producers' farm practices. The use of IPM has proven very beneficial to farmers by using multiple techniques for pathogen and pest control. This strategy combines biological, cultural, chemical, and genetic practices to aid in pathogen control all the while reducing the application of chemical products. It can assist with the management of several pathogens with similar control methods while at the same time helping to reduce input costs.

Botanical Control

Botanicals are plants capable of releasing substances that are toxic to several pests, theyare used these days because plants have a great number of chemicals that are biosynthesized and are considered the most important source of chemical compounds (Addor, 1995). Grainge and Ahmed (1988) reported that plants are nature's chemical factories which provide the richest sources of organic chemicals on earth. At the present time there are a number of botanical pesticides being marketed. Botanicals have been used as alternatives to synthetic pesticides in recent times. Some of these botanicals are already being used in insect pest management (Agnihotri, Walia & Gajbhyie., 1999). Botanicals contain compounds that mostly affect pests in their growth by inhibiting metamorphosis. The compound would either prevent metamorphosis from taking place at the right time or force pests to go through early stages of metamorphosis so that development takes place at a time not favourable for pests. Essential compounds from various plants have shown promise as potential sources for new nematicides. Several compounds at very low concentrations immobilized juvenile root-knot nematode juveniles and some also reduced hatching of eggs Oka *et al*., (2007). Abdi (1996) reported that botanicals also tend to protect the plant from diseases. Botanicals are easily available in many places and are often cheaper. Furthermore, crude extracts of the botanicals are easy to prepare by farmers. Addition of organic amendments to the soil stimulates microbial activity and increased accumulation of this matter from plant decomposition and microbial metabolites were deleterious to nematode population in the soil (Chitwood, 2002). Nematicidal properties of the botanicals have already been documented

earlier by various Scientists. The plant products in form of, leaf extracts (Netscher and Sikora, 1990; Akhtar, 1999), oil cakes Yadav, Y.S., Siddiqui, A.U. and Parihar, A. (2006), plant latex (Siddiqui and Alam, 1990), decomposed products of indigenous medicinal plants and their parts (Goswami & Vijayalakshmi, 1986; Jain & Hasan, 1984) are known to have antihelmintic properties.

Exploration of nematicidal potential of botanicals and their application is on the increase. Different plant species are being tested to identify the sources of nematicidal substances and many of them have shown promising results in the control of plant parasitic nematodes. *Azadirachta indica* exhibited pathogenicity effect on *Meloidogyne incognita* race 2 infesting cowpea. (Claudius-Cole, A.O. Aminu, A.E. & Fawole, B, 2010). Extracts from *Azadirachta indica* was found to be most effective in reducing the population of rice root knot nematode, *Meloidogyne graminicola*. The extracts significantly increased the growth of rice plant (Mukesh Dongre & Sobita Simon, 2013). *Vernonia amygodalina* and *Azadirachta indica* 5% concentration recorded nematicidal properties against *M. incognita in vitro* Wondimeneh Taye, Sakhuja, P.K. and Tadele Tefera. (2013).

Antagonistic Plants

Antagonistic plants are capable of releasing substances into the soil that are toxic to several plant parasitic nematodes. Some antagonist plants also increase plant growth and yield by improving plant nutrition. Some of these antagonistic plants include *Tagetes* spp (Kumari et.al., 1986), *Acacia albida* Dell, *Asparagus*, and *Allium sativa (L),* (Hasabo & Noweer, 2005);

Azadirachta indica (Rizvil *et al,* 2015). Mian and Rodriguez-Kabana (1982) in their study observed fewer galls on aqueous castor extract treated plants than the water treated plants and attributed this to the action of the toxic compounds released by castor bean. Khan (1990) revealed that leaves of *Azadirachta indica (L)* have strong nematicidal properties and their addition to soil negatively affected the development of *Pratylenchus zeae* and also improved plant growth in chilli. Toxic compounds accumulated by antagonist plants are more lethal to plant pathogens including nematodes, allowing better plant growth (Yasmin, Rashid, Uddin, Hossain & Ahmed, 2003).

Various researchers have reported on the management of root knot- nematodes on tomato by utilizing plant products. They revealed that the use of botanicals as nematicidal and nematostatic products are economical and eco-friendly (Goswami & vijaylakshmi, 1981; Khanna, 1997; Zaki & Bhatti, 1998 and Philippe, Sylivie, Miseille & Thierry, 2004).

Organic Amendments of Soil

Literature reveals that various organic amendments can be added to the soil to reduce the impact of nematodes on crop plants. Reductions in plant parasitic nematode populations in response to applications of organic amendments were reported by Muller and Gooch (1982). The incorporation of organic material into the soil has been shown repeatedly to reduce root-knot nematode densities (Stirling, 1991). Baby and Manibhushanrao (1993) found that organic amendments were associated with suppression of nematode populations through stimulation of antagonistic soil microflora during their decomposition. Organic soil amendments have been reported to possess nematicidal properties

in vitro and in vivo and also increase crop yields significantly (Parr, Papendick, Hornick & Colacicco, 1989). The amendments, which include peat, plant parts, manure, and composts, are useful for increasing the water and nutrient holding capacity of the soil, especially sandy soils (Perry, 2001). He further stated that because plants that are water stressed are more readily damaged by nematodes, increasing the soil capacity to hold water can lessen the effects of nematode injury. Likewise, more frequent irrigation can help reduce the damage cause by nematodes. In either case there will be just as many nematodes in the soil, but they will cause less damage. Nematode infections on plant arereduced by incorporating chopped plant parts into the soil before planting. The use of organic amendment is associated with reduced infection, or survival of nematodes and increased numbers of microbial antagonists of nematodes (Sitaramaiah and Singh, 1978). Plant based soil amendments such as castor, sesame, sorghum-sudangrass, velvet bean and zinnia incorporated into soil or applied as mulch controlled nematodes under greenhouse conditions, and were used as organic amendments to suppress root-knot nematodes when used in crop rotation (Mian and Rodrigeuz-Kabana, 1982b; Huang, 1984; Rodrigeuz-Kabana *et al*., 1988; Rich Rich, J. R., G. S. Rahi, C. H. Opperman, and E. L. Davis,1989; McSorley and Gallaher, 1993; McSorley *et al.*, 1994). Extracts of marigold (*Tagetes* spp), *Ricinuscommunis* (L) (Adegbite &Adesiyan, 2005), *Tridaxprocumbens* (Mani & Chitra, 1987) have all been reported to be highly toxic to root knot nematodes. Osei, Fening, Gowen and Jama (2010) also reported that application of green manures in the soil is not only beneficial to disease management but also improving the plant growth and productivity and application of green manure leads to build-up of

beneficial microflora, that keep the plant healthy and vigour, around the rhizosphere, which will help to reduce the plant parasitic nematodes in the soil.

Application of of some plant extracts not only reduced root galling in okra and tomato but also egg-laying capacity of the female nematode (Singh & Sitaramaiah, 1969). Application of oil cakes as soil amendments is an agronomic practice in many parts of the world. Saifullah and Gul (1990) observed reduction in infection by *Meloidogyne* spp. on tomato where mustard, linseed, sesame, castor and cotton seed were used. In one test, neem cake incorporated into soil completely blocked the development of the resting forms of *R. solani*, thereby interfering with the long term survival of other devastating fungus (Singh, Singh &Singh., 1980).

In addition to affecting root-knot nematodes, studies conducted showed that treating soil with *Azadirachta indica* can reduce the population of fungi in soil that attack and feed off plant roots (Singh, Pant, Khan & S.K.Saxena, 1985 and Sivakumar & Gunassekaran, 2011).

The use of nitrogenous organic matter as a soil amendment is a successful strategy for the management of *Meloidogyne* spp. and other plant parasitic nematodes in vegetables and other root-knot susceptible crops (Mian and Rodrigeuz-Kabana, 1982a; Rodriguez-Kabana, R., D. Boube, and R. W. Young, 1990). Oil cakes, sawdust, urea and bagasse also have been used in managing root-knot nematodes with some success (Sikora Sikora, R. A., K. Sitaramaiah, and R. S. Singh.1973).

Nematicidal Phytochemicals

The use of a crude phyto-chemical extract, instead of a purified or synthetic compound may result in beneficial effects beyond mere nematode control and thus may convey additional economic benefit. A crude extract may involve the extra expense of application of larger volumes of material and the cost of the manufacture of crude extract against synthetic materials is a function of the complexity involved. Direct *in vitro* tests must be complemented by *in vivo*, soil-based experiments in order to examine phytotoxicity (Chitwood, 2002).

Research on botanical compounds has revealed their antagonistic activities. Several factors such as phenological age of the plant, percent humidity of the harvested material, and the method of extraction have been identified as possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Lahlou, 2004). Nematicidal phytochemicals include essential oil, triterpenods, sapanins, glucosinolates, isothiocynates, cyanogenic glycosides, alkaloids, phenolics, flavonoids, polyacetylenes, polytheinyls, and pyrethrum etc.

Chemical Constituents of *AzadirachtaIndica, Bryophyllum pinnatum, Khaya S senegalensis, Tectona grandis* and *Vernonia amygdalina*

Azadirachta indica

Azadirachta indica commonly known as Neem is widespread, and, in addition is easily multiplied. Its extracts have a large activity range against deleterious insects and nematodes. Literature shows that the interest of *Azadirachta indica* for the control of plant pest was first demonstrated against insects due to its various biological effects on insect behavior or development including settling, oviposition or feeding, behaviours, metamorphosis, fecundity, egg-

sterility or vigour of insects. *Azadirachta indica* extracts has also since been applied in several ways in plant protection; foliar treatments with aqueous extracts and post harvest protection; stored grains most particularly against a wide range of caterpillars and the larvae of various beetle species (Schmuttere, 1990). It is interesting to know thatthe effectiveness of *Azadirachta indica* against nematode has also been shown since in different ways which include global toxicity of the soluble water extract, reduction of larvae hatching (Khan, Adhami, Siddiqui, & Saksens,1967), reduction of nematode mobility (Khan *et al.,* 1997),decrease of the egg laying capability and increase of resistance at the plant level to nematode invasions (Sitaramaiah & Ssingh, 1978), inactivation of larvae and reduction of root galling by root-knot nematodes(Gupta & Kali, 1981)reduction of nematode population (Haseeb, Pandey & Husain.,1988).

Azadirachtaindica products are hand characterized by anti-feedant, anti-oviposition, repellent and growth regulatory properties (Biol, 2006) and systematic properties (Radwanski, 1977) which provide a better selectivity toward non-phytophageous insects and other useful organisms (Rodriguez *et al.,* 1987). *Azadirachta indica* is not toxic to mammals and has been used in India for ages as traditional medicine and by farmers for its pesticidal, antifungal, and anti feedant properties (Jotwani& Srivastava, 1981). Brahmachari (2004) also reported that Indian farmers have used the leaves for hundreds of years as a pesticide and insect repellent. The *Azadirachta indica* tree has attracted the attention of many chemists and biologists all over the world during the past two decades, because of its efficacy against certain pests. *Azadirachta indica* products have revealed that some of them are

effective against insects and nematodes (Holyoke and Reese (1987), Skul, (1992), Byomakesh, Padhi & Dash (1998), Khanna and Sharma, (1998), Nanjegowda, Naik, Ravi, Reddy & Kumar, (1998) and Sharma (2000). *Azadirachta indica* oils affect at least 200 insect species some of which are resistant to conventional pesticides, it contains essential compounds that have been proven to have biological activity on insect pest behaviour, feeding, fumigant toxicity, knockdown activity and lethal toxicity via contact (Isman, 2000).

The insecticidal properties of *Azadirachta indica* products were first reported by Chopra (1956). When he observed that *Azadirachta indica* oil *in vitro* studies exerted an antibacterial effect and antifungal action against numerous pests.

Plant viruses also pose some of the most threats to world agriculture. Several successful test of *Azadirachta indica* against insect vectors of plant virus have been performed. Neem leaf extracts reduced the transmission of tobacco mosaic, a virus that seriously affects several vegetable crops (Sivakumar & Gunassekaran, 2011).

All parts of *Azadirachta indica*has been used as seed-coating and bare-root-dip treatments against nematodes Akhtar, Yeoung and Isman, (2008). Water extracts of *Azadirachta indica* has been identified to have nematicidal properties and have proven to better control soil pests, especially soil parasitic nematodes and also provides soil nutrients (Agbenin 2005 andAgyaiko, Kwakye, Bonso, Osie & Frimpong, 2006).

Neem based formulations and azadirachtin significantly suppress root-knot nematode (Meloidogyne incognita), on cucumber and cyst nematode

(*Globodera rostochiensis*), on potato Trifonova and Atansov (2011) The seed and leaf extracts of neem (*Azadirachta indica* A. Juss) caused 100% juvenile mortality of the root-knot nematodes and some free-living nematodes on potato.

Two concentrations of aqueous extracts of the neem leaves and seeds were evaluated on root-knot nematode, Meloidogyne incognita, on tomato plants. The neem extracts recorded reduction in nematodes population between 38 and 50% Taye, Sakhuja and Tefera (2012). Moreover, the use of dry neem leaves as incorporated into the soil reduced the root-knot nematode *Meloidogyne incognita* significantly and enhanced the weight of fruits/ plant by 19% of eggplants. Also, the neem leaf extract was effective against root-knot nematode *M. incognita* and inhibit the eggs hatching Khan, Mohiddin, Ejaz and Khan (2012a) Using formulated neem oil as seed treatment and seedling root dip against root knot nematode Meloidogyne incognita on tomato, chilli and brinjal was effective and reduced nematode population [92]. Besides, seed coatings with neem oil, neem formulations and products obtained from different plants, have also been used for the control of plant parasitic nematodes Sivakumar and Gunasekaran (2011); Ayeni and Adeleye (2011) and Javed *et al.*, 2009. The numbers of *Pratylenchus penetrans* and *Meloidogyne hapla* in tomato roots grown in 1% neem cake were reduced by 67 and 90%, respectively Abbasi, Riga, Conn and Lazarovits (2005) Active neem constituents can be absorbed through plant roots and systemically move upward through the plant's xylem tissues Gill and Lewis (1971); Nisbet, Woodford, Strang and Connoly (1993), which mean that it could be used to

manage plant parasitic nematodes as soil application, especially against those plants' root feeders.

On the other hand, in certain reports, the nematicidal mechanisms of neem were suggested and concluded that the involvement of phenolic compounds absorbed systemically by the roots of tomato plant might have induced tolerance against nematodes Mohan (2011). The narcotic effect of neem formulations could be due to by-products (ammonia, formaldehyde, phenols and fatty acids), released during their decomposition Khan, Alam and Ahmad (1974). It was claimed that direct toxicity of neem formulations due to nimbin, salanine, thionemone, azadirechtin and nimbidine Devakumar, Goswami, Mukherjee (1985). The neem leaf extract inhibited the eggs hatching of root-knot nematode *in vitro* Agbenin (2009). On the other hand, soil amended with oil-cakes of neem and other plant products have been successfully used for the control of plant-parasitic nematodes Alam (1990) and Mohan (2011). Whilst, fewer juveniles penetrated the roots of plants raised in neem cake amended soil compared to untreated plants Alam, Ahmad and Khan (1980).

Soil amended with plant parts from the *Azadirachta indica* tree inhibited root-knot nematode development the plant extracts provide nitrogen in a slow release form in addition to protecting plants against parasitic nematodes. The extract provides compounds that stimulate production of oxygen radicals which block the metabolic path ways of the nematodes (Gommers, Bakker & Nymbrg, (1982). Some of these compounds are synthetic metabolites and are more lethal to plant pathogens including nematodes, allowing better plant growth (Yasmin, Rashid, Uddin, Hossain & Ahmed, 2003). Similarly, *Azadirachta indica* cake, made from crushed *Azadirachta indica* seeds,

alsoprovides nitrogen in a slow-release form in addition to protecting plants against parasitic nematodes. It can be mixed with fertilizers such as composted manures, seaweed, and kelp. *Azadirachta indica* cake is toxic to plant-parasitic nematodes and not as detrimental to beneficial free-living soil organisms (Riga & Lazarovits, 2001).

Chemical analysis of plant tissues has shown that plants growing in *Azadirachta indica* cake treated soils contain greater concentrations of phenol and frequently also amino-acids, proteins and carbonhydrates. Phenolics content found in*Azadirachta indica* leaf has been reported by several researchers to be toxic to insects, fungi, bacteria, nematodes and weeds (Wu, Pratley & Haig, 2001; Chitwood, 2002; Simmonds, 2003 Simmonds & Stevenson, 2001; Koul, 2008; Carlsen & Fomsgaard, 2008; Popa, Dumitru, Volf,& Anghel, 2008). In addition, treatment with neem cake increases the content of phenolic compounds in the soil (Ahmad & Kkan, 1980).

Azadirachtaindica is a mixture of more than 100 limonoid compounds, including azadirachtin, salannin, and nimbin and their analogues provoking repellence, feeding deterrence and insect growth inhibition (Schmutterer, 1990). Limonoid triterpenes possess insecticidal and antifungal properties (Carpinella, Giorda, Ferrayoli& Palacios, 2003 and Akhtar *et al*., 2008). About one third ofLimonoids known today are obtained from Meliaceae species (*Azadirachta indica* and *Melia azedarach*). Aldhous, (1992) reported that azadirachtin compounds contain molecules, which cause decrease effect in insect growth regulation and development, while hydroxyl-furan fragment causes the anti-feedant effects. Recently it has been observed that azadirachtin provokes a rapid increase in the mitotic index of insect cells, induces the

appearance of many aberrant mitotic figures and prevents to some extent the polymerisatrion in vitro of mammalian tubulin (Salehzadeh *et al*., 2003).

Azadirachta indica also contains *Glucosinolates and Isothiocyanates* compounds which contain sulphur and nitrogen. Incorporation of glucosinolate containing plant material in soil, their bioactive hydrolysis products, named isothiocyanates are releasedVig, Rampal, Thind&Arora, (2009). These products can be used to control soil pests and weeds, a practice known as biofumigation. This practice is considered an ecological substitution of the soil fumigation with toxic fumigants, used in the past to suppress soil fungus, bacteria, nematodes and weeds, since it is considered fully biodegradable and less toxicand when released into the soil trigger the plant's defense mechanism produce toxins that kill the target organisms, and produce defensive barriers around the roots of the host plant thus preventing the harmful fungi from entering the host and in fungus *Isothiocyanates* inhibit oxygen uptake (Vig *et al*., 2009).

Polyacetylenes and *Polythienyls* compounds are also known to be present in *Azadirachtaindica*and possess insecticidal and nematicidal properties (Wat *et al*., 1981 and Chitwood, 2002).

Azadirachtin is the most active insecticidal component of *Azadirachta indica*, with a yield of about 5g from 2kg of seeds (Nanduri, Thunuguntla & Nyavanandi, 2003, Sastry, Suresh & Hari, 2006, Bandyopadhyay, Biswas & Chatterjee, 2002 and Genupur, Jesu, Srinivasan, Kamalakaran & Sundar, 2006). Azadirachtin proved highly active against *Meloidogyne incognita* in tomato plants, which reduced galls, egg masses and juveniles. Meanwhile, azadirachtin (Achook® 0.15% EC) recorded reduction in the presence of galls

on plant roots and juveniles in soil. Several reports found that azadirachtin, and/or neem extracts enhanced the plant growth, and increased the yield in different crops Trifonova and Atansov (2011). The actions of azadirachtin as insecticide are based on multiactions pathways such as toxicity, anti-mitotic effects, antifeedant activity, insect growth regulator, fecundity suppression, sterilization, oviposition repellency, including harmful effects on endocrine system and damages of the cuticle of larvae, preventing them from moulting Howard *et al.,* 2009. Azadirachtin is considered strong anti-feedant because of its effects on the insect's chemoreceptors, which deter the insect from consuming the plant. Moreover, azadirachtin not only blocks peptide hormone release that cause molting abnormalities, but also cause damage in insect's tissues, including muscle, fat and gut cells Aerts and Mordue (1997).

All parts of the tree yield a compound called beta-sitosterol. The leaves also contain quercetin, gallic acid, catechin, carotenes, and ascorbic acid, (Subapriya & Nagini, 2005). Low concentrations of aflatoxin in *Azadirachtaindica*leaves have been reported by several researchers (Boeke, Boersma & Alink, 2004 andDhongade, Kavade & Damle 2008).

Bryophyllum Pinnatum

Bryophyllumpinnatum (Crassulaceae) is commonly known as air plant, love plant, miracle leaf or life plant depending on the area it is found and has been accepted as a herbal remedy in almost all parts of the world, (Gupta, Lohani & Arora, 2010). It is a crassulescent herb of about 1 metre in height, with opposite, glabrous leaves (with 3–5 deeply crenulated, fleshy leaflets) distributed worldwide but growing primarily in the rain forest (Ursula,

Nathalie, Lukas & Roland, 2006). It grows widely and used is as folk medicine in tropical Africa, India, China, Australia, and tropical America, Madagascar, Asia and Hawaii. It is astringent, sour in taste, sweet in the post digestive effect. It is well known for its haemostatic and wound healing properties (Nielsen, Olsen & Moller., 2005, Lans, 2006).

The plant has gained considerable attention for their medicinal properties and finds application in folk medicine, as well as in contemporary medicine (Kamboj & Saluja, 2009). Preliminary phytochemical investigation of different parts of plant extracts of *B. pinnatum* shown that presence of alkaloids, phenols, flavonoids, saponins, tannins, carotenoids, glycosides and bufadienolides (Hossan & Yemitan, 2009). Saponins compound contains hydrophilic carbohydrates that provide them with surfactant properties, but they possess also significant anti-feedant and nematicidal properties (Chitwood, 2002; Koul, 2008 and Duke *et al*., 2003).

The Alkaloids bind to postsynaptic receptors and interfer with the transmission of signals in nerves, leading to a continuous firing of the neuro-receptor (Regnault & Philogéne, 2008). Flavonoids compounds, are distributed widely in vascular plants and Bryophytes and have been reported to possess feeding attractant and deterrent, antioxidant, and anti-inflammatory activity (Okwu, 2004). This shows that *Bryophyllumpinnatum* contain appreciable amount of bioactive compounds.

The bufadienolides which are active components of *Bryophyllumpinnatum* are known to possess antibacterial, anti-tumorous, cancer preventive and insecticidal actions. Bufadienolide (bryophillin C) compound have been

proven to have insecticidal properties (Supratman, Fujita, Akiyama & Hayashi., 2000).

The aqueous leaf extract from the medicinal plant *B. pinnata* (Crasaceae) contains compounds known to have anti leishmania activity (Misra & Dixit, 1979). The leaves are found to contain various chemical constituents including bryophyllin B (Quazi, Sayyed, Sheikh, Gomase& Choudhari, 2011) and Bryophyllin C (Veitch & Grayer 2007) which are known to have potent biological activity.Bufadienolides: bryophyllin A and bryophyllin C from *B.*(*pinnatum*) showed strong insecticidal activity against third instar larvae of the silkworm (Veitch & Grayer 2007). Tannins in the crude extract of *B. pinnata* have shown anthelmentic activity. The results reveal that chloroform; methanolic and aqueous extract of *B. pinnata* not only demonstrated paralysis but also caused deaths of worms (Majaz, Nazim, Asir, Shoeb & Bilal, 2011).

Khaya Senegalensis

The *Khaya senegalensis* commonly known as mahogany belongs to the *Meliaceae* and the genus *Swietenia,* and is part of the chinaberry family. The genus *Khaya* (A) Juss is the main source of African *Khaya senegalensis*, and is closely related to the South American genus Swietenia, the original source of *Khaya senegalensis*. The bark is very bitter and is traditionally used for medicinal purposes (Taylor, Adesogan &Chem, 2010). The fruit contains flavonoids and saponins.

Pharmacological screening has led to the conclusion that the essential oil of *Khaya senegalensis* (meliaceae) has an anti-inflammatory and partial antibacterial activity. The essential oils affect several targets at the same time,

because of their great number of constituents; this decreases the ability of the target organism to develop resistance or against them. Essential oils induce cytotoxicity, damage the cellular and organelle membranes, act as pro-oxidants on proteins and DNA and produce reactive oxygen species. Such activity is mostly induced by phenols, aldehydes and alcohols. In some cases essential oils and their components have demonstrated nuclear and cytoplasmic mutagenicity, acting on mitochondria and the respiratory system Bakkali, Averbeck, Averbeck and Idaomar (2008).

LD_{50} tests showed the seed oil is nontoxic to human. Some limonoids have been isolated from the stems, barks, leaves and flowers of *K. senegalensis*. They include phragmalin limonoids (khayanolides D and E), khayanosides, 2, 6-dihydrofissinolide and two mexicanolides named khayanone and 2-hydroxysenega- nolide (Nakatani et *al*., 2001, 2002). Abdelgaleil, Iwagawa, Doe & Nakatani (2004) also reported the isolation of three other limonoids compounds (seneganolide A), 2-hydroxyseneganolide A and 2-acetoxyseneganolide A. These limonoids have a wide range of biological activities, including insect antifeeding and growth regulating properties, and medicinal activities in humans and animals. They also possess antiviral, antifungal and anti bactericidal properties (Abdelgaleil *et al*., 2001; Abdelgaleil and Nakatani, 2003; Ademola, Fagbemi & Idowu, 2004). Phytochemical analyses of the ethanol leaf extract of *Khaya senegalensis* confirm the presence of the various classes of active chemical constituents including saponnins, flavonoids, tannins, alkaloids glycosides and carbohydrates and some of these active constituents have activity against micro-organisms. The tannin found in *Khaya senegalensis* have being used as

anthelmintics, antioxidants, antimicrobials, antiviral properties. The presences of these active chemical constituents are therefore indicators that the leaf extracts of *Khaya senegalensis* in posses antimicrobial activity (Ribera, Cotoras & Zúñiga, 2008 and Martin & Magunacelaya, 2005). The compound aldehyde furfural found in the leaves of *Khaya senegalensis* was the foremost nematicidal principle, exhibiting activity similar to that of the commercial nematicide fosthiazate. It is known to possess high nematicidal fumigant activity against *M. incognita*, tested in greenhouse and micro plot conditions (Rodriguez, Kabana, Morgan-Jones& Chet, 1993). The nematicidal activity of *Khaya senegalensis* reveals its potency of incorporation into IPM programs (Ntalli, Menkissoglu-Spiroudi & Giannakou, 2010).

Tectona Grandis

Tectona grandis commonly known as teak belongs to the *Lamiaceae* family and the genus *Tectona.* This species naturally occurs in deciduous forest, but is planted commonly along roadsides and in large plantations throughout the tropics.

It is commonly found in India and other South-East Asian countries (Kjaer, Lauridsen & Wellendorf, 1995).

All parts of *Tectona grandis* tree contain some chemical constituents. Tectoleafquinone **(**I,4,5,8-tetrahydroxy-2-isopentadienyal nthraquinone) and betulinic acid are found within the leaves of *Tectona grandis*; p-sitosterol, P-sitosterol glucoside, betulinic acid found in the stem bark; tectoquinone (2-methylanthraquinone) found in the wood and oil; betulinic acid, 2-methyl-mthraquinone (tectoquinone). Compounds like caoutchouc, squalene,

desoxylapachol, 1,4-dihydroxytectoquinone are fatty acids of kernel oil of *Tectona grandis*, y, y-dimethyl-l,4-naphthaquinone,2 (3,3-dimethylally1)-1, 4 naphthaquinone, 0-toly methyl ether, 1-4 polyisoprene, lapachonone, tectol, lapachol and dehydrotectol 2-hydroxymethyl anthraquinone and anthraquinone-2-carboxylic acid, tectoquinine, resin, anthraquinone-2-carboxaldehyde are chemical constituents found in the heart wood of *Tectona grandis*; 5-hydroxylapachol, lapachol, dehydro-a-lapachone, methylquinizarin, squalene are chemical constituents of the roots of *Tectona grandis*; anthraquinones, naphthaquinones, quinones, neutral compounds with naphthalene rings, fatty acids, squalene, betulic acid, a triterpene and triterpenoids are also chemical constituents of the wood of *Tectona grandis*; tectoquinone, caprylic, capric, lauric, mysistic, palmitic, stearic, oleic, linoleic acids are chemical constituents found in seeds of *Tectona grandis* (Dayal & Seshadri, 1979 and Anonymous, 1990).

The chemical constituents Tectoleaf quinone and betulinic acid of *Tectona grandis* leaves contains pharmacological activities like antiulcer, anti-inflammatory, antifungal, juvenoid activity, inter-ceptory activity, antimicrobial and cytotoxic activities Anonymous (2001). The leaves have antibacterial, antiulcer, antifungal, anthelmintic, and anti-inflammatory properties, (Oudhia, 2003).

Compounds such as lapachol, methyl quinizarin and squalane have been isolated from *Tectona grandis*, and have cytotoxic effect in experimental animals (Goel, Pathak, Biswas, Pandey & Sandal, 1987). Ethyl acetate extracted compound from Pyrethrum is a compound found in *Tectona grandis* and consists of mixture of substances that have the ability of controlling a

wide range of insects and mites. Affected insect presents hyperactivity followed by convulsions and finally death. Pyrethrins have a rapid knockdown effect but are known to have great instability to light, air, and moisture thus reducing considerably the risks related to their use, (Isman, 2008).

*Tectona grandis*has essential oils (Sherifat, Akinsola & Guido, 2013) and the biological activity of essential of oils and their components on contact with pest insects, comprising behaviour and feeding deterrance effects, fumigant toxicity, knockdown activity and lethal toxicity have been observed (Isman, 2000).

Vernonia Amygdalina

*Vernoniaamygdalina*commonly called bitter leaf is a tropical plant belonging to the family Compositae.It is used widely as vegetable and medicinal plant (Ibrahim, Abdurahman & Ibrahim, 2000). This plant contains complex active components that are pharmacologically useful. One of the most common medicinal uses of *Vernonia amygdalina* is in the treatment against intestinal worms including nematodes. Not only humans but also chimpanzees ingest the bitter pith of *Vernonia amygdalina* for the control of intestinal nematode infections (Huffman, 2003). The characteristic bitter taste of *Vernonia amygdalina*has been attributed to compounds such as alkaloids, saponins, tannins and glycosides.

Vernonia amygdalina extracts has been proven to have antibiotic action against drug resistant microorganisms and possess antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities. Sesquiterpene lactones (vernodalin, vernolepin and vernomygdin) and steroid glucosides

(vernoniosides) compounds of *Vernonia amygdalina* leaf have reported to have significant anti-parasitic activity, especially vernodalin and vernonioside B1. Vernodalin and vernomygdin have cytostatic activity.Such cytostaticaction against human breast cancer cells have been observed in aqueous extracts of *Vernonia amygdalina* (Kupchan, Hemingway, Karim & Werner, 1969).

Vernonia amygdalina is a control agent against diseases in plants. The ash from burnt branches has been used to control seed-borne fungi (*Curvularia*, *Aspergillus*, *Fusarium* and *Penicillium* spp.) thus ameliorating seed viability and germination capacity (Ibekwe, Nnanyere & Akujobi, 2001; Dutta, 1993).

Plants species belonging to the family Compositae contains saponins, flavonoids, tannins and anthraquinones was found to have very potent antibacterial as well as antifungal activitiesOgundare, Adetuyi and Akinyosoye (2006). These phytochemical constituents were further reported to be responsible for many antimicrobial activities of different plant species (Ghoshal, Prasad & Lakshmi, 1996 and Iwu, Duncan & Okunji, 1999). Flavonoids have been reported to be synthesized by plants in response to microbial infections and are good antibacterial agents; tannins have been demonstrated to have antibacterial activities (Akiyama, Fijii, Yamaski, Oono & Iwatsuki, 2001). D'Addabbo *et al.*, (2010) has proven that Saponins contains hydrophilic carbohydrates that provide them with surfactant properties, but they possess also significant anti-feedant, fungicidal and nematicidal properties. *Cyanogenic glycosides* compounds found in *Vernonia amygdalina* are known to be present in more than 2500 plant species. When applied to soil, they play a major role in plant defense against herbivores due

to their bitter taste and release of toxic hydrogen cyanide. They release toxins that cause tissue disruption that suppress insects, fungus, nematodes and weeds development (Zagrobely *etal*., 2004; Morant *etal*., 2007; Bjarnholt, Laegdsmand, Hansen, Jacobsend& Møller, 2008; Carlsen & Fomsgaard, 2008). *Vernonia amygdalina* also contains alkaloids compounds. Research has proven that they contain nitrogen atoms, and derives from various botanical families amongst which are the Solacaneae,and nicotine is undoubtedly the oldest alkaloid used in agriculture as well as the one of the first molecules used as insecticide. It is an acetylcholine mimic binding to postsynaptic receptors and interfering with the transmission of signals in nerves, leading to a continuous firing of the neuroreceptor. This overstimulation leads to depression of the central nervous system (Regnault & Philogéne, 2008).

CHAPTER THREE

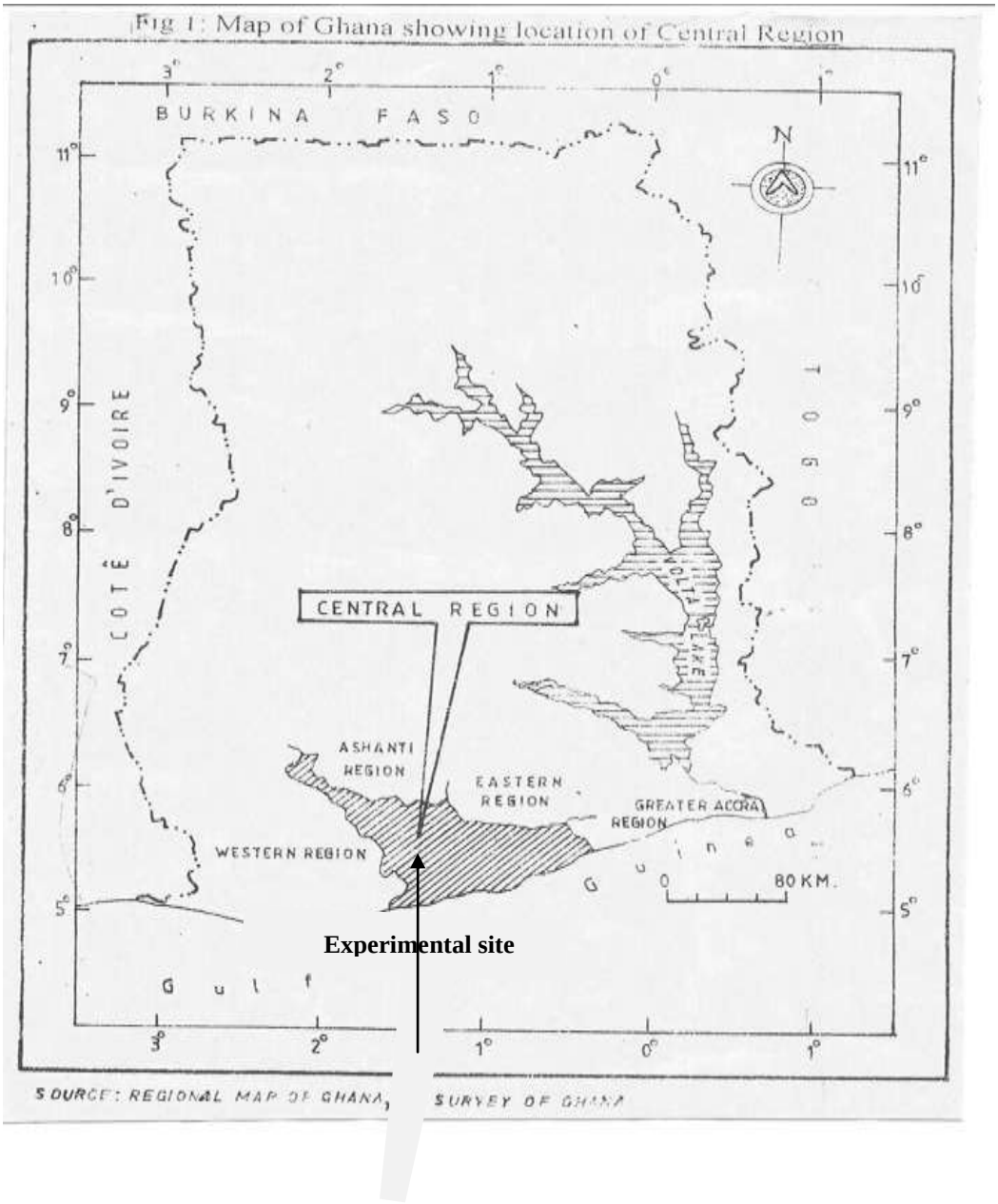
MATERIALS AND METHODS

Three studies were carried out. The first was a laboratory experiment to evaluate the effect of extracts from fresh leaves of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* on eggs and hatchability of juveniles nematicidal activity against second stage of *Meloidogyne*spp; the second was conducted in the field to evaluate the effect of aqueousleaf extracts of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* applied to soil as drench on shoot length, root length, number of gallsandyield of tomato and the third was conducted in potsto evaluate the application of leaf extracts of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* as mulch and soil admixture on growth and severity of nematode infection on tomato plants.

Experimental Site

The project was carried out at the Crop Science laboratory of the School of Agriculture and at the School of Agriculture Teaching and Research Farm of the University of Cape Coast, between July 2014 to April 2015. The site falls within the coastal savannah zones in the Central Region of Ghana (fig.1). The soil is well-drained, slightly acid soil (pH 5.5-6.5), deep and friable. It is sandy clay loam belonging to Atabadzi series (Asomah, 1973). Soil pH at planting was 6.5. The major rainy season is within March and July with the maximum in June while the minor rainfall occurs in September and November with the maximum in October. The coolest month is August. Abban (1985) also

reported that the temperatures are almost uniformly high throughout the year with mean annual minimum of about 25^0C.



Central Region

Figure 1: Map of Ghana Showing Location of the Experimental Site in the Central Region

Laboratory Studies

The laboratory studies were conducted in the laboratory of the School of Agriculture department of Crop science, University of Cape Coast. They were carried out to evaluate the effect of extracts from fresh leaves of *Azadirachta indica*, *Bryophyllum pinnatum*, *Khaya senegalensis*, *Tectona grandis* and *Vernonia amygdalina*on eggs and hatchability of juveniles nematicidal activity against second stage of *Meloidogyne* spp.

Source of Nematode Inoculum

For the laboratory studies, a culture of *Meloidogyne* spp was prepared from tomato plants infected with root-knot nematode collected from an infected plot on the Teaching and Research Farm of the School of Agriculture, University of Cape Coast.

Thirty tomato plants were collected at random from the infested field. In order to get all roots of the infected plants, the plants were watered and the soil soaked for ten minutes to ensure easy up-rooting of plants. The plants were slowly up-rooted, washed with tap water to removed adhering soils and were placed into paper bags and taken to the Crop Science Laboratory of the University of Cape Coast for extraction. Eggs were first extracted from the infested tomaato roots by a modified Hussey and Barker (1973) method. The washed roots of the tomato plants were chopped into pieces of about one centimeter in length with a pair of scissors. Ten grams of the chopped roots was put in an uncovered jar and enough of 0.5% sodium hypochlorite poured on them for four minutes. The resultant sodium hypochlorite-roots suspension was quickly passed through a 200 mesh sieve over 600 mesh sieve. Eggs

collected on the fine sieve were rinsed with tap water to remove the sodium hypochlorite. The roots were further rinsed with tap water to remove additional eggs which were collected by sieving. The collected eggs were topped with water to obtain the egg-water suspension and were kept in water at 10^0C in a refrigerator to prevent hatching.

Number of eggs in aqueous suspension was determined using a stereo microscope (45X). One milliliter of the egg-water suspension was pipetted after bubbling air through the suspension for homogenecity and dispensed into a petri dish with scored lines to prevent repeated counting of individuals. Counting was done three times and the mean number of eggs/ml estimated.

Plate 1: Counting *Meloidogyne* eggs and juveniles under stereomicroscope (Magnification: x3)

Acquisition of Leaves of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* for the Studies

Leaves were collected from mature plants of *Vernonia amygodalina* (Bitter leaf) *Bryophyllum pinnatum* (Life plant), *Azadirachta indica* (Neem) and*Tectona grandis* (Teak) at the School of Agriculture Teaching and Research farm, University of Cape Coast while leaves of *Khaya senegalensis* (Mahogany) were collected from the campus of the University. All botanicals studied were collected on the same day (August 2, 2014) during the early morning hours (6am-9am).

Toxicity Studies

These studies were carried out to find the concentrations of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* that were effective on *Meloidogyne* eggs and juveniles in the laboratory with the view of evaluating the most effective concentration of each of the botanicals in field experiment. The pot experiment was an evaluation of application of leaf extracts of *Azadirachtaindica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* as mulch and admixture on the growth and severity of *Meloidogyne* inoculated tomato plants.

Preparation of Aqeous Leaf Extracts of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina*

The aqueous leaf extracts of the five botanicals were obtained using the methods described by Adegbite & Adesiyan (2005) and Orisajo, Okeniyi,

Fademi and Dongo, 2007. The leaves of each of the botanicals were washed with tap water. The extracts were obtained by blending 20g of fresh leaves of each of the five botanicals species in an electric blender separately and 100 ml of distilled water was added in a 500 ml flask, mixed for two minutes and filtered through a cheese cloth and kept for 24 hours. Each extract was considered as a standard solution "S" (100% concentration equivalent to 100ml) and then kept in the refrigerator until use for laboratory studies. Suspensions of three concentrations of each of the five botanicals namely, 15%, 20% and 25% were prepared with distilled water (Orisajo *et al*., 2007).

Plate 2: Aqueous leaf extracts of Botanicals in beaker preparation (Magnification: x2)

Screening of Aqueous Leaf Extracts of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* for Egg Hatchability and Nematicidal Activity against Second Juveniles of *Meloidogyne* Spp

Hatchability Test

This test was conducted at the Crop Science Laboratory at the University of Cape Coast. For each of the botanicals, a suspension of *Meloidogyne* eggs were obtained by the modified method of Hussey and Barker (1973) one milliliter of the egg suspension (40-55 eggs/ml) was transferred into Petri dishes and allowed to settle at room temperature. The water was pipetted out and immediately replaced by 15%, 20% and 25% of standard solutions of the extracts respectively. The experiment was lain out in a completely randomized designwith three replicates. The Petri dish containing egg suspension and distilled water served as control (Muhammad, 2008). Observations were made daily. After three days of exposure, the number of eggs hatched were counted using a fine needle under a low power (4X) stereomicroscope and recorded.

The number of eggs hatched were observed under stereomicroscope of 45X and counted seventy two hours after the application of treatments. The formular calculating hatchability was:

$$\text{Hatchability} = \frac{\text{\# of initial egg - \# of juveniles}}{\text{Initial \# of egg}}$$

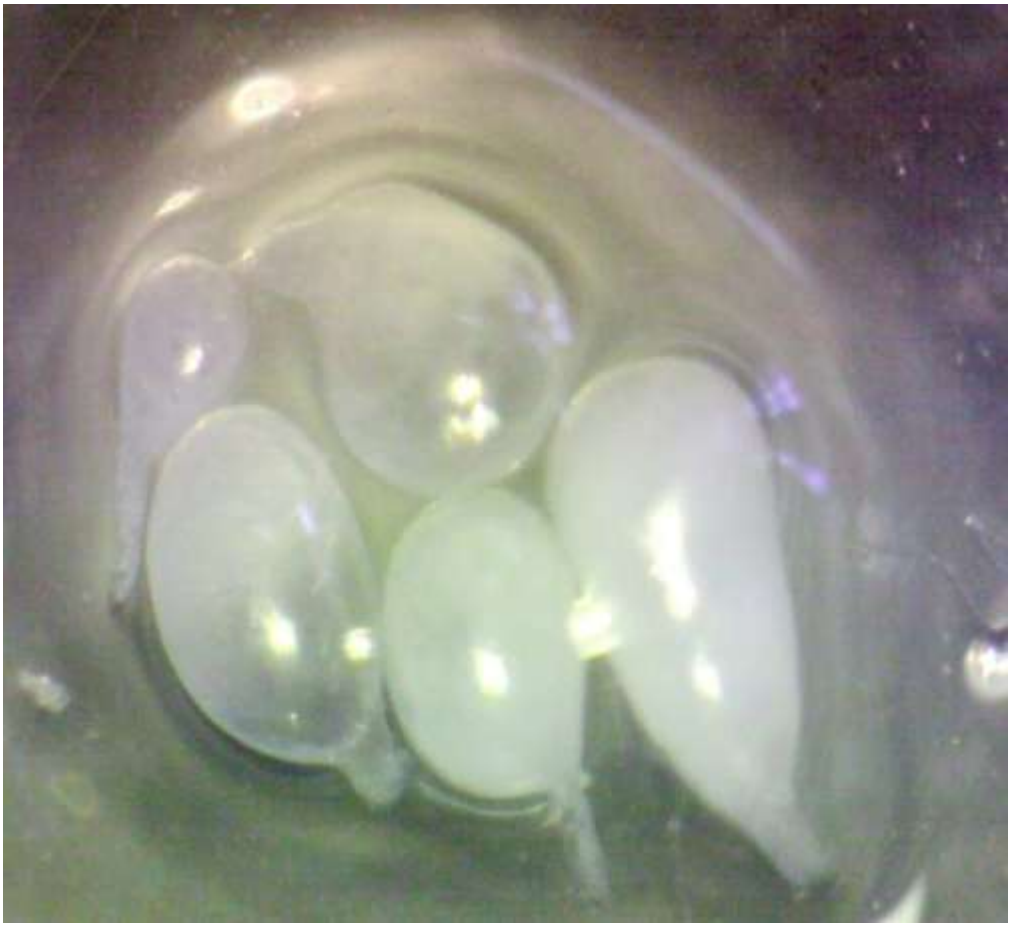


Plate 3: Meloidogyne female with eggs extracted from tomato roots at thecrop science lab (Magnification: x3)

Data Analysis

Analysis of variance($P<0.05$) was doneusing GenStat statistical package to test significance of the effect of the botanicals on egg hatchability of *Meloidogyne* spp. Duncan's multiple range test was used to compare means**.**

Mortality Test

This test was carried out at the Crop Science Laboratory of the University of Cape Coast. Five uniformly sized egg masses of *Meloidogyne*collected from infested tomato roots were transferred into sterilized Petri plates (5 cm

diameter) containing sterile distilled water. The experiment was laid out in completely randomized design (CRD) with three replications. The Petri plates containing the egg masses were counted using stereomicroscope and kept at room temperature (30 ± 2^0C) on laboratory bench to allow hatching. Second stage juveniles (J), obtained from the egg masses, were kept in sterile distilled water in cavity blocks, each containing 100 ± 10 J. Water was pipette out and immediately replaced by 15%, 20%,and 25%, of standard solution of the respective treatments, while juveniles in distilled water only served as control (Alam, 1985). Observation was made hourly and data was recorded. The *Meloidogyne* juveniles were observed under a light microscope and the immobile juveniles transferred to plate wells with tap water, which were placed on laboratory bench for 24 hour. The juveniles that did not recover their motility after incubation for 24 hours in water were counted as dead. They were those juveniles which could not move when probed with fine needle.

The number of dead juveniles was recorded 6 hour, 12 hour and 24 hour, after the application of various concentrations of the five botanicals.

Mortality=:$\frac{Dead\ Juveniles}{Total\ \#\ initial\ live\ Juveniles}\ x\ 100\%$

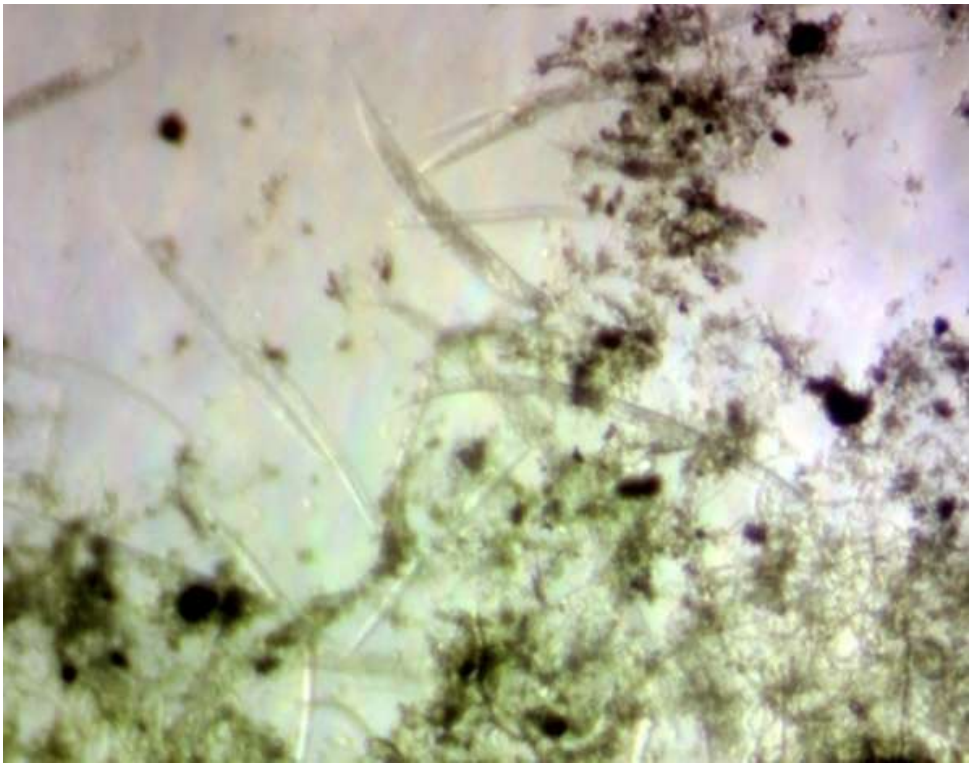


Plate 4: *Meloidogyne* juveniles in plant extract (Magnification: x3)

Data Analysis

Analysis of variance ($P<0.05$) was done using GenStat statistical package to test significance of the effect of the botanicals on second stage juvenile of *Meloidogyne* spp. Duncan's multiple range test was used to compare means**.**

Screening of Aqueous Leaf Extracts of *Azadirachta Indica, Bryophyllum Pinnatum, Khaya Senegalensis, Tectona Grandis* and *Vernonia Amygdalina Meloidogyne* Spp under Field Condition

The field experiment was carried out at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast to evaluate in the field the various concentrations of the five botanicals which were studied in the

laboratory. The aim of the experiment was to observe the effect of soil conditions on the toxicity of the five botanicals on *Meloidogyne* spp.

The field used for the study had 64 mature tomato plants which were uprooted one month before the experiment was carried out. A survey was however carried out to ascertain the levels of *Meloidogyne* juvenile and eggs in the study area. Both soil samples and infected tomato plants were collected and sent to the laboratory for an assay.*Meloidogyne* juveniles were extracted from soil using a modified Bearman's Tray technique while *Meloidogyne* infected tomato root galls were assessed for disease incidence and severity using scale as described byTaylor and Sasser.

The selected site of 109m in length and a width of 13m were marked off with a measuring tape. Ten tomato plants were selected at random across the field for root-knot infection assessment on the scale of 0-5 (Taylor & Sasser, 1978). Taylor and Sasser reported that infection rating based on root galling and nematode reproduction should be a good criterion because there is generally a positive correlation between nematode reproduction and crop damage.

Key for assessing nematode population

0= Free from galls

1= 1-2galls or egg masses

2= 3-10 galls or eggmasses

3= 11-30 galls or eggmasses

4= 31-100 galls or eggmasses

5>100 galls or eggmasses

Plate 5: Nematode infested tomato roots with root knots collected from infested field (Magnification: x3)

Nematode Extraction

The soil samples collected were put together and mixed to ensure homogenecity. A sample of 100cc of the mixed soil was taken for nematode extraction using a modified Bearman's Tray Technique. The modified technique involved a plastic sieve of diameter 25 cm and an aluminum plate. The plastic sieve was lined with a double ply papper, and placed in the aluminum plate. Two set ups were made and 100cc of the mixed soil from the experimental area was divided into two. The two halves of the soil were separately placed on the tissue paper in each set up and the tissue paper edges were fold above the top of the soil to prevent drying. Water was poured next

into the aluminum plate using a beaker and was absorbed through the tissue paper until the soil samples were moist and adequate amount of water was left in the aluminum plate for 72 hours. The wet state of the soil made live nematodes active and aid movement in the soil, some passed through the tissue paper and were trapped in the water in the aluminum plate. The water in the aluminum plate containing active nematodes was emptied into test tubes for six hours for nematodes to settle at the bottom. The top half of the water in the test tube was then poured out and the bottom half poured into a petri dish.

The petri dish was marked into four quadrants and numbered from one to four as described by (Daykin & Hussey, 1985) and nematodes counted. The same procedure was followed for the second set up. Nematodes were counted starting from quarter one through four under a stereoscopic microscope at a magnification of 30 to 45X. The objective was focused with care in order to pin point floating nematodes. The number of nematodes counted in the two set up were added together to get the total number of juveniles in the 100cc of soil for the experimental area.

Experimental Design and Field Layout

The field experiment was a Randomized Complete Block Design. The treatments consisted of two different concentrations (20% and 25%) of each aqueous extract of the five botanicals,a synthetic nematicide (Furadan) and an untreated control. There were five replications.

An experimental plot was 11 m long and 6 m wide and 1 m apart between plots. Each plot contained two planting rows and each row had five plants giving a total of ten plants per plot. The spacing between plots and between

blocks was 1 m each. The experimental field has long history of root-knot nematode infestation but to have a uniform distribution of root-knot nematode in the field, the field was artificially infested with chopped roots of nematode infested tomato plants spread uniformly at $10g/m^2$. Sowing was carried out in the month in January 2015 and harvesting occurring in April of 2015.

Four week old tomato seedlings (cv. Wosowoso) were transplanted and the extracts applied as soil drench around the root of the seedlings. Furadan (carboforan) treatment was applied (Basal ring method) at $5g/m^2$plant was done before transplanting.

Root samples were also collected from all experimental plots and assessed. Four soil samples were collected from each plot and taken for nematode extraction using sieving method as described by Hussey and Barker (1973) and count.

The data collected on tomato plants were:

Number of fruit per treatment

Nematode population per plant

Mean Shoot length per plant

Mean Root length per plant

Number of galls per plant

Data Analysis

Data were analysed using Analysis of variance ($P<0.05$) and Duncan's multiple range test was used to compare means**.**Data collected on galls was transformed using square root transformation and data collected on nematode population in soils was transformed by log transformation.

Screening of Leaf Extracts of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* Pathogenecity of *Meloidogyne* Spp

The experiment was carried out to evaluate the pathogenecity of *Meloidogyne.* A complete randomized block design with five replications was used. There were 12 treatments including two controls.

The treatments non inoculated soils, anuninoculated but untreated soil and aninoculated but untreated soil consisted of each of the five botanicals used as mulch and soil admixture.

A total of 60 perforated plastic buckets of 25cm in diameter were used for the pot study. Soils was first sieved to remove all debris and then sterilized for 15 minutes with hot boiling water to ensure nematode free status before inoculation.

Seeds of tomato variety, Wosowoso, were planted and nursed for four weeks and transplanted as a seedling per bucket. When the tomato seedlings were established in buckets they were inoculated with *Meloidogyne* juveniles at the rate of 100 ± 10 juveniles per plant. The inoculation was done by first removing the top soil around the seedling in a bucket and 5ml of a suspension of the 100 ± 10 juveniles in water injected into the soil around the seedling using a 5ml pipette. The top soil around each seedling which was initially removed was placed back.

Ten days after the inoculation, the treatments which were in the form of mulch and soil-admixture of each of the botanicals were randomly applied to the seedlings. For the mulch and soil-admixture treatments, 20g of the fresh leaves of each of each of the five botanicals was applied either asmulch or as

admixture. As mulch, the leaves were spread over the soil in the bucket close to the seedling while for theadmixture the leaves were groung and mixed with the soil in the bucket.

The controls were buckets with seedlings inoculated but not treated with any of the botanicals; and the other was the tomato seedling which were neither inoculated nor treated with botanicals. These types of control were included in the experiment to observe their performances relative to those of the inoculated tomato seedling treated with the various botanicals.

The tomato plants were watered regularly and after 50 days they were uprooted for data collection on the following parameters:

1. Shoot length perplant
2. Root length perplant
3. Number of Flowers per plant
4. Number of root galls per plant

6A


6B 
6C

Plate 6

A Inoculated untreated tomato plant (Magnification: x3)

B Photograph showing root-knot nematode assay on tomato plant (Magnification: x2)

C *Meloidogyne* infection on Inoculated untreated tomato plant (Magnification: x3)

Data Analysis

Data were analysed using Analysis of variance ($P<0.05$) on all parameters and Duncan's multiple range test was used to compare means. Data collected on galls and flowers were transformed using square root transformation.

CHAPTER FOUR

RESULTS

Preliminary Study

A preliminary study of the experimental field clearly showed that the site which was chosen for the field evaluation of the botanicals, was infested with root-knot nematode *Meloidogyne* species. From the field survey the incidence and severity of the root-knot disease were 8 and 4 respectively. The nematode population was 95/100cc of soil.

Eggs Hatchability of *Meloidogyne* Spp Exposed to Three Concentrations of Aqueous Leaf Extracts of *Azadirachta indica, Bryophyllum pinnatum, Khaya senegalensis, Tectona grandis* and *Vernonia amygdalina* for 72 Hours

When eggs of *Meloidogyne* spp were exposed to various concentration of aqueous leaf extracts from five medicinal plants for 72hr, the egg hatchability are shown in Table 1.

Table 1: Percent *Meloidogyne* eggs hatch after 72hr Exposure to various Concentrations of Aqueous leaf extracts of *Azadirachta indica, Khaya senegalensis, Tectona grandis, Vernonia amygdalina* and *Bryophyllum pinnatum*

Conc. of Extracts (%)	Botanicals				
	Azadirachta indica	***Khaya senegalensis***	***Vernonia amygdalina***	***Tectona grandis***	***Bryophyllum pinnatum***
25%	2.33	4.33	6.67	7.67	8.33
20%	4.33	6.00	7.33	8.33	9.67
15%	6.66	7.33	8.00	9.67	11.33
Control (0%)	28.33	28.33	28.33	28.33	28.33
Lsd (5%)	2.11				

Values were transformed using $\sqrt{(x+0.5)}$, where x is the mean number of eggs.

Table 1 shows the effect of the extracts of the five botanicals on the hatchability of *Meloidogyne* eggs 72hr after exposer to various concentrations of aqueous extracts of five medicinal plants. From the Table (Table 1) the highest mean egg hatchability of 28.33 was in the control were no extract had been applied. Generally the hatchability of the *Meloidogyne* eggs in the extracts of the five botanicals across all the concentrations was significantly lower than that of the control. However, among the botanicals, hatchability of *Meloidogyne* eggs in *Azadirachta indica* extract was lowest across all concentrations and that in the extracts of *Bryophyllum pinnatum*in each of its concentrations was highest. Even though the differences in the hatchability of *Meloidogyne* eggs in the control and those in the different concentrations of

the botanicals were significant, the differences among the various concentrations of the botanicals were not significant except between 15% and 20% of *Azadirachtaindica*, *Khaya senegalensis*, and *Bryophyllum pinnatum*, at 15% and 25%. There were also variations in the potency of the botanicals in reducing hatchability. There was a gradual decrease in egg hatchability with increase in extract concentration (Table 1).

Juvenile Survival of *Meloidogyne* Spp Exposed to Three Concentrations of Aqueous Leaf Extracts of *Vernonia amygdalina, Bryophyllum pinnatum, Khaya senegalensis, Azadirachta indica* and *Tectona grandis* for 6 Hour, 12Hour and 24Hour

When juveniles of *Meloidogyne* spp were exposed to various concentration of aqueous leaf extracts from five medicinal plants for three exposure periods, the mortality are shown in Tables 2,3,4,5 and 6.

Effect of *Vernonia Amygdalina* Extract on *Meloidogyne* Juveniles after Treatment with Different Concentrations under Three Exposure Periods

Table 2: Mortality of *Meloidogyne* Juveniles after Treatment with Aqueous Plant Extracts

Conc.of plant Extracts (%)	% mortality of *Meloidogyne* juvenile in three exposure periods		
	6hrs	12hrs	24hrs
25%*Vernonia amygdalina*	35.76	42.32	54.54
20% *Vernonia amygdalina*	35.24	41.70	48.64
15%*Vernonia amygdalina*	31.06	34.21b	37.07
0% Control	0.00	0.00	6.22
Lsd (5%)	4.56	1.86	0.79

Values were transformed using √(x+0.5), where x is the mean number of juveniles. Table 2 shows the effect of *Vernonia amygdalina* extracts on the mortality of *Meloidogyne* juveniles during three different exposure periods in the laboratory, namely, 6hrs, 12hrs and 24hrs.

It is clear from the Table that, generally, juvenile mortality increased in *Vernonia amygdalina* extracts as the period of exposure increased from 6 hours to 12 hours and 24 hours across the three different concentrations. It is interesting to note that during the 6hr exposure period the effect of different concentrations of *Vernonia amygdalina* extract were not significantly different from each other. However, over the period of 12 hours juvenile mortality in both 20% and 25% of *Vernonia amygdalina* extracts were significantly different from that in the 15%, and after 24 hours of exposure, juvenile mortality in the

three concentrations of *Vernonia amygdalina* extract was significantly different from each other.

It can be seen from the Table 2 that juvenile mortality in all the different concentrations over the three exposure periods was significantly higher than those in the control.

Effect of *Bryophyllum pinnatum* Extract on *Meloidogyne* Juveniles after Treatment with Different Concentrations under Three Exposure Periods

Table 3: Mortality of *Meloidogyne* Juveniles after Treatment with Aqueous Plant Extracts

Conc.of plant Extracts (%)	% mortality of *Meloidogyne* juvenile in three exposure periods		
	6hrs	12hrs	24hrs
25%*Bryophyllum pinnatum*	35.37	42.70	48.97
20%*Bryophyllum pinnatum*	31.76	38.62	40.46
15%*Bryophyllum pinnatum*	31.06	33.39	35.45
0% Control	0.00	0.00	6.22
Lsd (5%)	4.56	1.86	0.79

Values were transformed using $\sqrt{(x+0.5)}$, where x is the mean number of juveniles. Table 3 shows the effect of *Bryophyllum pinnatum* extract on mortality of*Meloidogyne* juveniles during three exposure periods; 6 hours, 12 hours and 24 hours.

The Table shows that during each of the exposure periods, juvenile mortality at thethree different concentrations of *Bryophyllum pinnatum* was significantly different from those in the control treatment.

Generally, the juvenile mortality in all the concentrations was least during the 6 hour exposure period ranging from 31.06 to 35.37 and highest during the 24 hour exposure period ranging from 35.45 to 48.97. The juvenile mortality during the 12 hour exposure period ranging from 33.39 to 42.70 fell between the two estimates. After exposing the *Meloidogyne* juveniles to *Bryophyllum pinnatum* extract treatment for six hours, the highest percentmortality of 35.37 was recorded for *Bryophyllum pinnatum* extract whilst juveniles in the control recorded the least juvenile percent mortality of zero (Table 3). The mortality of *Meloidogyne* juvenile in the 25%, 20% and 15% *Bryophyllum pinnatum* extracts was significantly higher than that of the control (Table 3).

However, during the different hours of exposure in *Bryophyllum pinnatum* extracts, mortality of *Meloidogyne* juveniles in the 25% concentration recorded the highest percent mortality whilstmortality of *Meloidogyne* juveniles in the 15% concentration recorded the least percent mortality.

Effect of *Khaya senegalensis* Extract on *Meloidogyne* Juveniles after Treatment with Different Concentrations under Three Exposure Periods

Table 4 presents the effects of three concentrations of *Khaya senegalensis* extract on the survival of *Meloidogyne* juveniles in the laboratory in three exposure periods. From the Table it can be seen that the juvenile mortality in the different concentrations of the *Khaya senegalensis* extracts were

significantly higher than those in the control during the three exposure periods and mortality was dependent.

Table 4: Mortality of *Meloidogyne* Juveniles after Treatment with Aqueous Plant Extracts

Conc.of plant Extracts (%)	% mortality of *Meloidogyne* juvenile in three exposure periods		
	6hrs	12hrs	24hrs
25% *Khaya senegalensis*	41.09	45.19	60.24
20% *Khaya senegalensis*	38.64	45.11	52.74
15% *Khaya senegalensis*	31.06	34.82	41.16
0% Control	0.00	0.00	6.22
Lsd (5%)	4.56	1.86	0.79

Values were transformed using $\sqrt{(x+0.5)}$, where x is the mean number of juveniles

It is clear from the Table that the three concentrations of the *Khaya senegalensis* extracts had varied effects on the survival of *Meloidogyne* juveniles. Except during the 24 hour exposure period when juvenile mortality in the different concentrations were significantly different from each other, during the 6 hour and 12 hours exposure periods juvenile mortality in 20% and 25%, respectively were not significantly different. However, mortality at both concentrationswas significantly different from mortality in the 15% *Khaya senegalensis* extracts.

Effect of *Azadirachta indica* Extract on *Meloidogyne* Juveniles after Treatment with Different Concentrations under Three Exposure Periods

Table 5: Mortality of *Meloidogyne* Juveniles after Treatment with Aqueous Plant Extracts

Conc.ofplant Extracts (%)	% mortality of *Meloidogyne* juvenile in three exposure periods		
	6hrs	12hrs	24hrs
25%*Azadirachta indica*	38.03	47.49	65.16
20%*Azadirachta indica*	35.76	41.32	54.54
15% *Azadirachta indica*	35.24	39.20	42.70
0% Control	0.00	0.00	6.22
Lsd (5%)	4.56	1.86	0.79

Values were transformed using $\sqrt{(x+0.5)}$, where x is the mean number of juveniles

Table 5 shows the effect of *Azadirachta indica* extract on the survival of*Meloidogyne* juveniles in the laboratory during three exposure periods. It is evident from the Table that the juvenile mortality caused by the three concentrations of the *Azadirachta indica* extract during the three exposure periods was significantly higher than observed in the control. It can be noted that the mortality caused by the 15%, 20% and 25% *Azadirachta indica* extracts, respectively, were not significantly different from each other after 6 hour of exposure. During the 12 hour exposure period even though the juvenile mortality caused by 15% and 20% *Azadirachta indica* extracts were

not significantly different, they were significantly different from that observed in 25% *Azadirachta indica* extracts.

From the Table it is clear that the juvenile mortalityobserved in the three concentrations of *Azadirachta indica* extract over 24 hour were significantly different from each other.

Effect of *Tectona grandis* Extract on *Meloidogyne* Juveniles after Treatment with Different Concentrations under Three Exposure Periods

Table 6: Mortality of *Meloidogyne* Juveniles after Treatment with Aqueous Plant Extracts

Conc.of plant Extracts (%)	% mortality of *Meloidogyne* juvenile in three exposure periods		
	6hrs	12hrs	24hrs
25%*Tectona grandis*	31.06	42.70	48.90
20% *Tectona grandis*	31.06	34.82	42.64
15% *Tectona grandis*	35.76a	34.21b	37.07c
0% Control	0.00	0.00	6.22
Lsd (5%)	4.56	1.86	0.79

Values were transformed using $\sqrt{(x+0.5)}$, where x is the mean number of juveniles

In Table 6 are presented juvenile mortality observed in three different concentrations of *Tectona grandis*extract during the three exposure periods in the laboratory.

Generally the juvenile mortality observed in the three concentrations of *Tectona grandis* extract over the three exposure periods were significantly higher than that observed in the control.

It is evident from the Table that juvenile mortality in the 15%, 20% and 25% *Tectona grandis* extract were not significantly different from each other during the first 6 hours of exposure. Within the 12 hours of exposure percent mortalityof 34.21% in 15% and 34.82% in 20% *Tectona grandis* extracts were similar butboth were significantly lower than that in the 25% *Tectona grandis* extract (42.70%). Mortality in the different concentrations of *Tectona grandis* extract was significantly different from each other during 24 hour exposure period. Also, 24 hour of exposure of juvenile mortality in the concentrations were significantly different from each other with the highest of 48.90 occurring at 25% and the least of 37.07 at 15%.Mortality at 20% was 42.64%.

Effect of Extracts of *Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis, Vernonia amygdalina* and *Azadirachta indica* on Growth and Yield of Tomato Plants in Root-Knot Nematode Infested Field

When tomato plants were drenched with various concentrations of aqueous leaf extracts from five medicinal plants in root-knot nematode infested field growth and yield are shown in Tables 7 and 8.

Table 7: Growth Parameters of Tomato Plants cultivated in Nematode Infested Soil Drenched with various Concentrations of five Plant Extract

Botanicals	Mean shoot length/plant(cm)	Mean Tap root length/plant(cm)
Control (0%)	33.48	2.34
***Bryophyllum pinnatum* (20%)**	41.48	2.68
***Bryophyllum pinnatum* (25%)**	45.62	2.78
***Tectona grandis* (20%)**	40.22	2.96
***Tectona grandis* (25%)**	49.58	3.30
***Khaya senegalensis* (20%)**	41.82	3.30
***Khaya senegalensis* (25%)**	50.20	3.50
***Vernonia amygdalina* (20%)**	41.00	3.02
***Vernonia amygdalina* (25%)**	48.98	3.12
***Azadirachta indica* (20%)**	43.28	3.36
***Azadirachta indica* (25%)**	52.70	3.60
Furadan	61.14	4.40
Lsd (5%)	6.30	0.22

Table 7 shows the effect of *Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis, Vernonia amygdalina*and *Azadirachta indica*extracts as soil drench on the growth of *Meloidogyne* infected tomato plant on the field.*Meloidogyne* infected tomato plants treated with Furadan, a synthetic nematicide, had the longest mean shoot length of 61.14 cm and the control plants had the shortest mean shoot length of 33.48cm. It is clear from the Table that the mean shoot length of the *Meloidogyne* infected plants treated

with the two concentrations of the five botanicals was significantly different and longer than those of the control plants.

Among the two concentrations of each botanical, except the two concentrations of *Bryophyllum Pinnatum* 20% and 25% the effects of thetwo concentrations of each of the other botanicals on the mean shoot length were significantly different from each other.

Generally the mean shoot length of the *Meloidogyne* infected plants treated with 25% of all the botanicals which were 45.62, 49.58, 50.20, 48.98 and 52.70 for*Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis, Vernonia amygdalina, Tectona grandis* and *Azadirachta indica* respectively, were significantly longer than those of the 20% of each of the botanicals.

Mean tap root length was also significantly affected by the plant extracts at the concentrations applied (Table 7). Control plants had the shortest mean tap roots of 2.34 and those of the Furadan treated plants had the longest mean tap root of 4.40cm.

Table 8 shows the effect of *Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis, Vernonia amygdalina* and *Azadirachta indica*extracts as soil drench on *Meloidogyne* infection soiland fruit yield ontomato plant in the field.

Generally, the number of galls formed on plants drenched with the five botanicals across all the concentrations was lower than that of the control.

Among the botanicals, the number of galls formed on plants drenched with *Azadirachta Indica* 25% concentration was the least across all the five botanicals. The differences in the number of galls of the control and those in the different concentrations were significant; the differences in the number of

galls among the various concentrations of *Tectona grandis*, *Azadirachta indica*, *Khaya senegalensis*, *Bryophyllum pinnatum* and *Vernonia amygdalina* 20% & 25% were not significantly different from each other.

Table 8: Mean Number of Galls and Yield of Tomato Plants after Treatment with Plant Extracts Concentrations

Botanicals	Mean no.of galls/plant	Mean no.of fruit/plant
Control (0%)	32.60	16.40
Bryophyllum pinnatum (20%)	22.60	18.60
Bryophyllum pinnatum (25%)	20.80	20.20
Tectona grandis (20%)	20.00	18.80
Tectona grandis (25%)	17.80	20.40
Khaya senegalensis (20%)	18.40	21.20
Khaya senegalensis (25%)	17.60	24.20
Vernonia amygdalina (20%)	19.40	20.60
Vernonia amygdalina (25%)	17.60	21.80
Azadirachta indica (20%)	17.60	24.40
Azadirachta indica (25%)	11.60	28.00
Furadan	0.00	32.00
Lsd (5%)	6.39	1.25

Fruit yield of the control plants were also significantly different from those drenched with different concentrations of the five botanicals except between *Bryophyllum pinnatum* at 20% and *Tectona grandis* at 20%.

Similarly, number of fruit among tomato plants drenched withthe various concentrations of *Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis* and *Vernonia amygdalina*at 20% and 25% were not significantly different from those of *Azadirachta indica* at 20% and 25% concentrations.

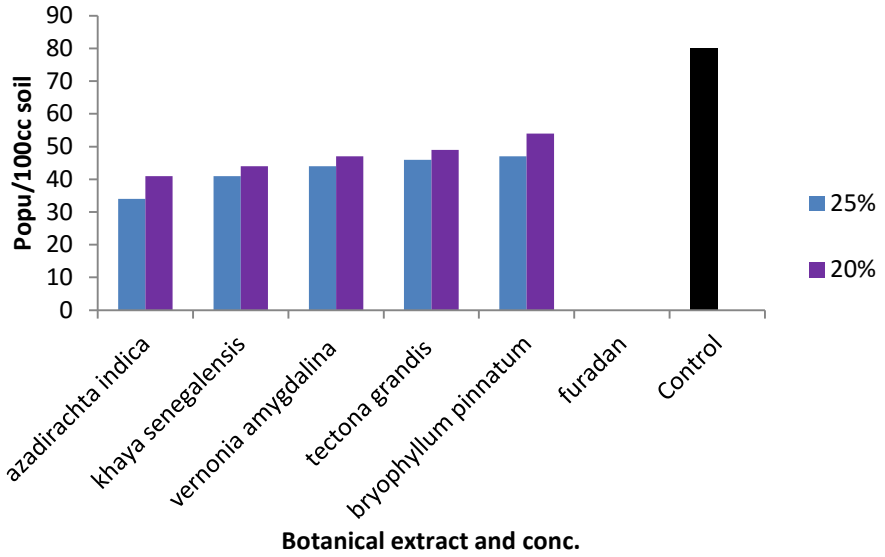


Figure 2: Population of *Meloidogyne* spp on the field after Drenching Soil with different Botanical Extracts

Figure 2 shows the effect of extracts concentrations of *Azadirachta Indica, Khaya senegalensis, Vernonia amygdalina, Tectona grandis* and *Bryophyllum pinnatum* on *Meloidogyne* population in infested field.

Among the botanicals, the lowest *Meloidogyne* population of 34 and 41 occurred in soils treated with 25% and 20% aqueous extracts respectively. The highest *Meloidogyne* populations of 47 and 54 occurred in the soils treated with 20% and 25% *Bryophyllum pinnatum* extracts respectively. In between these two extremes fell the populationin the plots treated with the extracts of *Khaya Senegalensis, Vernonia Amygdalina* and *Tectona grandis*.

Generally it is clearly seen from the Figure that 25% concentrations across all five botanicals had lower *Meloidogyne* populations than the 20% concentrations. The highest *Meloidogyne* population of 87 in the study occurred in the control. The *Meloidogyne* population in the plots treated with Furadan was zero.

Table 9: Correlation Coefficients of Relationship between Root Galling, Growth Characteristics and Yield of Tomato Plant in the Field

	# of Galls/plant	*# of fruits/plant*	*Shoot length/plant*	*Root length/plant*
# of Galls/plant	1			
# of fruits/plant	-0.91903**	1		
Shoot length/plant	-0.90228**	0.879583**	1	
Root length/plant	-0.71408**	0.827468**	0.689693**	1

*Significant at P=0.05
**Significant at P=0.01

Table 9 shows the correlation among root galling, growth parameters and yield of tomato. Number of fruits per plant, shoot length per plant and root length per plant were negatively correlated with number of galls per plant. The number of fruits per plant, however, contrasted by been positivelycorrelated with shoot length (0.87) per plant and root length (0.82) per plant. The correlation between shoot length per plant and root length per plant was also found to be positive and highly significant.

Effect of *Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis, Vernonia amygdalina* and *Azadirachta indica* Applied as Mulch and Soil-Admixture on the Growth of Tomato on *Meloidogyne* Infested Soil

Growth and yield parameters of tomato plants that has received five botanical applied as mulch and soil admixtures to*Meloidogyne* infested soil.

Table 10: Shoot Length and Root Length of *Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis, Vernonia amygdalina* and *Azadirachta indica* applied as mulch and Soil Admixture, on the Growth of *Meloidogyne* Infected Tomato Plants

Botanicals	Mean shoot length/plant	Mean Tap root length/plant
Uninoculated untreated control	55.40	4.48
***Bryophyllum pinnatum* mulch**	53.60	4.44
***Bryophyllum pinnatum* soil admixture**	65.20	4.96
***Tectona grandis* mulch**	55.20	4.66
***Tectona grandis* soil admixture**	61.60	5.34
***Khaya senegalensis* mulch**	61.20	4.64
***Khaya senegalensis* soil admixture**	66.00	4.54
***Vernonia amygdalina* mulch**	58.00	4.92
***Vernonia amygdalina* soil admixture**	63.00	5.20
***Azadirachta indica* mulch**	68.40	4.96
***Azadirachta indica* soil admixture**	76.80	5.52
Inoculated untreated control	34.30	3.24
Lsd (5%)	5.01	0.36

Table 10 has mean shoot lengths and mean root tap lengths of infected tomato plants mulched or in soils admixed with the five botanicals. From Table 10 it is evident that the mean shoot and tap root lengths of infected tomato plants in soil admixed with each of the five botanicals were significantly longer than those of the infected tomato plants which were mulched. The mean shoot lengths in the soil admixtures ranged from 65.20 cm in *Bryophyllum pinnatum* soil ad-mixture to 76.80cm in *Azadirachta Indica* soil-admixture, while with the mulch, mean shoot length ranged from 53.60cm for *Bryophyllum pinnatum* mulch to 68.40 cm for *Azadirachta indica* mulch.

A similar trend can be seen in the mean lengths of the tap roots of the infected tomato plants grown in mulched and those grown in admixed soils. The mean tap root lengths of mulched plants ranged from 4.44 cm for *Bryophyllum pinnatum* mulch to 4.92 cm for *Azadirachta indica* soil ad-mixture. The effects of the mulch and soil ad-mixtures of *Tectona grandis, Khaya senegalensis* and *Vernonia amygdalina* on shoot growths and tap roots of infected tomato plants fell between the extreme lengths of these parameters.

It is interesting in Table 10 that mean shoot lengths and tap root lengths of the inoculated untreated tomato plants which were 55.40cm and 4.80cm respectively, were not significantly different from those of tomato plants with *Bryophyllum Pinnatum* mulch (53.60cm, 4.44cm) and *Tectona grandis* mulch (55.20cm, 4.66 cm).

Generally, the inoculated untreated tomato plants had the shortest mean shoot lengths and tap root lengths, which were 34.30 cm and 3.24 cm respectively. The growth of infected Tomato plants in the soil admixtures was significantly higher than those which were mulched.

Table 11: Mean Number of Galls on Roots of Tomato Plants in *Meloidogyne* Infested Soil

Botanicals	Mean no.galls/plant
Uninoculated untreated control	0.00
***Bryophyllum pinnatum* mulch**	13.40
***Bryophyllum pinnatum* soil admixture**	11.20
***Tectona grandis* mulch**	14.00
***Tectona grandis* soil admixture**	10.60
***Khaya senegalensis* mulch**	14.20
***Khaya senegalensis* soil admixture**	12.00
***Vernonia amygdalina*mulch**	12.40
***Vernonia amygdalina*soil admixture**	12.00
***Azadirachta indica* mulch**	10.60
***Azadirachta indica* soil admixture**	9.60
Inoculated untreated control	42.42
Lsd (5%)	3.28

InTable 11are presented mean numbers of galls formed on inoculated Tomato plants which were treated with extracts of *Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis, Vernonia amygdalina* and *Azadirachta indica* applied either as mulch or soil admixture. Mean numbers of galls formed on the roots of the inoculated tomato plants either as mulched or planted in soils admixed with botanicals were significantly lower than those on the roots of inoculated untreated tomato plants, being 42.42.

Among the botanically treated tomato plants, the mean number of galls on the roots of the infected Tomato plants planted in soils admixed with botanicals were significantly lower than those mulched with botanicals. The least mean number of galls being 9.60 was formed on the roots of tomatoes plants planted in *Azadirachta indica* soil-admixture. The highest mean number of gallswas formed on the roots of the infected tomato plants mulched with *Khaya senegalensis*. This was followed by infected tomato plants mulched with *Tectona grandis* with mean galls number of 14.0.

Uninoculated untreated tomato plant had no galls on their roots. From Table 12, it is evident that the highest mean number of flowers of 32 per plant was produced by the tomato plants uninoculated and also untreated with any of the botanicals and least mean of 10.2 flowers per plant was produced by tomato plants inoculated with *Meloidogyne* but untreated. These two extremes were significantly different and between them fell mean number of flowers per plant produced by *Meloidogyne* infected tomato plant treated with various botanicals either as mulch or soil admixture.

Generally the botanicals, whether as mulch or soil admixture, significantly affected flower production in the *Meloidogyne* infected tomato plant, but their mode of application had no significant difference on flowers production. From Table 12, number of flowers per plant produced by *Meloidogyne* infected tomato plant mulch with *Bryophyllum pinnatum* (10.60), *Tectona grandis* and *Khaya senegalensis* (11.40) and *Vernonia amygdalina* (13.60) were not significantly different from that produced by *Meloidogyne* infected tomato plant in soil-admixed with *Bryophyllum pinnatum* (12.20).

Table 12: **Growth effect of *Bryophyllum pinnatum, Tectona grandis, Khaya senegalensis, Vernonia amygdalina* and *Azadirachta indica applied* as Mulch or Soil-admixture on Flowers Production on Tomato Plants Grown in *Meloidogyne* Infested Soil**

Botanicals	Mean no. flowers/plant
Uninoculated untreated control	32.00
***Bryophyllum pinnatum* mulch**	10.60
***Bryophyllum pinnatum* soil admixture**	12.20
***Tectona grandis* mulch**	12.00
***Tectona grandis* soil admixture**	16.40
***Khaya senegalensis* mulch**	11.40
***Khaya senegalensis* soil admixture**	16.40
***Vernonia amygdalina* mulch**	13.60
***Vernonia amygdalina* soil admixture**	15.80
***Azadirachta indica* mulch**	19.40
***Azadirachta indica* soil admixture**	23.40
Inoculated untreated control	10.20
Lsd (5%)	5.87

Similarly, the flower production in *Meloidogyne* infected tomato plants in soil-admixed with *Tectona grandis* (16.40) and *Khaya senegalensis* (16.40) were not significantly different but they were significantly different from that produced by *Meloidogyne* infected Tomato plant in *Vernonia amygdalina* soil admixture (15.80).

It is clear from Table 12 that flowers produced by *Meloidogyne* infected tomato plants grown in soil-admixture with *Azadirachta indica* (23.40) was significantly higher from of those in *Azadirachta indica* mulched (19.40). Flower production by *Meloidogyne* infected tomato plants in soil-admixtures of the five botanicals was more than those mulched.

Table 13: **Correlation Coefficients of Relationship between Root Galling, Growth Characteristics and Yield of Tomato Plant in *Meloidogyne* Infested Soil**

	# of Galls/plant	*# of flowers/plant*	*shoot length/plant*	*root length/plant*
# of Galls/plant	1			
# of flowers/plant	-0.60845**	1		
shoot length/plant	-0.71483**	0.365824**	1	
root length/plant	-0.47305**	0.805114**	-0.09617*	1

*Significant at P=0.05
**Significant at P=0.01

It is evident from Table 13 that correlation between number of galls per plant and characters such as number of flowers per plant, shoot length per plant as well as root length per plant were all negatively correlated but highly significant. The correlation between shoot length per plant and number of flowers per plant was found to be positively correlated and highly significant with value of 0.36.

Number of flowers per plant positively correlated with root length per plant with value of 0.80 and was also highly significant. Furthermore root length per plant negatively correlated with shoot length per plant and was significant.

CHAPTER FIVE

DISCUSSION

The results of laboratory studies on the five botanicals revealed their nematicidal properties. The varying degrees to which they affected the hatchability of nematode eggs and the survival of nematode juveniles probably suggest that the five botanicals contain different chemical constituents which affected differently the various aspects of *Meloidogyne* development and survival. This observation is in agreement with findings of Khan (1990), who reported that many wild and cultivated medicinal plants have been shown to possess nematicidal properties against several plant parasitic nematodes. It further confirms findings of Lahlou (2004), who observed botanicals to contain compounds that have antagonistic activities. Lahlou (2004) further listed some of the nematicidal phytochemicals as essential oil, triterpenod, sapanins, glucosinolates, isothiocynates,cyanogenic glycosides,alkaloids, phenolics, flavonoids, polyacetylenes, polytheinyls, limonoids, pyrethrumamong others. These phyto-chemicals with some nematicidal properties have severally been reported to be part of chemical constituents of *Tectona grandis* (Anonymous 2001 and Sherifat, Akinsola & Guido, 2013), *Khaya senegalensis* (Nakatani et *al*., 2001 and Pavela, 2009), *Azadirachta indica* (Simmonds & Stevenson, 2001 and Koul, 2008), *Vernonia amygdalina* (Ogundare, Adetuyi and Akinyosoye 2006 and D'Addabbo *et al.*, 2010) and *Bryophyllum pinnatum* (Okwu, 2004 and Regnault & Philogéne, 2008).

The generally low percentages of egg hatch in the high concentrations of the extracts of the five botanicals probably suggest that some of their chemical constituents have some ovicidal properties. The least percentages of egg hatch

in *Azadirachta indica* extracts is suggestive of the presence of very potent ovicidal phyto-chemicals in the *Azadirachta indica* plants. Aldhous (1992) has reported that azadiractin has been repoted to have compounds with molecules which effect insect growth regulation and development. It is likely these azadiractin compounds in the *Azadirachta indica* extract could have inhibited the growth of the embryo in the unhatch eggs and hence the low hatchability observed in the *Azadirachta indica* extracts. It was also observed that percentages of egg hatch decreased with increasing concentration of the extract with the least recorded in the botanicals. This observation agrees with findings of Adegbite and Adesiyan (2005), who worked with root extracts of *Azadirachta indica, C. odorata, R. communis* and *Jatropha curcas* and recorded increased inhibition with concentration of the extract.

The high juvenile mortality rate in the extracts of all botanicals across their concentrations is suggestive of their nematicidal potential. This is in conformity with earlier findingsof Majaz, Nazim, Asir, Shoeb & Bilal (2011) who reported that the lannins in the extract of *Bryophyllum pinnatum* showed significant antihelmintic activity. Aldehyde Furdurate in the leaves of *Khaya senegalensis*has also been reported to exhibit activity similar to that of the commercial nematicide Fosthiazate Rodriges *et al.*, (2011).

Oudhial (2003) has observed that *Tectona grandis* leaves has some antihelminticproperties. Life plant has been severally reported to release toxins that cause tissue disruption and suppressed insects, fungi, nematodes and weed development (Zagrobely *et al*,. 2004 and Carslsen & Fomrgaard, 2008). *Azadirachta indica* has been reported to have Isothiocyanate, Polyacetylens and polytheinyls which are known to have nematicidal properties. The

presence of several compounds with nematicidal properties probably explains the highest juvenile mortalities rates observed in the two concentrations of *Azadirachta indica* and also during the three periods of exposure compared with those of the other botanicals.

The least juvenile mortality rates in *Bryophyllum pinnatum* extracts probably suggest fewer presences of the compounds with nematicidals properties or their less potency. The various juvenile mortality rates of the extracts of *Tectona grandis*, *Khaya senegalensis* and *Vernonia amygdalina* confirm the presence of compounds with nematicidal properties and hence their varying potentials as nematicides. Juvenile mortality also increased with increase in exposure of time. This agrees with Joymatti *et al.* (1998), who reported that juveniles exposed to extracts of *Melothria purpusilla Cogn* for a longer period of time decreased in their rate of hatching as compared to those exposed to a shorter period of the same extracts.

The high juvenile mortality effect by the botanicals according to Adegbite and Adesiyan (2005) might be due to the chemical properties present in the extracts that possess antihelmintic properties. It was also suggested that botanicals with nematicidal properties affect the developmental stages or kill juvenile. Probably these properties increase with increase in time hence, the increased mortality rates as exposure period increased**.**

Generally the lengths of the shoots and roots of the Tomato plant treated with the various botanical extracts being longer than that of the control but shorter than that of the Furadan treated plant confirms the effect of the botanicals in the field.

Among the botanicals, tomato plants treated with 25% of each of the botanical had longer shoots and tap-roots than those of 20% is suggestive that with increasing concentrations the efficacy of each of the botanicals checking *Meloidogyne* root-knot disease could probably be improved upon. The improvement in efficacy of each of the botanicals, is however, likely to be influenced by their phyto-chemical constituents. The longest shoots and tap-roots produced by tomato plants treated with 25% *Azadirachta indica* extracts is suggestive of *Azadirachta indica* having the greatest potential as a nematicide. This assertion is confirmed among the botanically treated plants by the least number of galls formed on the roots of infected tomato plants treated with 25% *Azadirachta indica* extracts and the highest number of fruits produced by them.

Lower*Meloidogyne* populations in plots drenched with various botanicals, is further indication of the nematicidal properties of *Tectona grandis, Khaya senegalensis Vernonia amygdalina,* and *Bryophyllum pinnatum* and particularly that of *Azadirachta indica* in the field.

The considerable difference observed between the application of the botanicals as mulch and soil admixtures on the parameters of tomato studied probably suggests that the nematicidal effect of *Tectona grandis*, *Khaya senegalensis*, *Vernonia amygdalina*, *Bryophyllum pinnatum* and *Azadirachta indica* could probably be influenced by their mode of application. The lengths of shoots and tap-roots of infected tomato plants in the soils admixed with each of the botanicals were generally longer than those of the infected plants mulched with the botanicals. The lengths of the shoots and tap-roots of infected tomato plants treated with *Azadirachta indica* either as mulch or soil

admixture, were longest among the botanicals. It is most likely that generally, the soil admixture because they were grinded before being mixed with the soils in the pots decomposed faster than mulch and added some plant nutrients to the soil in addition to controlling *Meloidogyne* Oka *et al.*, (2007). This confirms the findings that *Azadirachta indica* apart from controlling some plant pest also improves the soil nutrient to the advantage of the attacked plant as observed by (Hasabo and Noweer, 2005). It is possible that *Tectona grandis, Khaya senegalensis, Vernonia amygdalina*and *Bryophyllum pinnatum*also improved the nutrient status of the soils in the pots in which they were applied but not to the extent of *Azadirachta indica*.

The considerable differences between the number of galls formed on the roots of the infected tomato plants mulched and those on the roots of plants in the soils-admixed with the botanicals is suggestive of the influence of the mode of application on the nematicidal properties of the botanicals. Probably the grinded botanicals when admixed with the soil released more compounds with nematicidal properties than their mulch (Yasmin *et al.*, 2003).

Generally, the number of flowers produced by the botanically treated *Meloidogyne* infected tomato was lower than that of Uninoculated and untreated tomato plants and higher than that of inoculated untreated tomato plant. This is also indicative of the nematicidal potential of the *Tectona grandis*, *Khaya senegalensis*, *Vernonia amygdalina*, *Azadirachta indica* and *Bryophyllum pinnatum*. The number of flowers of the botanically treated tomato plant was higher than that of the inoculated untreated tomato plant. This could be due to the role the botanical play as plant nutrient provider in addition to being toxic to *Meloidogyne.*

The differences in correlations among the parameters studied both in the fieldand pots could probably be due to environmental factors and mode of application of the botanicals in the two studies.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

In vitro study showed that the *Azadirachta indica, Khaya senegalensis, Vernonia amygdalina, Tectona grandis* and *Bryophyllum pinnatum* extracts used caused a significant reduction in root knot nematode eggs hatchability as well as higher juvenile mortality. It was also observed that *Azadirachta indica, Khaya senegalensis, Vernonia amygdalina, Tectona grandis* and *Bryophyllum pinnatum* extracts are nematicidal at higher concentrations. Also, it was observed that a reduction in egg hatch as well as a higher juvenile mortality by the extracts was dependant on exposure period.

Pot experiments revealed that lesser number of galls was found on the roots of the *Azadirachta indica, Khaya senegalensis, Vernonia amygdalina, Tectona grandis* and *Bryophyllum pinnatum* extract-treated plants as compared to the inoculated untreated control plants. In all the pot experiment, it was observed that the shoot length and root length of the extracts treated plants were longer compared with the inoculated untreated control.

The field study results, however, showed that no differences were found between shoot lengths and root length recovered from the tomato roots treated with the extracts. However, mean number of galls formed in both the extract treated plants and control plants were significantly different.

Conclusions

It was evident from the study that *Azadirachta indica, Khaya senegalensis, Vernonia amygdalina, Tectona grandis* and *Bryophyllum pinnatum* have some nematicidal effects on *Meloidogyne* spp in tomato.

Application of extracts of the botanicals as soil drench on tomato plants in the field reduced root galling and increased the growth and yield oftomato.

Their application of all the botanicals as soil admixtures was more effective than when applied as mulch.

The leaf extracts of *Azadirachta indica, Khaya senegalensis, Vernonia amygdalina, Tectona grandis* and *Bryophyllum pinnatum* if further studied have the potentials as substitutes for synthetic nematicides.

Recommendations

Further studies should be carried out on other modes of application of *Azadirachta indica, Khaya senegalensis, Vernonia amygdalina, Tectona grandis* and *Bryophyllum pinnatum* to come out with most effective mode of application for the control of *Meloidogyne* in organic farming.

The Ministry of Food and Agriculture should educate farmers on the importance and use of botanical as nematicide so as to enable farmers rely on the use of naturally occurring pesticides which are effective, less expansive and pose nothreat to their health and the environment.

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APPENDIXES

APPENDX A

Analysis of variance table for number of egg hatch at 72hr

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BOTANICAL	5	1416.424	283.285	58.36	<.001
BOTANICAL.CONC	10	64.222	6.422	1.32	0.261
Residual	32	155.333	4.854		
Total	47	1635.979			

APPENDX B

Analysis of variance table for number of juvenile mortality at 6hr

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MORTALITY. TEST	5	5563.73	1112.75	88.17	<.001
MORTALITY_TEST.CONC	10	302.56	30.26	2.40	0.028
Residual	34	429.11	12.62		
Total	49	6295.40			

APPENDX C

Analysis of variance table for number of juvenile mortality at12hr

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MORTALITY_TEST	5	7409.475	1481.895	170.44	<.001
MORTALITY_TEST.CONC	10	764.035	76.403	8.79	<.001
Residual	34	295.607	8.694		
Total	49	8469.117			

APPENDX D

Analysis of variance table for number of juvenile mortality at 24hr

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MORTALITY_TEST	5	8651.701	1730.340	1087.79	<.001
MORTALITY_TEST.CONC	10	2249.179	224.918	141.40	<.001
Residual	34	54.084	1.591		
Total	49	10954.963			

APPENDX E

Analysis of variance table for number of fruit per plant of field experiment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	6	966.883	161.147	38.68	<.001
TREATMENT.CONC	5	71.300	14.260	3.42	0.010
Residual	48	200.000	4.167		
Total	59	1238.183			

APPENDX F

Analysis of variance table for number of galls per plantof field experiment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	6	2948.90	491.48	19.45	<.001
TREATMENT.CONC	5	119.90	23.98	0.95	0.458
Residual	48	1213.20	25.27		
Total	59	4282.00			

APPENDX G

Analysis of variance table for shoot length per plantof field experiment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	56.01	14.00	0.57	
REP.*Units* stratum					
BOTANICAL	6	2049.17	341.53	13.97	<.001
BOTANICAL.CONC	5	818.48	163.70	6.69	<.001
Residual	44	1075.99	24.45		
Total	59	3999.65			

APPENDX H

Analysis of variance table for root length per plantof field experiment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	0.2360	0.0590	0.48	
REP.*Units* stratum					
BOTANICAL	6	14.4783	2.4131	19.69	<.001
BOTANICAL.CONC	5	0.6130	0.1226	1.00	0.429
Residual	44	5.3920	0.1225		
Total	59	20.7193			

APPENDX I

Analysis of variance table for number of flowers per plant of potted plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	70.77	17.69	0.83	
REP.*Units* stratum					
TREATMENT	11	2268.80	206.25	9.72	<.001
Residual	44	934.03	21.23		
Total	59	3273.60			

APPENDIX J

Analysis of variance table for number of gallsper plant of potted plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	50.433	12.608	1.90	
REP.*Units* stratum					
TREATMENT	11	5306.133	482.376	72.60	<.001
Residual	44	292.367	6.645		
Total	59	5648.933			

APPENDIX K

Analysis of variance table for shoot length per plantof potted plant (mulch and soil-admixture)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	276.94	69.24	2.22	
REP.*Units* stratum					
BOTANICAL	6	8208.77	1368.13	43.81	<.001
BOTANICAL.medium	5	735.30	147.06	4.71	0.001
Residual	54	1686.36	31.23		
Total	69	10907.37			

APPENDIX L

Analysis of variance table for root length per plantof potted plant (mulch and soil-admixture)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	1.4420	0.3605	2.22	
REP.*Units* stratum					
BOTANICAL	6	26.5357	4.4226	27.27	<.001
BOTANICAL.medium	5	2.8370	0.5674	3.50	0.008
Residual	54	8.7580	0.1622		
Total	69	39.5727			