CHAPTER ONE

1.1 INTRODUCTION

Tomato is an important cash crop in the forest, transitional and savannah zones of Ghana (Norman, 1992). Total land area for production in Ghana increased from 28,400ha in 1996 to 37,000ha in 2000 (GIPC, 2001). Tomato contains important chemical compounds that play important roles in the prevention of cancer, heart disease, cataracts and many other health problems (Beecher, 1998).

The production of tomato, however, is confronted with a lot of problems which include limited availability of improved planting material, high cost of labour operations such as land preparation, staking, weeding, harvesting, storability, pests and diseases. Root-knot nematodes (*Meloidogyne* spp.), according to De Lannoy (2001), are a major pest of tomato. Hemeng (1981) reported an average yield loss of 73-100% in the Guinea Savannah zone of Northern Ghana due to root-knot nematodes. According to Amer-Zareen *et al*. (2003), root knot nematodes have a wide host range and are considered the greatest threat to global agriculture. *Meloidogyne* spp. attack more than 2000 host plants (RPD, 1993).

As a result of their ability to attack many crops, root knot nematodes are liable to cause losses wherever there is intensive cultivation of susceptible crops, when precautions against population build up are not taken. In heavily infested soils, the root system of the plant is reduced and feeder roots do not appear. This damage extends from simple mechanical damage to highly involved nematode-plant interaction caused by chemicals introduced by the nematode (Caveness and Ogunforowa, 1985).

The nematode infection acts as energy sink absorbing photosynthate needed by the plant for growth and fruit production, hence crop yields are reduced and harvested produce is of poor quality and reduced storage life. Root-knot damage leads to symptoms similar to those caused by nutrient deficiency, water stress or soil-borne diseases. Heavily infested plants do not respond to water and fertilizer because the

nematodes severely damage the conducting tissues of the roots. The damage predisposes the crop to other pathogens by the leaching of nutrients into the soil which favours the growth of bacteria and fungi (Sasser, 1989). Agrios (1997) observed that root knot nematodes increase the incidence and severity of *Fusarium* wilt and bacteria wilt by providing an infection court for the pathogens.

According to Kinloch (1982), the growth of a plant is inversely proportional to the initial population density of *Meloidogyne* species; hence as nematode population rises above the economic threshold, control becomes more difficult. It is, therefore, important to control the potential rise of root-knot nematodes so that they remain below levels at which they reduce the yield of crop plants (Bridge, 1996a).

Various control strategies are, therefore, employed to manage the root knot nematode. Some of these have been the use of nematicides, biological agents and resistant varieties.

Indiscriminate use of chemical leads to phytotoxicity, environmental pollution and nematodes resistance (Adegbite and Adesiyan, 2005). It also has the disadvantage of being toxic to man and animals when used improperly (Luc *et al*., 1990). According to Chitwood (2002), the economic cost of research and registration of new chemicals is an enormous hurdle for a prospective new chemical nematicide to overcome as compared to a phytochemical which requires much less data to register.

Sukul (1992), reported that many plants belonging to 57 families possess nematicidal properties and it is possible to use these plants to control root knot nematodes. Adegbite and Adesiyan (2005), observed that root extracts of *Chromolaena odorata* (L.) and *Azadirachta indica* (L.) exhibited 100% inhibition of egg hatch and larval mortality of root knot nematodes. Aqueous extracts of basil leaves (*Ocimum basilicum*) *(*L.), marigold leaves (*Tagetes* spp.), neem seed (*Azadirachta indica*) and China berry leaves (*Melia azedarach*) (L.) all affected the survival of root knot nematode juveniles under laboratory conditions and also reduced second stage juveniles of the same pest in soils and roots of egg plant under

field conditions (Hasabo and Noweer, 2005). Korayem and Hasabo (1994) observed that juveniles of *Meloidogyne* species exposed to standard solutions of bulb extract of *Allium sativum* (L.) were killed 24 hours after exposure.

Seed extracts of *Jatropha curcas* (L.) and *Lantana camara* (L.) had an inhibitory effect on egg hatch and juvenile mortality of *Meloidogyne incognita* (Kofoid and White) Chitwood (Joymati *et al*., 1998). Khan (1990), revealed that leaves of *Azadirachta indica*, *Albizia adianthifolia* (Schum), *Acacia albida* (Del.) and *Tamarinda indica* (L.) have strong nematicidal properties and their addition to soil adversely affected the development of *Pratylenchus zeae* Graham and also improved plant growth in chilli.

According to Gommers *et al.* (1982), plant extracts act by producing compounds that stimulate production of oxygen radicals which block the metabolic pathways of the nematodes. Some of these extracts, for example, mature seeds of *Azadirachta indica* synthesize more metabolic substances such as azadirachtin and other closely related metabolites such as vepaol, isovepaol and nimibiden (Sankaram *et al*., 1986). Such synthesized metabolites accumulate in the seed and are more lethal to plant pathogens including nematodes, allowing better plant growth (Yasmin *et al*., 2003).

The addition of organic residues strongly had impact on the physical and biological properties of soils and promoted an environment favourable to nematode antagonistic microorganisms (Stirling, 1991). Incorporation of plant parts or extracts into the soil alone or with bio-control agents have been suggested as an alternative, safe and effective control method for the management of plant parasitc nematodes (Siddiqui and Alam, 1985).

There is the need, therefore, to develop effective and environmental friendly nematicides which are less toxic to man and animals but are potent against nematodes of tomatoes as synthetic ones. Following this, the nematicidal potential of some botanicals have been evaluated and some found to be toxic against the root knot nematodes (Adegbite and Adesiyan, 2005).

This study seeks to evaluate the nematicidal potential of crude aqueous extracts of castor bean in the management of root knot nematodes on tomato plants.

The objectives of the study, therefore, were to:

1. evaluate the effect of the castor bean aqueous extracts on root knot nematodes egg hatch and juvenile mortality *in vitro,*
2. determine the minimum effective concentration of the extracts in Petri dishes for controlling root knot nematodes *in vitro* and *in vivo,*
3. determine the efficacy of extracts by root dip, soil drench, and both on root-knot nematodes and
4. find out the effect of the extracts on root knot nematodes under planthouse and field environments.

CHAPTER TWO

LITERATURE REVIEW

2.1.0 **Nutrient composition and benefits of tomato**

Edible part of the tomato represents about 94% of the total weight of the fruit (De Lannoy, 2001). A 100g tomato contains 93.8g water, 1.2g protein, 4.8g carbohydrate, 7mg calcium, 0.6mg iron, 0.5mg carotene, 0.06mg thiamine, 0.04mg riboflavin, 0.6mg niacin and 23mg vitamin C (De Lannoy, 2001).

Tomatoes are also very rich in all three important vitamins A, B and C (Norman, 1992) while most vegetables are deficient in one or more. It is a rich source of many important nutrients and contains as much vitamin C as many citrus fruits, with a normal sized tomato providing up to 40 percent of the recommended daily intake of this important nutrient (Collins, 2007). According to Beecher (1998), tomatoes also contain vitamin E, trace elements, flavonoids, phytosterols, and several water-soluble vitamins.

Tomatoes are known to contain a great many important compounds such as lycopene that play an important role in the prevention of cancer, heart disease, cataracts and many other common health problems (Collins, 2007).

2.1.1 **Tomato production and constraints in Ghana**

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), tomato is the most important vegetable grown in Ghana and a wide range of areas are suitable for its 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).

2.1.2 **Tomato cultivars grown in Ghana**

The varieties grown in the country are those which have evolved from varieties introduced by the Portuguese. The fruits are of irregular shape, multisided and poor post harvest qualities. On the contrary, fruits of improved varieties are better shaped and good handling qualities. These cultivars however, are more susceptible to diseases and pest and these include Power, Pectomec, Roma, Royal and Mongal.

2.1.3 **Nematodes associated with tomatoes**

Nematodes have been identified as one of the major pests affecting tomato production throughout the world, particularly, in the tropical and sub-tropical regions (Berlinger, 1986). According to Belinger (1986), over 60 different species representing 19 genera of plant parasitic nematodes attack tomato but the most destructive nematode according to Norman (1992) is the root-knot nematodes which belongs to the genus *Meloidogyne*.

Most widespread and devastating species are the *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood.

These species can cause complete crop loss under adverse growing conditions (Belinger, 1986). There has been a reported average yield loss of between 73100% in the Guinea Savannah zone of Northern Ghana (Hemeng, 1981).

Rotylenchulus reniformis (Linford and Oliveira) has been reported on tomato in Australia, Nigeria and other West Africa countries. These pests feed on the phloem or pericycle causing damage to the vascular tissue. Some species of *Pratylenchus* including *P. penetrans* (Cobb), *P. brachyurus* (Filipjev and Seht. Stek) *and P. coffeae* (Zimmerman) have been reported on tomato although the symptoms are nonspecific and appear as stunted growth, leaf discolourisation and reduced yield.

2.2.0 ***Meloidogyne* species**

These are obligate parasites of major economic importance as a plant parasite in the tropics. According to Sasser and Carter (1985), almost all plants that account for the majority of the world's food supply are susceptible to infection by this pest. According to Mai and Abawi (1987) *M. incognita* with *M. javanica* and *M. hapla* accounted for about 99% of all population of root knot nematodes found on cultivated crop plants in agricultural soils.

The four common root knot nematode species, *M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria* have a very extensive host range, including most of the commonly grown agronomic crops and weeds that belong to many plant families (Hussey, 1985). Although there are different species of this pest, Netscher (1978) and Fargette (1987) indicated that almost all infection is as a result of mixed population.

2.2.1 **Biology and life cycle of *Meloidogyne* species**

The life cycle of *Meloidogyne* spp. involves five developmental stages (Mai and Abawi, 1987). Embryonic development in the egg results in the formation of the vermiform first stage larva which later moults into the second stage larva still within

the egg. The second-stage larvae hatch from the egg by breaking the egg shell with repeated thrusting of their stylets. Hatching, according to Mai and Abawi (1987), does not depend on root diffusates. The second-stage juveniles migrate in the soil and thus are the infective stage.

The juveniles are attached to roots by various stimuli such as carbon dioxide (Coomans, 1979; Green, 1971). Root penetration is accomplished through mechanical action by thrusting of the stylet and possibly enzymatic activities (Bird and Loveys, 1975; Hussey, 1985). Juveniles in roots migrate intercellularly through the cortical tissues toward the region of cell differentiation. They finally become sedentary with their heads inserted in the periphery of the vascular tissues while their bodies are in the cortical region parallel to the long axis of the root (Mai and Abawi, 1987).

The sedentary larvae continue to enlarge during the formation of giant cells and galls completing the second and third moults and differentiating the sexes. Fourth moult produces the males and females. Depending on the host and soil temperature, the entire life cycle may be completed in 17 to 57 days (Widmer and Abawi, 2000).

2.2.2 **Survival and dissemination of root knot nematodes**

Root knot nematodes survive in soils as eggs and larvae. Eggs are deposited by mature females in an egg sac consisting of a gelatinous matrix secreted by the females to protect the eggs from dehydration (Pattison, 2007; Orion and Franck, 1990). The length of survival of root knot nematode in the soil depends on the species, soil aeration and other factors (Sasser and Carter, 1985; Taylor and Sasser, 1978).

Dissemination among fields and between production areas is through irrigation water, vegetative plant parts and soils infested with eggs and larvae that adhere to farm implements, animals and man (Mai and Abawi, 1987).

2.2.3 **Symptoms and damage of root knot nematode**

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 and 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 and Abawi, 1987).

2.2.4 **Disease complexes involving root-knot nematodes**

Root knot nematodes 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) observed that *Fusarium* wilt of cotton was more severe in the presence of root-knot nematodes *(Meloidogyne* spp.). *Meloidogyne-Fusarium* interaction has been described on several hosts (Powell, 1979), and has been studied in detail, especially on tomatoes and cotton. The presence of root-knot nematodes increases the incidence, rate of development, and/or the severity of wilt on *Fusarium-susceptible and Fusarium-tolerant* crop cultivars (Agrios, 1997).

In most interactions involving fungi, the nematode usually assisted the fungus by altering the incidence and speed of the development of the pathogen and thus the severity of disease it caused. In certain situations, the nematode has been responsible for breaking disease resistance to *Fusarium* wilt.

Root knot nematodes have also been observed to increase the incidence and severity of *Phytophthora parasitica* var. *nicotiana* (L.), the causal agent of black shank of tobacco (Johnson *et al*., 2005), and peanut (Diamonde and Beute, 1981) *Macrophomina phaseolina* (Tassi) Goid and on cowpea (Devi *et. al*.1992) *Rhizoctonia solani* (Khun).

Infection of plant roots by root-knot nematodes may also affect the incidence of other pathogens on above-ground plant parts. In tobacco, for instance, infection by *M. incognita* predisposed plants to brown spot caused by *Alternaria alternata* (Fries) (Shepherd, 1990), while rice plants infected with *Ditylenchus angustus* (Butler) Filipjev and *Aphelenchoides besseyi* Christie were more susceptible to rice blast caused by *Pyricularia oryzae* Cav. and stem rot caused by *Sclerotium oryzae* Catt. (McGawley *et al*., 1984).

2.3.0 **Nematode Disease Management Options**

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.

2.3.1 **Antagonistic plants to nematodes**

These are plants capable of releasing substances from their roots into the soil that are toxic to several plant parasitic nematodes. Some of these antagonistic plants

include *Tagetes* spp (Kumari et.al., 1986), *Azadirachta indica* (Alam and Saxena,1977), *Crotalaria* spp (L) (Yuhara,1971; Subamaniyan and Vadivelu, 1990), *Datura sp* (L) (Kumari *et al*.,1986) *Ricinus communis* (L) (Zaki and Bhatti, 1989), *Cannabis sativa* (L), *Cassia fistula* (L), *Jatropha curcas* (L), *Lantana camara* (L) (Joymati *et al.*,1998), *Acacia albida* Dell, asparagus, and *Allium sativa* (L), (Hasabo and Noweer, 2005).

2.3.2 **Organic Amendments of soil**

According to Siddiqui and Alam (1987), infection of *M. incognita* and *Rotylenchulus reniformis* on tomato was reduced by incorporating chopped plant parts of water hyacinth into the soil before planting. Water extract of water hyacinth also showed nematicidal and nemato-static properties. Aqueous root extracts of *C. odorata* also inhibited the hatching of *M. incognita* eggs (Adegbite and Adesiyan, 2005). Aqueous extracts of neem leaves was found to be efficacious for the control of *M. incognita* on tomato (Akhtar and Alam, 1990; Rao and Bajaj, 1984), and on okra (Zaiyd, 1977). Extracts of marigold (*Tagetes spp*), *Ricinus communis* (L) (Adegbite and Adeiyan, 2005), *Tridax procumbens* (Mani and Chitra, 1987) have all been reported to be highly toxic to root knot nematodes.

Latex bearing plants have also been found to have great potential in controlling nematodes (Siddiqui *et al.,* 1987). Siddiqui *et a*l. (1987) reported of good control of *M. incognita* and *Rotylenchulus reniformis* on tomato and eggplant when chopped shoot parts of *Ficus elastica* were incorporated into the soil as mulch.

Sikora *et al. (*1973) observed that the addition of sugar cane baggase to soil before planting tomatoes caused 22% reduction in galling. Efficacy of *Tagetes* spp against many phytonematodes have also been described. Growing of marigold intermixed with several crops or during intervening period between the crops has been found to be most effective against *M. incognita* (Alam and Saxena., 1977).

Oil cakes are generally rich in mineral ingredients such as nitrogen and phosphorus (Akhtar and Alam, 1990). Water extracts of oil cakes of mustard, neem, sesame,

groundnut and castor showed inhibitory effect on larval emergence of *M. incognita* (Khan *et al.,* 1967). Application of neem, castor, groundnut, linseed and mustard seed cakes not only reduced root galling in okra and tomato but also egg-laying capacity of the female nematode (Singh and Sitaramaiah, 1969).

Application of oil cakes as soil amendments is an agronomic practice in many parts of the world. Saifullah and Gul. (1990) found reduction in infection by *Meloidogyne* spp. on tomato where mustard, linseed, sesame, castor and cotton seed were used. Addition to soil of castor cake before sowing gave better results than neem leaf cake whereas a combination of these amendments gave maximum control of nematodes (Saifullah and Gul, 1990). Siddiqui and Alam (1988) reported the potential of root-dip treatment of neem leaf extract against root knot nematode and other parasitic nematodes.

2.3.2 **Refined natural extracts as nematicides**

Sulfur-containing nematicidal substances such as allygrin from *Allium grayi* Regal, was active against *M. incognita* (Tada *et al.,* 1988). Thiosulfinates isolated from ether extract of *Allium fistulosum* var. *caespitosum* (L), was found to possess nematicidal (and antibacterial) activity against the root knot nematode.

Allicin, a major component of *Allium sativum* (L), inhibited hatching of *M. incognita* at concentrations as low as 5% and was toxic to juveniles at 25% (Gupta and Sharma, 1993). Immersion of tomato roots in allicin solutions as a prophylactic measure was beset with problems of phytotoxicity and lack of nematotoxicity, but a 5-minute immersion in 2.5% allicin inhibited penetration of roots by juveniles by 50% and was not phytotoxic. Application of concanavalin A, a lectin from the jackbean, *Canavalia ensiformis* (Fabaceae), resulted in substantial reduction of *M. incognita* on tomato in growth chamber, greenhouse, and microplot experiments, possibly by binding to nematode chemoreceptors (Marban-Mendoza *et al.,* 1987).

2.3.3 **Soil solarisation**

Solarisation is very effective in controlling nematodes in soils (Egunjobi and Larinde, 1975). A transparent polyethylene film covering the soil surface generates

and retains intense heat in the soil that kills all stages of nematodes. However, the method is expensive for large-scale use, but for use on small areas such as seedbeds could be economically accessible. Its detrimental effect on potential biological control agents in the soil has to be considered, although this is thought to be minimal (Gaur and Perry, 1991).

2.3.4 **Burning stubble**

Burning vegetation after a bush fallow in slash-and-burn farming does not itself provide effective control of nematodes in soil (Lowe, 1992) and a very rapid burn has even less chance of killing soil nematodes. Burning the stubble after harvest can give excellent control of the nematodes, but there must be complete burning to eliminate them. Burning trash on seed beds before planting is effective in controlling nematodes at the nursery, hence healthy seedlings.

2.3.5 **Flooding**

Flooding effectively kills soil nematodes through poor aeration but it is a costly and uneconomic means of controlling nematodes (Stover, 1979), even for commercial farmers. However, use of land that is naturally flooded or that has been previously artificially flooded, is a highly effective strategy for nematode control.

Populations of root knot nematodes were lower on susceptible dry-season crops following flooding. Flooding maintained for 66 days according to Bridge (1996b) reduced the damage caused by *M. incognita* on vegetable crops.

2.3.6 **Crop rotation**

The aim of any rotation is to allow a sufficient interval to elapse after a susceptible host crop so that nematode populations can decline to a level that will allow the next susceptible crop to grow and yield at an acceptable rate (Travedi and Barker, 1986).

Rotation with resistant or nonhost crops for two to three years generally provides excellent control of root-knot nematodes (RPD, 1993). It is important, however, to keep these crops free of weeds or volunteer plants susceptible to the species involved, since their presence nullifies the use of rotation.

2.3.7 **Resistant varieties**

Use of resistant varieties is perhaps the best method of controlling root-knot nematodes (Bridge, 1996b). However, these varieties are usually resistant to only one or two species of *Meloidogyne*. Therefore, this method is limited to situations in which one or two *Meloidogyne* species are present. Resistance may not provide protection against even one species, since numerous intraspecific races and biotypes are known to exist in nature.

2.3.8 **Heat treatment of propagation material**

Plant parts infected with root-knot nematodes can be disinfected by placing them in hot water. The temperature and period of exposure involved depend on the plant being treated. The temperature must be controlled critically and is usually just below that which injures plant tissues. Hot water treatment at 53-55^{O}C for 20 minutes has been recommended for crops such as banana (Broadley, 1979)

2.3.9 **Biological control**

Instances of suppressive soils to plant-parasitic nematodes, due to fungi, bacteria, or other antagonistic organisms, have been documented (Bird and Bird, 1986). However, only a few commercial biocontrols agents such as Pasteuria peretrans and *Trichoderma hanianum* Rafai have been used successfully.

2.3.10 **Chemical management**

Management of root knot nematodes has traditionally relied on the use of nematicides. Control by traditional nematicides such as the fumigants 1, 3dichloropropene and non-fumigants such as the carbamates, aldicarb and oxamyl

and the organophosphates, fenamiphos give good control when applied correctly. Although a range of non-fumigant nematicides have acceptable levels of efficacy, most of these compounds such as fenamiphos has been detected in groundwater (Luc *et al.*, 1990).

2.4.0 **Prospects of phytochemicals**

According to Chitwood (2002), the search for nematicidal phytochemicals has not yet reached maturity; efforts have largely consisted of basic and descriptive research.

In addition, most plant nematological studies have begun with plants or plant compounds known to be active against other pests and pathogens. Nonetheless, the few investigations of phytochemicals with biological activity against nematodes have yielded a wide variety of structurally diverse compounds.

Most investigators have pointed out that inadequate attention has been given in most cases to the concentrations of many of these compounds within plants, or their cellular or subcellular locations. Also, the mode of action of most nematicidal phytochemicals is largely unknown.

With respect to efficacy of phytochemicals, none of the compounds have successfully exhibited activity as low as commercial nematicides. Although efficacy of specific compounds may be high *in vitro* against some developmental stages of some nematodes, the behavior of the compounds in soil or other considerations have limited the agricultural use of specific phytochemicals.

The usefulness of phytochemicals as control tools is a function of economics, which in turn is a function of the alternative chemical and nonchemical methods for nematode management available to a specific grower. Specific phytochemicals may be expensive to purify or synthesize chemically.

The use of a crude phytochemical 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; however, the cost of crude versus synthetic materials is a function of the complexity involved in the manufacture of each. Utilization of green manures or nematotoxic rotation crops is another method of incorporating phytochemicals into management practices. The benefit of green manuring, apart from nematode management often includes weed suppression.

Direct *in vitro* tests must be complemented by *in vivo*, soil-based experiments in order to examine phytotoxicity. (Chitwood, 2002).

2.5.0 **Description and active ingredients in castor bean**

Castor bean (*Ricinus communis*) is a member of the family Euphorbiaceae. Cassava (*Manihot esculenta*), rubber tree (*Hevea brasiliensis*), ornamental poinsettias (*Euphorbia pulcherrima*), are other important members of this family.

The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein. The seed coat contains ricin, a toxin, which is also present in lower concentrations throughout the plant. Every part of the plant is toxic, but the most dangerous are the seeds which contain high concentration of a type 2 ribosomeinactivating enzyme, ricin, which is one of the deadliest natural poisons.

Ricin which is a lectin, also termed a toxalbumin is a toxic plant-derived compound that combines with carbohydrate and protein components. Taken orally, ricin is readily absorbed from the stomach and intestine.

CHAPTER THREE
MATERIALS AND METHODS

3.1 **Experimental site**

The experiments were conducted in the Plant Pathology laboratory and Plant house of the Department of Crop and Soil Sciences of Kwame Nkrumah University of Science Technology (KNUST), and a farmer's field at Kotei, a vegetable farming community near KNUST, Kumasi.

3.2 **Source and extraction of root knot nematodes eggs from infested tomato roots**

Tomato plants infested with root knot nematodes were collected from tomato fields at Kotei, near KNUST. Eggs were extracted from the tomato infested roots by modified Hussey and Barker (1973) method. Washed roots of tomato were chopped with a pair of scissors. About 10g of the chopped roots was put in a jar and enough 0.5% sodium hypochlorite poured on it for four minutes. The resultant sodium hypochlorite-roots suspension was quickly passed through a 200 mesh sieve over 500 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 for *in vitro* studies and plant house inoculation.

3. 3 **Counting of root knot nematodes eggs**

Number of eggs in aqueous suspension was determined by using a stereo microscope. Two millilitres of the egg-water suspension was pipetted after bubbling air through the suspension for homogenecity and dispensed into a counting tray. Counting was done two times and the mean number of eggs/ml estimated.

3. 4 **Extraction and counting of root knot nematodes juveniles**

Extraction of root knot nematode juveniles was from infested roots of tomato, using modified Baermann tray method (Whitehead and Hemming, 1965). The roots were chopped with a pair of scissors and 10g of each entry in the study were placed separately in a plastic sieve lined with a two- ply tissue paper placed in a plastic plate. Tap water was poured carefully into the plastic plate in which the sieve was resting until the tissue became moist. The set up was left for 48h and were then poured separately into beakers and left for about 24h for the juveniles to settle at the bottom. The volume of each suspension was standardised to 50ml. Aliquot of 1ml of each suspension was taken with a pipette into a counting tray and counting done with the aid of a stereo microscope. Each suspension was homogenized by blowing air through with a pipette.

3. 5 **Source and preparation of castor bean seeds aqueous extracts**

Dried castor beans were collected from Kotei, near KNUST decorticated by pressing each seed gently in between the thumb and fore finger to get the beans for use. Fifty grammes of the thoroughly washed castor beans was ground in 100mls of tap water in an electric blender at high speed. This was filtered through cheese cloth and the filtrate served as the standard solution or crude extract. The extract obtained was then diluted further with water to 10 % (v/v), 20 % (v/v), 30 % (v/v) and 40 % (v/v). The crude extract and dilutions were used as treatments.

3.6 **Experimental designs**

The laboratory and pot studies were set up in complete randomised design (CRD) with four replicates for each of the study. However, randomised complete block design (RCBD) with four replicates was used for the field experiment.

3.7 **Statistical Analysis**

Data collected were analysed, using the Genstat statistical package. Least significant difference (Lsd) at 5% was used for comparing mean differences. All count data were transformed using square root transformation of $\sqrt{(x+0.5)}$ to ensure a normal distribution.

3.2.0 **Experiment 1: Effect of Castor bean aqueous extracts on root knot nematode eggs and juveniles *in vitro***

This experiment was conducted at the Plant Pathology laboratory of the Department of Crop and Soil Sciences, Faculty of Agriculture, KNUST.

3.2.1 **Application of treatments**

Twenty milliliters of each of the aqueous castor bean extracts and water was dispensed into Petri dishes. One hundred root knot nematode eggs and juveniles were put into separate Petri dishes containing the different extract treatments. Water was used as the control. The set ups were kept on laboratory benches at room temperatures.

3.2.2 **Parameters measured**

The number of eggs hatched were observed and counted on the third, sixth and ninth day after application of treatments. Number of dead juveniles (mortality) and their nature were observed and recorded 24h, 48h and 72h after the application of treatments. Juveniles were considered dead when they were found to have lost their body content and were inactive.

3.3.0 **Experiment 2**: **Effect of root-dip of potted tomato seedlings in aqueous extract of castor bean on root knot nematodes**

This pot trial was carried out in the Plant house at the Department of Crop and Soil Sciences, KNUST.

3.3.1 **Source of tomato seeds**

The tomato cultivar, Pectomec, used for the experiment, was purchased from the Obek Agro Services in Kumasi.

3.3.2 **Soil preparation and sterilisation**

The soil for the experiment was prepared by mixing three parts of riversand with one part of loam soil. The soil mix was sterilised, using a metal barrel steam steriliser. The steam steriliser has two chambers, the lower chamber contained water and the upper part the soil mix. The soil was covered with wet jute sacks to conserve steam in the chamber. Fire wood was used as the source of heat. The sterilised soil was allowed to cool before use.

3.3.3 **Raising of tomato seedlings**

The tomato seeds were nursed in sterilised top soil in a seed box. The seedlings were transplanted into one litre size pots filled with 850mls sterilised topsoil three weeks after germination.

3.3.4 **Application of treatments**

The roots of the test plants were dipped in 50mls of the different concentrations of the castor bean aqueous extracts for six hours before transplanting them into pots filled with the sterilised soil mix. The control plants were dipped in equal volume of water for the same period. The 10% of the crude extract (v/v) was replaced with 60% crude extract because it showed no significant difference with the control when used in the laboratory experiment.

3.3.5 **Inoculation of tomato seedlings**

The potted seedlings were inoculated with 1000 eggs of root knot nematodes two weeks after transplanting. Three holes were made in a triangular form, 2cm from the tomato plant. The eggs in water suspension were then dispensed into the holes and covered with the soil.

3.3.6 **Determination of plant growth parameters**

The test plants were harvested eight weeks after inoculation. To ensure easy removal of the plants from the soil, the sides of the plastic pots were pressed to loosen the soil. The soil was then removed from the roots by gently shaking the plants.

3.3.7 **Parameters measured**

The following parameters were measured;

- Plant height at harvest,
- Stem girth at harvest,
- Fresh shoot weight,
- Number of root knot nematode juveniles /5g of tomato roots,
- Root gall score (Bridge and Page, 1980) (Appendix 9)
- Egg mass of *Meloidogyne* spp.
- Fresh root weight

3.3.8 **Assessment of egg mass and root knot nematode galls**

The roots of the harvested tomato plants were each washed separately and dabbed dry with tissue paper. The entire root system of each test plant was then immersed in Phloxine B stain for five minutes and the number of egg masses counted. Galling was scored using the rating chart by Bridge and Page (1980), as shown in Appendix 9.

3.4.0 **Experiment 3: Effect of soil drenching with aqueous castor bean extracts on root knot nematodes of tomato**

This study was carried out in the Plant house at the Department of Crop and Soil Sciences, KNUST.

The same source of tomato seeds, method of soil preparation and sterilisation, raising of tomato seedlings and inoculation of potted tomato seedlings as described for experiment 2 was used.

3.4.1 **Application of treatments**

Before inoculation of the potted tomato plants, the roots of the plants were exposed by creating three holes in a triangular form 2cm from the stem. The soil around the root zone was then drenched with 50mls of each extract separately. The roots were then covered with the dug soil. The same procedure was done for the control, using water. The test plants were drenched with their respective treatments weekly for two months until the end of the experiment.

3.4.2 **Assessment of plant growth and root knot nematode galls and egg mass**

Harvesting and assessment of root knot nematode egg mass and galling of inoculated tomato seedlings were done eight weeks after inoculation as described in section 3.3.8. Also the same parameters in section 3.3.7 were measured.

3.5.0 **Experiment 4: Effect of root dip plus side drenching of aqueous castor bean extracts on root knot nematode of potted tomato**

This trial was conducted in the Plant house at the Department of Crop and Soil Sciences, KNUST.

The source of tomato seeds, method of soil preparation and sterilisation, raising of tomato seedlings and inoculation of potted tomato seedlings followed the same procedure as described in experiment 2.

3.5.1 **Application of treatments**

The roots of the test plants were initially dipped in 50mls of the different concentrations of the extract for six hours before growing them into pots filled with sterilised soil inoculated with about 1000 root knot nematode juveniles. The roots

of the tomato plants were exposed by creating a hole 2cm from the plant triangularly. Each plant was then drenched with 50mls of the extracts. The roots were then covered with the dug soil. The roots of the test plants were drenched with their respective treatments weekly for two months until the end of the experiment.

3.5.2 **Assessment of plant growth and root knot nematodes egg mass and galls**

Harvesting of tomato plants and plant growth parameters measured and assessment of root knot nematode egg mass and galling, for this study are the same as described in sections 3.3.6, 3.3.7, and 3.3.8.

3.6.0 **Experiment 5: Effect of aqueous castor bean extracts on root knot nematode under field environment**

This experiment was carried out at Kotei, a vegetable farming community near KNUST.

3.6.1 **Experimental site and land preparation**

The site used for the experiment was a farmer's field at Kotei previously used for the cultivation of tomato before. Beds each measuring 6m x 1m and separated from each other by 2m were prepared for the experiment after the weeds on land had been cleared by slashing.

3.6.2 **Root knot nematodes population on experimental site**

The initial population of root knot nematode on the field was assessed by sampling soils from the field. Root knot nematode juveniles were extracted and counted as described above in section 3.4.

3.6.3 **Application of treatments**

Because of the initial presence of root knot nematode on the field, the tomato roots were not inoculated again. However, the soil around the root zone was drenched with 50mls of each extract separately. The roots were then covered with the dug soil. The same procedure was done for the control, using water. The test plants were drenched with their respective treatments weekly until for two months until the end of the experiment.

3.6.4 A**ssessment of plant growth and root knot nematode eggs and galling**

Harvesting of tomato plants and plant growth parameters measured and assessment of root knot nematode egg mass and galling, for this study is the same as described in sections 3.3.6, 3.3.7, and 3.3.8.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 **Experiment 1 Effect of Castor bean aqueous extracts on root knot nematode eggs and juveniles *in vitro***

Table 4.1 Effect of aqueous castor bean extracts on root knot nematode egg hatch at different periods

Treatments (v/v)	Mean egg hatch at different periods (transformed)*

	Day 3	Day 6	Day 9
Castor crude extract	1.47	1.22	0.71
40 % crude extract	1.80	1.68	0.90
30 % crude extract	2.80	2.03	0.71
20 % crude extract	2.92	2.81	2.53
10 % crude extract	3.56	3.13	2.18
Water (Control)	3.90	6.54	8.65
Lsd (5%)	0.25	0.14	0.19
CV (%)	2.60	4.40	`12.40

*√(x+0.5), where x is the mean number of eggs.

Three days after application of the extracts to root-knot nematode eggs, the mean egg hatch ranged from 1.47 to 3.90 (Table 4.1). The highest egg hatch was observed in the water treatment whilst the lowest was observed in the crude extract. There was significant difference (P = 0.05) between 40% extract, 30% extract and the crude extract (Table 4.1). On the sixth day, mean egg hatch was between 1.22 and 6.54. The highest egg hatch was found in the control whilst the least hatch was in the crude extract. There was significant difference (P = 0.05) between all the treatments (Table 4.1).

On the ninth day with the exception of the 40%, 30% and crude extracts which showed no difference, there was significant difference (P = 0.05) between all the other treatments.

Table 4. 2 Effect of castor bean aqueous extract on root-knot nematode juvenile mortality *in vitro* at different period

Treatments (v/v)	Mean number of juvenile mortality at different periods (transformed)*

	24h	48h	72h
Castor crude extract	4.88	6.86	8.45
40 % crude extract	3.76	5.76	8.19
30 % crude extract	3.62	5.89	8.09
20 % crude extract	3.62	5.22	8.08
10 % crude extract	3.75	5.13	7.35
Water (Control)	3.14	4.47	5.94
Lsd (5%)	0.25	0.22	0.18
CV (%)	4.50	2.70	1.50

*$\sqrt{(x+0.5)}$, where x is the mean number of eggs.

A day after exposing the juveniles to the treatments, the mean number of juvenile mortality of 4.88 was recorded for the crude extract whilst the control recorded the least mean number of juvenile mortality of 3.14 (Table 4.2). There was, however, no difference (P=0.05) between 40% 30%, 20%, and 10% extracts but there was significant difference between the crude extract and all the other treatments (Table 4.2).

Two days after exposure, mean number of juvenile mortality increased and ranged from 4.47 to 6.86 for the crude extract and control treatments, respectively. There was no difference (P=0.05) between 40% and 30% extracts (Table 4.2). Also there was significant difference (P=0.05) between the crude extract and other treatments.

As the exposure period increased, juvenile mortality also increased. On the 3rd day, the control treatment recorded the least mean mortality of 5.94 whilst the crude extract recorded the highest mean mortality of 8.45. There was no difference (P=0.05) between 30% and 20% extracts but the crude extract was significantly different from all treatments (Table 4.2).

According to Khan (1990), many wild and cultivated medicinal plants have been shown to possess nematicidal properties against several plant parasitic nematodes. The results of the

study showed that water extract of castor bean had an effect on the root knot nematode *in vitro* by inhibiting egg hatch and increasing juvenile mortality at different concentrations of the extract.

It was also observed that inhibition of egg hatch increased with increasing concentration of the extract with the highest recorded in the crude extract. This observation agrees with the 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.

Ameer-Zareen *et al*. (2003) reported of similar findings against root knot nematode eggs *in vitro* when aqueous extract of ginger was used. The study also agrees with Barker (2003) that nematode egg hatch was influenced by the exudates from its environment.

Egg hatch inhibition also increased with increase in exposure of time. This agrees with Joymatti *et al.* (1998), who reported that eggs exposed to extracts of *Melothria purpusilla* (Blume) 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 inhibitory effect according to Adegbite and Adesiyan (2005), might be due to the chemical properties present in the extract that possess ovicidal properties. It was also suggested that botanicals with nematicidal properties affect the embryonic development or kill the eggs. Presumably these properties increase with increase in time hence, the increased inhibition as exposure period increased.

The extract was also found to have a killing effect on root knot nematode juveniles (Table 4.2). This result agrees with the observations of Khan *et al*. (1967) that water extract of oil cakes such as groundnut, sesame, mustard and castor showed inhibitory effect on larval emergence of *M. incognita*. Also, aqueous extract of castor root was reported by Adegbite and Adesiyan (2005) to cause mortality of *M. incognita* juveniles.

The study also revealed that juvenile mortality increased with increase in concentration of the extract (table 4.2). This is in agreement with Hasabo and Noweer (2005) who found that the mortality effect of an extract on nematode is concentration dependant. Again Ameer-

Zareen *et al*. (2003) and Joymatti *et al.* (1998) whilst working with aqueous extract of ginger and *Jatropha curcas* also recorded similar results as was observed in the present study. Juvenile mortality was also found to increase with increase in exposure time. This observation agrees with the findings of Akhtar and Mahmood (1993), Lashien (2002), El-Nagdi and Mansour (2003).

The toxicity of botanicals, according to Ameer-Zareen *et al.* (2003), is due to biologically active constituent which, according to Knoblock *et al*. (1989), is characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cell and their functional groups interfering with the enzyme protein structure. Ricin, the principal toxin in castor seed, is a member of the toxalbumins and these are protein phytotoxins that are capable of inhibiting protein synthesis. Konstantopoulo *et al*. (1994) reported that plant extract activity may include denaturing and degrading of proteins and inhibition of enzymes.

4.2 **Experiment 2: Effect of bare root dipping of potted tomato seedlings in aqueous castor bean extracts on root knot nematodes**

Table 4.3 Effect of bare root dipping in aqueous castor bean extracts on plant height and stem girth of *Meloidogyne* inoculated tomato plants in pots

Treatments (v/v)	Mean plant height	mean stem girth
Castor crude extract	19.02	1.85
60% crude extract	17.36	1.70
40 % crude extract	17.42	1.68
20 % crude extract	15.68	1.45
Water (control)	15.54	1.45
Lsd (5%)	1.45	0.19
CV (%)	5.70	7.70

The plant height ranged from 15.4 cm to 19.02 for the control and crude extract respectively (Table 4.3). The application of the extracts had effect on the height of the plants. There was no difference between the 60% and 40% crude extracts and also between the 20% crude extract and the water. The crude extract was significantly different (P=0.05) from all the treatments (Table 4.3). The mean stem girth length ranged from 1.45 cm to 1.85 cm (Table 4.3). There was no difference (P=0.05) between the crude extract and 60% crude extract treatments. However, the differences (P=0.05) between the crude extract and control treatments were significant.

Table 4.4 Effect of bare root dipping on fresh shoot and root weights of *Meloidogyne*-inoculated tomato plants in pots

Treatments (v/v)	Mean fresh weight (cm)	
	Shoot	Root

Castor crude extract	9.95	1.52
60% crude extract	7.70	1.57
40 % crude extract	7.39	1.72
20 % crude extract Water (control)	6.95	2.63
	5.43	2.73
Lsd (5%)	1.90	0.69
CV (%)	17.40	22.60

The crude extract treatment recorded the largest fresh shoot weight of 9.95g whilst the control had the smallest fresh weight of 5.43g. The crude extract was significantly different from all the other treatments (Table 4.4). The root weight recorded indicated that crude extract treatment had the least weight of 1.52g whilst the control had the heaviest weight of 2.73g. There was no difference (P= 0.05) between the crude extract and the 60% and 40% crude extract but the difference (P=0.05) between the crude extract and 20% crude and the control were significant(Table 4.4).

Table 4.5 Effect of root dipping in aqueous castor bean extract on number of root knot nematode egg mass on potted tomato plants

Treatments (v/v)	Mean number of egg mass/5g of tomato
Castor crude extract	0.57
60% castor crude extract	0.83
40% castor crude extract	1.93
20% castor crude extract	2.18
Water (control)	2.24
Lsd (5%)	0.14
CV (%)	17.60

More egg masses were found on the roots of the water treated plants as compared with the tomato plants treated with the extracts (Table 4.5). There was no difference between the crude and 60% extracts but both treatments were significantly different (P=0.05) from all the other treatments.

Table 4.6 Effect of root dipping of tomato in aqueous castor bean extracts on root knot nematode juveniles and galling in potted tomato plants

Treatments (v/v)	mean gall score (0-10)	Mean number of juveniles/5g roots*
Castor crude extract	2.00	3.82
60 % crude extract	2.50	4.81
40 % crude extract	5.25	5.39
20 % crude extract	6.25	5.68
Control (water)	6.50	5.61
Lsd (5%)	0.73	0.29
CV (%)	10.70	3.80

* √ (0.5+ x) where x is mean counts, scale (0-10) see appendix 9

More juveniles were recovered from the roots of the control treated plants than from the plants treated with the extracts (Table 4.6). There was significant difference (P=0.05) between the crude extract and all the other treatments.

The roots of the control plants had the largest gall infestation as compared to the aqueous treated plants. There was no significant difference (P=0.05) between the 60 % and crude extract but both were significantly different (P=0.05) from all the other treatments (Table 4.6).

The aqueous castor bean extract was found to be potent in the management of root knot nematode juveniles (Table 4.6). This observation agrees with that of Hasabo and Noweer (2005), who reported that dipping of the egg plant cv. Baladi in water extracts of basil, marigold, China berry and neem affected the survival and reduced the population of *M.*

incognita juveniles. It was also observed that the mortality of root knot nematode juveniles was concentration dependant (Table 4.6) and this agrees with Lashein (2002) who reported a similar trend whilst working with *Jatropha curcus.*

Fewer galls were recorded on the aqueous castor extract than the water treated plants (Table 4.6) According to Mian and Rodriguez-Kabana (1982) this could be attributed to the action of the toxic compounds released by castor bean. The increase in plant height in the extract treated plants could be due to the reduction in the activities of the root knot nematode juveniles and less galls formed on the roots. Heavily root knot nematode infested plants according to Sidiqque and Alam (1987), exhibit stunted growth.

The larger shoot weight recorded in the aqueous extract treated plants could be attributed to the preventive ability of the aqueous castor bean. According to Hussey (1985), an increase in shoot weight was due to the uptake and transportation of water and nutrients which is dependent on the health of the roots.

4.3 **Experiment 3: Effect of soil drenching with aqueous castor bean extracts on root knot nematodes on tomato plants**

Table 4.7 Effect of soil drenching with castor bean extract on plant height and stem girth of root knot nematode inoculated tomato plants in pots

Treatments (v/v)	Mean plant height	mean stem girth
Castor crude extract	6.55	2.00
60% crude extract	5.89	2.00
40 % crude extract	4.95	1.85
20 % crude extract	3.66	1.72
Water (control)	3.64	1.45

Lsd (5%)	1.81	0.11
CV (%)	14.10	14.20

Soil drenching with the extracts had an effect on the height of the tomato plants. The control had the shortest plants of 3.64 cm whilst the crude extract recorded a mean height of 6.55 cm (Table 4.7). There were no significant differences between the crude extract and the 60% crude extract but each was significantly different (P=0.05) from all the other treatments. The greatest mean stem girth of 2.00cm was recorded for the crude extract and 60% crude extract whilst the water treatment recorded the smallest of 1.45cm (Table 4.7). There was no significant difference (P=0.05) between the crude extract and 60% crude extract, however, the difference (P=0.05) between the two and all the other treatments (Table 4.7), were significant.

Table 4.8 Effect of soil drenching with castor bean extracts on fresh shoot and root weights of root knot nematode-inoculated tomato plants in pots

Treatments (v/v)	Mean fresh weights (g)	
	Shoot	Roots
Castor crude extract 60	17.85	3.64
% crude extract	17.13	3.66
40 % crude extract	13.55	4.95
20 % crude extract	13.46	5.89
Control	12.98	6.55
Lsd (5%)	3.02	1.83
CV (%)	13.40	24.50

The crude extract recorded the largest weight of 17.85g whilst the control treatment recorded the least weight of 12.98g (Table 4.8). There was, however, no significant

difference (P=0.05) between the crude extract and 60 % extract. The least mean root weight of 3.64g was recorded for the crude extract whilst the control treatment had the heaviest weight of 6.55g (Table 4.8). With the exception of the crude extract and 60% crude extract which showed no significant differences, all the other treatments showed significant differences.

Table 4.9 Effect of soil drenching with castor bean extracts on mean number of root knot nematode egg masses produced on tomato plants in pots

Treatments (v/v)	Mean number of egg mass/5g of tomato
Castor crude extract	0.71
60 % extract	1.65
40 % extract	2.06
20 % extract	2.12
Control (water)	2.24
Lsd (5%)	0.16
CV (%)	16.20

Roots of the control and lower concentration treatments were found to be more favourable to root knot nematode activities (Fig.4.9). More eggs were, therefore, deposited on their roots than roots of plant treated with the higher extract concentrations.

Table 4.10 Effect of soil drenching with castor bean extracts on root knot nematode juveniles and galling of inoculated tomato plants in pots

Treatments (v/v)	Mean galling (0-10)	Mean number of juveniles/5g tomato roots*

Castor crude extract		2.25	4.00
60 % extract		4.75	4.74
40 % extract		6.25	5.34
20 % extract		6.50	6.15
Control (water)		7.00	6.37
Lsd (5%)	CV (%)	0.72	0.16
		8.70	16.20

* √ x+0.5, where x is mean number of root knot nematodes juveniles counted Scale (0-10) see appendix 9

More juveniles were counted from the control treated plants compared to those treated with the castor bean extracts (Table 4.10) and the control plants had the highest galling score of 7.0 whilst the plants treated with castor bean extracts had the least, the crude extract recording 2.25 (Table 4.10).

Results from the study conducted showed that the castor extract caused a reduction in the number of root knot nematode juveniles and egg masses (Tables 4.9 and 4.10). This observation is in agreement with Alashalaby and Noweer (2003), who reported that aqueous neem extract significantly reduced the total number of root knot nematode juveniles and inhibited egg hatch in peanut roots and soil. Mean number of juveniles recovered was low in the highest concentration compared to the control treatment (Table 4.10). This result corresponds with the findings of Joymatti *et al*., (1998) who reported that juvenile mortality was concentration dependent whilst working with extract of ginger.

Also, it was observed that the number of galls was less in castor bean extract-treated plants as compared to the control plants (Table. 4.10). This phenomenon, according to Gommers *et al.* (1982), may be due to the action of the extract releasing substances into the soil which inhibits the entry of root knot nematodes into the roots of plants.

Plant under the control treatments were taller than the castor bean extract-treated plants and finding of the study agrees with the findings of Couch and Van Staden (1993) who recorded significant increase in plant height and a corresponding reduction in *M. incognita* infestation when *Ecklonia maxima* extract was applied as soil drench. The increased shoot weight in the aqueous extract castor bean-treated plants may be due to the ability of the roots to absorb more nutrients as compared to the water-treated plants whose roots, were highly infested or galled. Heavily infested roots according to Hussey (1985), reduce the uptake and transportation of nutrients. It was also observed that the root weight of the water-treated plants was heavier than the castor extract-treated plants and this may be due to higher number of galls formed on their roots.

4.4 **Experiment 4**: **Effect of root dip plus soil drenching with aqueous castor bean extracts on root knot nematodes of potted tomato plants**

Table 4.11 Effect of root dipping plus soil drenching on plant height of root knot nematode-inoculated tomato plants in pots

Treatments (v/v)	Mean plant height of tomato (cm)
Castor crude extract	23.16
60 % crude extract	21.77
40 % crude extract	20.19
20 % crude extract	18.18
Water (control)	18.54
Lsd (5%)	0.88
CV (%)	12.40

Plant height ranged from 18.54 cm for the control treatment to 23.16cm for the crude extract (Table 4.11). There was a significant difference (P=0.05) between the crude extract and all the other treatments. There was however no difference between the 40% crude extract, 20% crude extract and the control treatments.

Table 4.12 Effect of root dipping plus soil drenching with aqueous castor bean extracts on fresh shoot and root weights of root knot nematode- inoculated tomato plants

Treatments (v/v)	Mean fresh weights (g)	
	shoot	Roots
Castor crude extract	11.86	2.02
60 % extract	11.77	2.05
40 % extract	8.34	2.32
20 % extract	8.03	2.47
Control	7.84	2.85
Lsd (0.05)	1.78	0.65
CV (%)	12.40	18.50

The crude extract recorded the largest fresh shoot weight of 11.86 g whilst the control had the least of 7.84g. There was a significant difference (P=0.05) between the crude extract and water treatments. The crude extract recorded the least root weight of 2.02g whilst the water had the heaviest of 2.85g (Table 4.12). There was a significant difference (P=0.05) between the crude extract and water treatment.

Table 4.13 Effect of root dipping plus soil drenching with aqueous castor bean extracts on root knot nematode juveniles and galling of inoculated tomato plants.

Treatments (v/v)	Mean galling (0-10)	Mean number of juveniles/5g of tomato roots *

Castor crude extract 60 % crude extract 40 % crude extract 20 % crude extract Control (water)	2.00 2.25 3.25 4.25 5.00	3.67 3.76 4.76 5.80 5.90
Lsd (0.05) CV (%)	0.24 13.30	0.80 15.90

* √ x+0.5 where x is mean number of juveniles/ g of tomato roots

Scale (0-10) -appendix 9

The mean number of root knot nematode juveniles recovered from the roots of the control plants were greater than the other treatments (Table 4.13). The control recorded the largest gall score of 5.0 whilst the crude extract recorded the least of 2.0 (Table 4.13) Table 4.14 Effect of root dipping plus soil drenching with aqueous castor bean extracts on mean number of root knot nematodes egg mass on tomato plants in pots

Treatments (v/v)	Mean number of egg mass/g tomato roots
Castor crude extract 60 % crude extract 40 % crude extract 20 % crude extract Water (control)	0.71 0.83 1.31 1.56 1.79
Lsd (5%) CV (%)	0.29 15.90

Roots of water and lower concentration extract treatments were found to be more favourable to root knot nematode activities (Table.4.14). More eggs were deposited on their roots compared with the higher concentrations.

In the present study castor bean extract was found to have a nematotoxic effect on root knot juveniles and eggs (Table 4.13). A reduction in the root knot nematode juveniles, according to Bunt (1975), could be attributed to poor root penetration and later retardation in activities such as feeding and reproduction. According to Akhtar and Mahmood (1993), castor bean extracts have a systemic activity against nematodes. The nematicidal constituents of castor were thereby probably absorbed by the roots of plants which in turn had adverse effects on the root knot nematodes.

The mortality of root-knot nematode juveniles was found to depend on the concentration of the extract (Tables 4.13). Mean number of juveniles recorded was least in the highest concentration as compared to the control treatment.

Plant height was found to be highest in the extract-treated plants. The increase in height according to Pattison (2007), is due to the reduction in the number of galls and decrease in the activities of the root-knot nematode juveniles.

According to Caveness and Ogunforowa (1985), root-knot nematode-infested plants were seriously affected by their reduced uptake and transportation of water and nutrients which, in turn, affected their shoot weight. Similar observation was made with regard to fresh shoot weight in this study (Table 4.12).

A considerable increase in stem girth was also recorded for the castor bean aqueous treated plants. The increase in stem girth, according to Gommers *et al.* (1982), was due to the translocation of water and nutrients to the shoots. It was, however, observed that the root weight of the control plants were considerably heavier than the castor extract-treated plants, and this might have been due to the higher number of galls formed on the roots.

4.5 **Experiment 5**: **Effect of aqueous castor bean extracts on root knot nematodes on tomato plants sunder field environment**

Table 4.15 Effect of castor bean extract on galling of tomato under field conditions

Treatments (% v/v)	Mean gall score (scale 0-10)
Castor crude extract	6.50

60 % crude extract	7.00
40 % crude extract	7.25
20 % crude extract	6.75
Water (Control)	7.00
CV (%)	19.00

Scale (0-10) appendix 9

The roots of the 40% extracted treated plants had the largest gall infestation as compared to all the other plants. However, there was no significant difference (P=0.05) between all the treatments (Table 4.15).

Table 4.16 Effect of castor bean extracts on plant height of root knot nematode- inoculated tomato plants under field environment

Treatments (% v/v)	Mean plant height (cm)
Castor crude extract 60	20.50
% crude extract	21.25
40 % crude extract	21.25
20 % crude extract	22.00
Water (Control)	20.50
CV (%)	12.30

It can be observed that the 20% crude castor extract had the highest height of 22.0 cm whilst the water treatment had the lowest height of 20.50cm (Table.4.16)

Table 4.17 Effect of castor bean extracts on root knot nematode juveniles recovered from inoculated tomato plants under field environment

Treatments (% v/v)	Mean number of juveniles/5g of tomato roots

Castor crude extract 60 %	17.10
of extract	17.10
40 % of extract	17.25
20 % of extract	17.23
Control	17.50
CV (%)	11.30

Mean number of root knot nematode juveniles recovered from roots of tomato ranged from 17.10cm to 17.50cm for the crude extract and water treatments, respectively. Although the control treatment recorded the highest number of juveniles, there was no significant difference (P=0.05) between the treatments.

Results from the field trial did not show any difference between the treatments in terms of plant height (Table. 4.16), number of root-knot nematode juveniles (Table 4.17) and number of galls (Table 4.15). This result corresponds with the results of Osei (2000), who recorded similar findings whilst working with African marigold. According to Chitwood (2002), naturally occurring compounds are often more readily degraded in the environment than synthetic compounds and this may have accounted for the poor performance of the castor bean extracts used. El-Nagdi and Mansour (2003) observed that the potency of botanicals was affected by exposure time and this may not be an asset in the use of botanicals if the target nematode is to be exposed to the extract for a long period of time as on the field.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

As the existing practice of chemical control is too costly for poor farmers, as well as its harmful effect responsible for air, soil and water pollution (Alam, 1987), this study was conducted to find the effect of aqueous castor bean extracts on root knot nematode of tomato.

An *in vitro* study showed that the castor bean extract used caused a significant reduction in root knot nematode eggs hatched as well as higher juvenile mortality. It was also observed that castor bean aqueous extract is nematicidal at higher concentrations. Also, it was observed that a reduction in egg hatch as well as a higher juvenile mortality by the extract was dependant on exposure period.

Pot experiments revealed that lesser number of juveniles were recovered from the roots of the aqueous castor extract-treated plants as compared to the water-treated plants. Also, the mean score of root knot nematode galls in the water was higher than was found in the castor extract treated-plants for all the pot experiments. In all the pots experiments, it was observed that the plant height, stem girth and shoot weight of the castor extracts treated-plants were greater compared with the control treatment. It was, however, observed that the root weight of the control plants were considerably heavier than the aqueous castor extract treated plants.

The field study results, however, showed that the castor bean aqueous extract could not perform well under field conditions. No differences were found between plant height, root knot nematodes juveniles recovered from the tomato roots and mean gall scores formed in both the castor extract-treated and water-treated plants.

Generally, the aqueous crude castor bean extract was potent and efficient in managing root knot nematodes *in vitro* and in potted tomato. However, it was not effective in the field.

5.2 Recommendations

- Farmers can use aqueous castor bean extract for the management of root knot nematodes at the nursery stage to reduce their build-up before transplanting to the field.

- A study using other extraction solvents such as alcohol and ether to determine their effect on root-knot nematode eggs hatch and juvenile mortality should be conducted

- Future study should incorporate mashed or ground or whole castor bean into soil as mulch to find its effect on root knot nematodes, plant growth and development.

References

Abawi, G.S, and Barker, K.R. (1984). Effect of cultural, soil temperature and population levels of *M. incognita* on root necrosis and *Fusarium* wilt of tomato. *Phytopathol.* 74:433-38.

Adegbite, A.A. and Adesiyan, S.O. (2005). Root extracts of plants to control root knot nematode on edible soybean. *World Journal of Agric Science* 1(1): 18-21.

Agrios, G.N. (1997). Plant Pathology. 4th Edition. Academic Press. USA. pp. 635

Akhtar, M. and Alam, M. M. (1990). Evaluation of nematicidal potential in some medicinal plants. *Int. Nematol. Network Newsl*. 6:8-10.

Akhtar M. and Mahmood, I. (1993). Control of plant parasitic nematodes with "Nimin" and some plant oils by bare- root dip treatment. *Nematol. Medit*., 21; 89-92.

Alam, M.M. (1987). Pollution free control of plant parasitic by soil amendment with plant waste. *Biol. Wastes* 22:75-79.

Alam, M. M. and Saxena, S.K. (1977). Persistent action of plant parasitic nematodes as a result of the application of organic amendments to the soil. Vrole of phenolic compounds. *Ind. Jnl Nematol* 9:136-42.

Alashalaby, E. M. and Noweer, E .M. A. (2003). Effect of five plant extracts on the reproduction of root knot nematode *Meloidogyne incognita* infested peanut under field condition. *J. Agric Science*,Mansoura Uni 28(12) 74477454.

Ameer-Zareen, Zaki, J. M. and Javed, N. (2003). Nematicidal activity of ginger and its effect on the efficacy *Pasteuria penetrans* for the control of root knot nematodes. *Asian Journal of Plant Science* 2(11):858-860.

Barker, A. D. P. (2003). Novel approaches to potato cyst nematode control. In Abstracts Conference on Potato Production - Living with Pesticide Changes, York, 25 November 2003.

Beecher, G. R. (1998). Nutrient content of tomatoes and tomato products. *Proc. of the society for experimental boil. And med*. 218: 98-100.

Berlinger, M. J. (1986). Pest. In: *The tomato crop*: A scientific basis for improvement. Atherton J.G and Rudich J (eds). Chapman and Hall, London. pp. 391-432.

Bird, A. F. and Loveys, B. R. (1975). The incorporation of photosynthates by *M .javanica. J. Nematol*. 7:111-113.

Bird, A. F. and Bird, J. (1986). Observations on the use of insect parasitic nematodes as a means of biological control of root-knot nematodes. *International Journal of Parasitology*. 1986; 16:511–516.

Bridge, J. (1996a). Imported and indigenous plant nematodes of different crops and cropping systems in Africa. *Afr. Plant Prot.* 2:67-74.

Bridge, J. (1996b). Nematode management in sustainable and subsistence agriculture. *International Institute of Parasitology*, UK. Pp. 203-209.

Bridge, J. and Page, S.L.J. (1980). Tropical Pest Management. 26 Number 3.

Broadley, R.A (1979). Nematicide treatment of banana planting material. *Australian Journal of Experimental Agriculture and Animal Husbandry* 19(100) 631 – 633.

Bunt, J.A. (1975) Effect and mode of action of some systemic nematicides. Meded.lando Hogesch, Wageningen 75:1-128

Caveness, F.E. and Ogunforowa, A.O. (1985). Nematological studies worldwide. In: Cowpea Research Production and Utilization. (eds.) Singh S.R and Rachie K.O. Wiley and Sons. pp. 273-285

Chitwood, D.J. (2002). Phytochemical based strategies for nematode control. *Ann. Review of Phytopathology*. 40. Pp. 221–249.

Collins, K. (2007). Benefits of eating tomatoes. www.msnbc.msn.com retrieved 06/06/09

Coomans, A. (1979). The anterior sensilla of nematode. *Rev. Nematol* 2:259283.

Couch, J.J. and Van Staden, J. (1993). Effect of seaweed concentrate from Ecklomia maxima (Osbeck) Papenfuss on *M. incognita* on tomato. *J. Appl. Phycol.* 5:37-43

De Lannoy, G. (2001). *Vegetables*. In: Crop production in tropical Africa. Romain H. Raemaekers (ed.) DGIC, Brussels. pp. 467-75.

Devi, T.P. and Gowani, B.K. (1992). Interrelationships of root knot nematode *M. incognita and Macrophomina phaseolina* with cowpea. *Ann. Agric Res.* 13:267-68.

Diamonde M, and Beute, M.K. (1981). Relation of *M. hapla* and *Macroposthonia ornate* population to *Cylindrocladium* black rot in peanuts. *Plt. Dis.* 65:339-42.

Egunjobi, O. A. and Larinde, M.A. (1975). Nematodes and maize growth in Nigeria II Effects of some amendments on population of *pratylenchus brachyurus* on growth and production of maize *(zea mays)* in Nigeria. *Nematol. Mediterr.* 3:65-73.

El-Nadgi, W.M.A. and Mansour, A.F.A. (2003). Management of the root knot nematode *M incognita* infecting sugar beet by certain medicinal plant oil products. *Egypt. J. agric Res. NRC* 28: 361-67.

F.A.O. (2005). www.fao.org

Fargette, M. (1987). Use of the esterase phenotypes in the taxonomy of the genus *Meloidogyne 2.* Esterase phenotype observed in West Africa population and their characterissation. *Reveue de Nematologie* 10:45-56.

Ghana Investment Promotion Council (GIPC). (2001). www.gipc.org.gh 23/05/08

Gommers, F.L. Bakker, J. and Nymbrg, H. (1982). Dithiophenes as single oxygen sensitizers. *Phytochemistry and Photobiology* 35: 615-619.

Green, C.D. (1971). Mating and host finding behaviour of plant nematodes. In :plant parasitic nematodes. Vol.II. BM Zuckerman, W.F Mai and Rhode R.A. (eds.) Academic Press. New York. 347pp.

Guar, H.S. and Perry, R.S. (1991). The use of soil solarisation for the control of plant parasitic nematodes. *Nematol. Abstr*. 60(4) 153-67.

Gupta, R. and Sharma, N.K. (1993). A study of the nematicidal activity of *Allicin*- An active principle in garlic, *Allium sativum* L against root knot nematode *Meloidogyne incognita* (Kofoid and white, 1919) Chitwood, 1949. *Int Jnl. Pest management* 39:390-92.

Hasabo, A. S. and Noweer, E.M.A. (2005). Management of root knot nematode *M incognita* on egg plant with some plant extracts. *Egypt. J. Phytotopathol* 33 (2) 65-72.

Hemeng, O.B. (1981). Efficacy of selected nematicides for the control of root knot nematode, *Meloidogyne spp.* on tomato in Ghana. *Ghana Jnl Agric. Sci*. 13:37-40.

Hussey, R.S. (1985). Host-parasite relationship and associated physiological changes. In: Advance treatise on *Meloidogyne.* Vol. 1. Biology and control. Releigh, North Carolina State Uni. 143-53pp.

Hussey, R.S. and Barker, K.R., (1973). A comparison of methods of collecting inocula of *Meloidogyne spp*. including a new technique. *Plant Dis. Rep*.57, 1025–1028.

Johnson, C.S. Way, J. and Barker, K.R. (2005). Nematode parasite of tobacco. In plant parasitic nematode in subtropical and tropical agriculture. (eds.) Luc M, Sikora R.A and Bridge J. Wallingford, CAB International. pp 675-708.

Joymatti, L., Dhanachand, C. and Devi, L.S. (1998). Effect of plant extracts on *M. incognita. Indian J.Nematology* 28:225-230.

Khan, A. F. (1990). Nematicidal potential of some naturally growing plants against *Pratylenchus* zeae. *Reveue nematol*.13 (4): 463-465.

Khan, A.M., Adhami, A.. Siddiqui, Z.A. and Saxsena, S.K. (1967). Effect of different oil cakes on hatching of larvae and on the development of root-knot caused by *Meloidogyne incognita. All India NematoL Symp.* Aug. 169 (Abstr.)

Knoblock, K. Weis, N. and Wergant, R. (1989). Mechanism of antimicrobial activity of essential oils. *37th Ann. Cong. Med. Plt Res. Braunscwig* pp 5-9.

Konstantopoulo, I., Vassilopoulo, L., Mawogantisi, P. and Scouras, G., (1994). Insecticidal effect of essential oils: a study of essential oils extracted from eleven Greek aromatic plants on *Drosophila auroria. Experimentia,* 48: 616-19.

Korayem, A.M. and Hasabo, S.A. (1994). Phytonematoxic properties in the extracts of some indigenous plants. *J. Union Arab Biol, Botany I (B):* 88-89.

Kumari, R. Verma, K.K. Dhindsa, K.S. and Bhatti, D.S. (1986). *Datura, Impomea, Tagetes and Lawsonia* as control of *Tylenchulus semipenetrans and Anguina tritici. Ind. Jnl. Nematol*. 16:236-240.

Lashien, A.M.S.A., (2002). Biological control of root knot nematode in some vegetables. MSc Thesis, Fac. Agric Cairo Uni. pp 107.

Lowe, J., (1992). Nematological Research at IITA 1969-1988. A summary of investigations conducted by F.E Caveness. *Plt. Health Management Res. Monogr*. 2 IITA, Ibadan, Nigeria. 52pp.

Luc, M. Sikora, R.A. and Bridge, J. (1990). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International Wallingford, U.K.pp.629

Mai, W.F. and Abawi, G.S. (1987). Interactions among root knot nematodes and *Fusarium wilt* fungi on host plants. *Ann. Rev. Phytopathol*. 25:317-38.

Mani, A. and Chitra, K.C. (1987). Toxicity of certain plant extract to *Meloidogyne incognita. Nematol Medit.* 17:43-44.

Marban-Mendoza, N., Jeyaprakash, A., Jansson, H.B., Daman, R.A. and Zuckerman, B.M. (1987). Control of root knot nematode tomato by lectins. *J. Nematol* 19:331-35.

McGawley, E.C., Rush, M.C. and Hollis, J.P. (1984). Occurrence of *Aphelenchoides besseyi* in Louisiana rice seed and its interaction with *Sclerotium oryza* in selected cultivar. *J. Nematol*. 1:318-20.

Mian, I.H. and Rodriguez-Kabana, R. (1982). Survey of the nematicidal properties of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria. Nematropica*, 12:235-246

Netscher, C. (1978). Morphological and physiological variability of *Meloidogyne* species in West Africa and implication for their control. *Mededelingen Landbouuwhogeschool* Wageningen. 46p.

Norman, J.C. (1992). Tropical vegetable crops. Arthur H. Stockwell Ltd Ilfracombe, Great Britain. 52-77pp.

Orion, D. and Franck, A. (1990). An electron micro study of cell walls hydrolysis by *Meloidogyne javanica* gelatinous matrix.*Rev. Nematol* 13:105107.

Osei, K. (2000) Effect of African marigold (*Tagetes erecta* L) on Root knot nematodes (Meloidogyne incognita) Chitwood, 1949 MSc thesis submitted to Faculty of Agric. KNUST. pp.88

Pattison, T. (2007). Tomato root knot nematode: biology, and control. Department of Primary Industry and Fishries. Queensland, Australia.pp.50

Powell, N.T. (1979). Internal Synergisms among organisms inducing diseases. In: Plant diseases. An Advance Treatise (eds.) Horstall, J.G. and Cowling E.B 4: 113-3, NY Academic 466p.

Report on pests and diseases (RPD). (1993). Uni. Of Illinois , Extension RPD 1101 , Illinois.

Rodriguez-Kabana, R. (1986). Organic and inorganic nitrogen amendments to soil as nematode suppressants. *J. Nematol* 18(2) 129-35.

Saifullah, Z.M, and Gul A, (1990). Organic amendments as control of root knot nematodes. *Int. Nemaatol. Network Newsletter* 7:22-24.

Sankaram, A.V.B., Marthnda, M., Murthy, K., Bhaskaraiah, M., Subramanyam, N., Sultana H.C., and Leushner, K. (1986). Chemical, biological activity and utilization of some promising neem extracts. *Third Int.Neem Conf.* Nairobi,Kenya. 1986

Sasser, J.N. (1989). Plant Parasitic Nematodes; the farmer's hidden enemy. North Carolina State Uni. Raleigh N. Carolina. 115p.

Sasser, J.N. and Carter, C.C. (1985). An Advance Treatise on *Meloidogyne Vol 1*, Biology and control North Carolina State Uni. Raleigh N. Carolina. 178p.

Shepherd, J.A.B.K.R. (1990). *Nematode* parasite of tobacco. In: Plant parasitic nematode in subtropical and tropical agriculture. (Ed). Luc M, Sikora R.A. and Bridge J. Wallingford, CAB International. pp. 493-517.

Siddiqui, M.A. and Alam, M.M. (1988). Effect of latex seed dressing on *Rotylenchulus reniformis* and plant growth of some vegetables. *Nematol. Medit.* 16:129-130.

Siddiqui, M.A. and Alam, M.M. (1987). Efficacy of seed dressing with extracts of neem and Persia lilak against *M. incognita and Rotylenchulus reniformis. Nematol. Mediterr*. 15:399-403.

Siddiqui, M.A. and Alam, M.M. (1985). Evaluaion of nematicidal properties of different parts of Margosa and Persian Lilac. *Neem Newsletter*, 2:1-4.

Siddiqui, M.A., Haseeb, A. & Alam, M. M. (1987). Evaluation of nematicidal properties in some latex bearing plants. *Indian J. Nematol,* 17: 99-102

Sikora, R.A., Singh, R.S. and Sitaramaiah, K. (1973). Control of root knot through organic and inorganic soil amendments 3. Effect of rice husk and sugar cane bagasse. *Haryana J. Hort. Sci*. 2:123-127.

Singh, R.S. and Sitaramaiah, K. (1969). Control of root knot nematode through organic and inorganic amendments of soil. I. Effect of oil cakes and saw dust. *First All India Nematology Sympossium*. New Delhi. 1969.

Stirling, G.R. (1991). Biological control of plant parasitic nematodes: Progress, Problems and Prospects. Wallinford, UK. CAB Int. 282p.

Stover, R.H. (1979). Flooding of soil for disease control. In soil disinfestation (Developments in Agriculture and Managed Forest ecology) (ed) D. Mulder 6:19-28 Amsterdam. The Netherlands: *Elsevier*

Subramaniyan, S. and Vadivelu, S. (1990). Effect of *Crotalaria speectarillis extracts on Meloidogyne incognita. Int. Nematol. Network Newsl*. 7:8.

Sukul, N.C. (1992). Plants antagonistic to plant parasitic nematodes. *Ind. Rev Sci.*12:23-52.

Tada, M., Hiroe, Y. Kiyohara, S. and Suzuki, S. (1988). Nematicidal and antimicrobial constituents from *Allium grayi Regel and Allium fistulosum L var. caespitosum* . *Agric Biol Chem* 52: 2383-85.

Taylor, A.L. and Sasser, J.N. (1978). Biology, identification and control of root knot nematode (*Meloidogyne* species). A co-operative publication of North Carolina State University and USAID. 111p.

Tuyagi, S.A., Siddiqui, M.A. and Alam, M.M. (1986). Toxicity of an insect repellent plant to plant parasitic nematodes. *Int. Nematol. Network Newsletter* 3; 16-17.

Travedi, P.C. and Barker K.R. (1986). Management of nematodes by cultural methods. *Nematropica* 16:213-36.

Whitehead, A.G. and Hemming J.R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biol., 55: 25-38

Widmer, T.L. and Abawi, G.S. (2000). Mechanism of suppression of *M. hapla* and its damage by green manure of sudan grass. *Plt Dis*. 84:562-68.

Yasmin, L., Rashid, M.H., Uddin Nazim, M., Hossain, M.E. and Ahmed M.U., (2003). Use of neem extract in controlling root knot nematode of sweet- gourd. *Pakistan Journal of Plant Pathology* 2(3):161-168.

Yuhara, I. (1971). Effect of soil application of chopped plant material of *Crotalaria* or Marigold on *Meloidogyne hapla* population. *Ann. Report.Soc.Pl Prot.* 22:62.

Zaiyd, M. (1977). Effect of organic soil amendments on the incidence of root knot nematode on Bhindi plants. *Proc. Bihar Acad. Sci*. 25:23-26.

Zaki, F.A. and Bhatti, D.S. (1989). Phytotherapeutic effect of some leaves on *M.javanica* infecting tomato plants. *Nematol. Medit*. 17:71-72.

APPENDIX

Appendix 1. Summary Anova for effect of root dipping on stem girth of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments	4	0.480	0.120	7.580	0.002
Error	15	0.237	0.015		
Total	19	0.717			

Lsd (0.05) CV (%)	0.1896 7.7

Appendix 2 Summary anova for effect of root dipping on fresh weight of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments Error Total	4 15 19	47.640 24.070 71.710	11.91 1.610	7.420	0.002
Lsd (0.05) CV (%)	1.9 17.4				

Appendix 3 Summary anova for effect of root dipping on root weight of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments Error Total	4 15 19	5.639 3.173 8.810	1.409 0.212	6.660	0.003
Lsd (0.05) CV (%)	0.69 22.60				

Appendix 4 summary anova for effect of drenching on plant height of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments Error Total	4 15 19	70.389 21.549 71.938	17.591 1.437	12.250	0.001
Lsd (0.05) CV (%)	1.81 14.1				

Appendix 5 Summary anova for the effect of drenching on root weight of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments Error Total	4 15 19	27.395 22.035 49.430	6.849 1.469	4.660	0.012
Lsd (0.05) CV (%)	1.83 24.50				

Appendix 6 Summary anova on the effect of drenching on egg mass of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments Error Total	4 15 19	6.258 0.178 0.436	1.564 0.012	13.214	0.001
Lsd (0.05) CV (%)	0.16 16.20				

Appendix 6 Summary anova on the effect of drenching on galls of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments	4	59.300	14.823	68.420	0.001
Error	15	3.2500	0.2167		
Total	19	62.550			
Lsd (0.05)		0.72			
CV (%)		8.70			

Appendix 7 Summary anova on the effect of dipping with drenching on egg mass of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments	4	3.450	0.863	22.790	0.001
Error	15	0.568	0.038		
Total	19	4.019			
Lsd (0.05)		0.29			
CV (%)		15.90			

Appendix 8 Summary anova on the effect of dipping with drenching on galling of root knot nematode-inoculated tomato plants

Source of variation	df	Sum of squares	Mean squares	F-value	F-probabilty
Treatments	4	26.300	6.575	22.790	0.001
Error	15	4.250	0.283		
Total	19	30.550			
Lsd (0.05) CV (%)	0.80 15.90				