T. R.
KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

THE EFFECT OF SOME PLANT EXTRACTS ON ROOT-KNOT NEMATODE *Meloidogyne incognita* POPULATIONS ON PEPPER AND TOMATOES

REBIN ABDALRAHMAN QADIR

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Bioengineering and Sciences

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BAZI BİTKİ EKSTRAKTLARININ DOMATES VE BİBERDEKİ *Meloidogyne incognita* KÖK-UR NEMATODU POPULASYONLARINA ETKİSİ

(YÜKSEK LİSANS TEZİ)

REBIN ABDALRAHMAN QADIR

ÖZET

Kök-ur nematodu, *Meloidogyne incognita*, Dünya'da bir çok üründe büyük zarar yapan önemli zararlılardan bir tanesidir. Domates ve Biber konukçularına bulaştırılan *Meloidogyne incognita*'ya karşı beş ayrı bitki uçucu yağ veya ekstraktlarının (Soğan, QL Agri35, Defne, Okaliptüs, Hardal) Nematisit etkileri araştırılmıştır. Üç nematot seviyesi (0, 1000 ve 2000 J2 /bitki), üç bitki uçucu yağ seviyesi (0, 100 ve 250 µL /bitki) ve dört tekerrürden oluşmuş olup iki ayrı bitkide (domates ve biber) tamamen tesadüfî bloklar deseninde bir sera denemesi kurulmuştur. Çalışmada, nematot seviyeleri ile kullanılan bitki yağ seviyeleri arasında istatistiksel olarak belirgin bir fark bulunmamıştır. Ancak, tüm bitki ekstrakt muameleleri her iki domates ve biber konukçu bitkilerinde mevcut nematot popülâsyonlarının büyümesini sınırlandıkları gözlemlenmiştir. Bitki uçucu yağlar arasında Okaliptüs muamelesinin olduğu domates (34.20±2.9 cm) ve biber (29.55±3.4 cm) konukçu bitkilerinin her ikisinin de en yüksek bitki boylarına sahip olduğu görülmüştür. Ayrıca, muameleler arasında, Okaliptüs'ün topraktaki ikinci dönem nematot larva (J2) sayılarını (Rf) hem domatesde (0.70±0.1) hem de biberde (0.10±0.2) belirgin bir şekilde azalttığı gözlemlenmiştir. Kullanılan beş bitki uçucu yağ muamelesinde, saksı/bitki başına 100 µL uygulama oranının kök-ur nematot kontrolünde en iyi sonucu verebildiği ve bu oranın halı hazırdaki nematot kontrol yöntemlerine alternatif olarak kullanılabileceği düşünülmektedir. Bitki ekstrakt veya uçucu yağlarının nematot kontrolünde bir alternatif olarak kullanılması için daha fazla çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: *Meloidogyne incognita***,** bitki uçucu yağları**,** nematot kontrol, bio-nematisit

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THE EFFECT OF SOME PLANT EXTRACTS ON ROOT-KNOT NEMATODE *Meloidogyne incognita* POPULATIONS ON PEPPER AND TOMATOES

(M. Sc. THESIS)

REBIN ABDALRAHMAN QADIR

ABSTRACT

The root-knot nematode, *Meloidogyne incognita,* is one of the major pathogen causing great losses in many crops worldwide. Nematicidal activity of five plant essential oils (onion, QL Agri35, bay tree, eucalyptus, mustard) against *M. incognita* were investigated in tomato and pepper. Experiment was designed as randomized complete block design with three nematode inoculums (0, 1000 and 2000 J2 per plant) and three essential oil volumes (0, 100 and 250 µL per plant) replicated four times. There were no significant differences between nematode inoculums level and essential oil volumes used. However, all plant extract treatments restrained nematode populations in both tomatoes and pepper host plants. Among the essential oils, eucalyptus sustained the highest plant heights of 34.20±2.9 cm and 29.55±3.4 cm for tomato and pepper, respectively. Among all treatments, eucalyptus reduced the number of second-stage juvenile in the soil (Rf) significantly in both tomatoes (0.70±0.1) and pepper (0.10±0.2). For all five plant essential oils, application of a rate of 100 µL per pot could give the best results in root-knot nematode control and be an alternative to the some current control methods. Further studies are needed in the area of using plant extracts or plant essential oils as an alternative to other nematode control tactics.

Key words: *Meloidogyne incognita***,** plant essential oil**,** nematode control, bio-nematicide

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SYMBOLS AND ABBREVIATIONS

J2 : Second stage juveniles

Mm : Millimeter

EST : Esterase

Mdh : Malate dehydrogenase

AMF : Arbuscular mycorrhizal fungi

mL : Milli litter

mg/kg : Milligram per kilogram

µL : Micro litter

cm^3 : Cubic centimeter

g : Gram

% : Percent

w/w : Weight/Weight

w/v : Weight/Volume

m^2 : Square meter

kg : Kilogram

hr : Hour

ppm : Part per million

RKN : Root-knot nematode

µg : Micro gram

cm : Centimeter

NaOCl : Sodium hypochlorite

µm : Micro mesh

˚C : Celsius temperature

EMI : Egg mass index

GI : Galling index

Rf : Reproduction factor

N : Nematode level

T : Treatment

L : Treatment level

SPSS : Statistical Packages for the Social Science

1. INTRODUCTION

Root-knot nematodes, *Meloidogyne* species, are placed in the class *Scernentea*, order *Thylenchida*, and family *Meloidogynidae*. Root-knot nematodes were first discovered parasitizing greenhouse cucumber in England in 1855 (Mitkowski and Abawl, 2003; Perry et al., 2009). The name *Meloidogyne* was first used by Göldi in 1887 to describe the current *Meloidogyne exigua* species that causes galling in coffee (Perry et al., 2009).

After the investigation of Göldi (1887) on *Meloidogyne* species parasitizing coffee trees in Brazil, root-knot nematodes were assigned *Anguillula marioni* by Cornu (1879) as the name to describe these pathogens (Perry et al., 2009). Several names were later given to this genus until Chitwood (1949) reverted to *Meloidogyne* as the genus name in describing the four widely distributed *Meloidogyne* species; *M. incognita. M. javanica*, *M. arenaria* and *M. hapla*. Since then, many species names (over 92) have been assigned to this genus (De Waele and Elsen, 2007; Adam et al., 2007; Dhandaydham et al., 2008).

Various *Meloidogyne* species are distributed worldwide, some occurring in the tropics, subtropics and others in temperate regions where they cause serious problems both to the quality and quantity of crop yield (Sasser, 1980). *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* are found in cool temperate regions, while *M. arenaria*, *M. incognita* and *M. javanica* are more common in warm temperate, tropical and subtropical regions of the world (Perry et al., 2009). Among the dominant tropical *Meloidogyne* species, *M. incognita* is considered to be the most destructive pathogen that highly damages crops.

Root-knot nematodes can cause great damage to the major crops losses in yield. It is complicated to guess yield suppression caused by plant parasitic nematodes due to wide spacious species (Cetintas et al., 2010). It is reported that root-knot nematodes responsible for 12.3% yield loss of the world's major crops (Sasser, 1998).

Meloidogyne species are polyphagus plant parasites which parasitize up to 5500 different high plant species (Sasser, 1980; Trudgill and Blok, 2001). These plant species include vegetables, ornamental and even weeds. Although most *Meloidogyne* species have a wide host range some *Meloidogyne* species, such as *M. incognita* and *M. arenaria* can be categorized into races based on host specificity (Taylor and Sasser, 1978).

Tomato (*Lycopersicon esculentum*) is one of the most popular and widely used vegetables in the world (Norman, 1992). The crop has developed into a huge number of cultivated types suitable to different environments, method of production and food uses. Its

tact in fresh or processed form has played a main role in its rapid and widespread adoption as an important food commodity (Kasem and Siemonsma, 1999). Ecological and geographical condition in Turkey allows producing good quality tomatoes in lot quantities. Tomatoes production in Turkey estimated to be around 10.7 million metric tons in 2009 (Anonymous, 2009). Tomato production is affected by the root-knot nematode which reduce yield by 30-50% (Sasser and Freckman, 1987; Jonathan et al., 2001; Saravanpriya and Sivakumar, 2005). Root-knot nematodes cause 20-30% yield loss (Aalders et al., 2009; Khan, 2009; Sasser, 1989).

Pepper (*Capsicum annuum var manderes*) is one of the most important vegetables in Turkey. Most common pepper varieties are susceptible to the root-knot nematode *Meloidogyne incognita*. *Meloidogyne incognita* causes damage to the root system and reduces the production.

The accurate identification of root-knot nematodes to species and host races is essential for their control or management. Many *Meloidogyne* species are easily identified based on distinct morphological characters. Several species are difficult to identify due to their similarity to other species and poor taxonomic descriptions. The four most common root-knot nematode species, composing 98% of all worldwide populations are *M. incognita*, *M. javanica*, *M. hapla and M. arenaria* (Hussey, 1985). Since the reevaluation of *Meloidogyne* spp. by Chitwood in 1949, female perineal patterns became the dominant diagnostic character of the four most common *Meloidogyne* species. The perineal pattern presents several benefits that render it a valuable diagnostic tool. Aside from minor variations, perineal patterns are constant within populations and their source (females) is abundant in infected host roots. Other diagnostic features used in taxonomic identification include female stylets, male heads and stylets, and second-stage juvenile (J2) heads and stylets.

Isozymes electrophoresis, Polyacrylamide gel electrophoresis (PAGE) has been widely used in studies of taxonomy, systematic and population genetics and it has proved to be a very useful technique in the identification of species of *Meloidogyne* (Esbenshade and Triantaphyllou, 1985). First assay to demonstrate some species-specific proteins were by Dickson et al., (1971) that could be used in separation of important *Meloidogyne* species and some other plant-parasitic nematodes.

All methods for control of plant parasitic nematodes can be categorized under one or more principles. All of the various tactics for control of nematodes suitable within one of these principles (Perry et al., 2009). Management of nematodes involves the manipulation of nematode densities to non-harmful or sub-economic threshold levels using several measures in relation to the whole production system, whereas control means the use of a single measure to reduce or eliminate nematode pests, which in most cases is not possible (Thomason and Caswell, 1987). Maintenance of diversity is a goal of management but not of control, and of increasing importance is the further need to take into view the impact of the pest management tactics on biodiversity and the ecological balance in the soil.

The modification of existing agricultural practices in order to manage nematode populations is one of the most acceptable alternatives to chemical control for both the small and large scale farmers in the tropics (Starr et al., 2001). Crop rotation decreases the potential for substantial yield losses due to nematode (Luc et al., 1990; Whitehead and Hemming, 1965) and provides at least short-term suppression of nematode population densities. The magnitude of these benefits is generally positively correlated with the number of cropping seasons between the planting of susceptible crops. However, most of the rotation schemes in operation have been designed to prevent disease outbreaks or increase available nutrients, and are not always compatible with nematode control (Luc et al., 2005).

Biological control of nematodes is defined as a reduction of nematodes by the action of living organisms, which occurs naturally, through the manipulation of the environment, or by the introduction of antagonisms (Stirling, 1991). Biological control is one of the promising non-chemical methods to control root-knot nematodes.

Exploitation of resistance in crops is one of the most effective and eco-friendly components of integrated pest management and inclusion of this property ensures increased crop yield in the presence of nematode (Khan and Mukhopadhyay, 2004). Screening for resistance remains a major goal as new diseases achieve significance or new races of existing pathogens become established. Nematode resistance in host plant is manifested by reduced rates of nematode reproduction and, consequently, lower nematode population densities in the crop rhizosphere than that of a susceptible one (Medina-Filho and Tanksley, 1983).

Although not used by resource-poor farmers as such, methyl bromide phasing out in developed countries by 2005 and in developing countries by 2015 (Haydock et al., 2006) has further massive the search for alternatives that can be used by these farmers, such as phytochemicals with bio-nematicidal properties (Chitwood, 2002; Ferraz and de Freitas, 2004). A number of alternative fumigants, such as 1.3-dichloropropene, iodemethane and propargyl bromide, have been recommended as alternatives but are inappropriate for subsistence farmers due to their toxicity, environmental problems and human animal health concern, high cost (Haydock et al., 2006) and unsuitable package sizes. Since the application of phytochemicals has been used with success to reduce root-knot nematodes across a range of crops (Chitwood, 2002; Ferraz and de Freitas, 2004), there is possible for their use in resource poor agriculture. Availability and cost-activity of bionematicides will, however, determine their applicability.

Additionally, bionematicides have advantages over synthetic products, in that they:

- Contain incoming compounds that plant-parasitic nematodes are not yet able to inactivate.
- Are less concentrated and therefore less toxic than synthetic compounds.
- Biodegrade comparatively rapidly.
- Are derived from renewable sources (Chitwood, 2002; Ferraz and de Freitas, 2004).

Application of ore phytochemicals by means of cover, green manure or rotation crops, as opposed to synthesized formulations of these products, will most probably be the most viable option for resource-poor farmers to apply against root-knot nematodes. The formulation of synthesized/purified phytochemicals as pre-applied seed/tuber coatings may, however, constitute a significant contribution in assisting resource-poor farmers in the continuous battle against *M. incognita.*

Chemical compounds with nematicidal properties have been identified from a range of plants (Chitwood, 1992, 1993, 2002; Ferraz and de Freitas, 2004). Various bio-nematicides of a plant nature continue to be screened and evaluated, but are also beginning to work their way on to the market (Haydock et al., 2006). Some phytochemicals have hostile, suppressive or repellent effects on plant-parasitic nematodes, while others are toxic (Viaene et al., 2006).

In this study, five essential oils extracts from five different plants such as: Onion (*Allium cepa*), QL Agri 35 (*Quillaja saponaria*), Bay tree (*Laurus nobilis*), Eucalyptus (Eucalyptus sp), and Mustard (*Brassica* sp.) were tested. Five essential oil application rates was: control (0 µL\plant), low (100 µL\ plant) and high (250 µL\plant).

2. LITERATURE REVIEW

2.1. History

The nematode species that parasitic on plants are of considerable significance in the field of agriculture. They exhibit three different kinds of parasitic behaviors: ecoparasitism, semi-endoparasitism and endoparasitism that lead to enormous crop losses. Among endoparasites, the species of *Meloidogyne, Heterodera* and *Globodera* are the major pests of agricultural importance. Four species of *Meloidogyne*, *M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria* described by Chitwood (1949) are considered as most destructive and widespread because of their cosmopolitan occurrence and extensive host range (Sasser, 1989).

Plant parasitic nematodes are notable antagonists in the yield of crops in agriculture system. It has been estimated that 10% of world crop production is lost as a result of damage caused by plant parasitic nematodes. This represents one third of the total loss attributed to pests and diseases (Whitehead, 1998). Among the plant parasitic nematodes, the root-knot nematode (*Meloidogyne incognita)* is an obligatory endoparasite that causes considerable damage to economically important crops worldwide (Bhatti and Jain, 1977; Eisenback and Triantaphyllou, 1991; Khan and Akram, 2000).

Root-knot nematode causes different morphological and anatomical responses in different plants and even in various parts of a particular plant and different species can cause different responses in the same plant (Krusberg, 1963).

2.1.1. Biology and life cycle of root-knot nematode

Most species of plant parasitic nematodes have a comparatively simple life cycle consisting of the egg, 4 juvenile stages and the adult (male and female). The root-knot nematodes complete their life cycle within their host roots (Mai and Abawi, 1987).

A first-stage juvenile develops and molts while still in the egg to become a second-stage juvenile which hatches from the egg. After hatching, root-knot nematodes move through the soil to find place on plant roots to feed.

The nematodes survive from the stress environment in soil as eggs and also J2. Females of root knot nematodes produce more than a 1000 eggs in a gelatinous matrix (egg mass) which can be observed linked to the prominent posterior end of the adult females on the root surface (Mai and Abawi, 1987). This mass protects the eggs from dryness

(Pattison, 2007). The infective second stage juvenile hatch from the eggs and move through the soil in search of roots of suitable host plants (Davis et al., 2004). The juveniles usually penetrate roots just behind the root tip region and launch their special constant feeding locations (giant cells or gall) in the vascular tissues of the root (Mai and Abawi, 1987). The giant cells supply nutrients for the sedentary nematodes which keep on feed and molting three more times. Root cells around the feeding locations are also induced to expand and form galls and often extensive secondary root formation and branching of the main root. Depending to the host and soil temperature, the whole life cycle completed in 17 to 57 days (Hussey, 1989) (Figure 2.1).

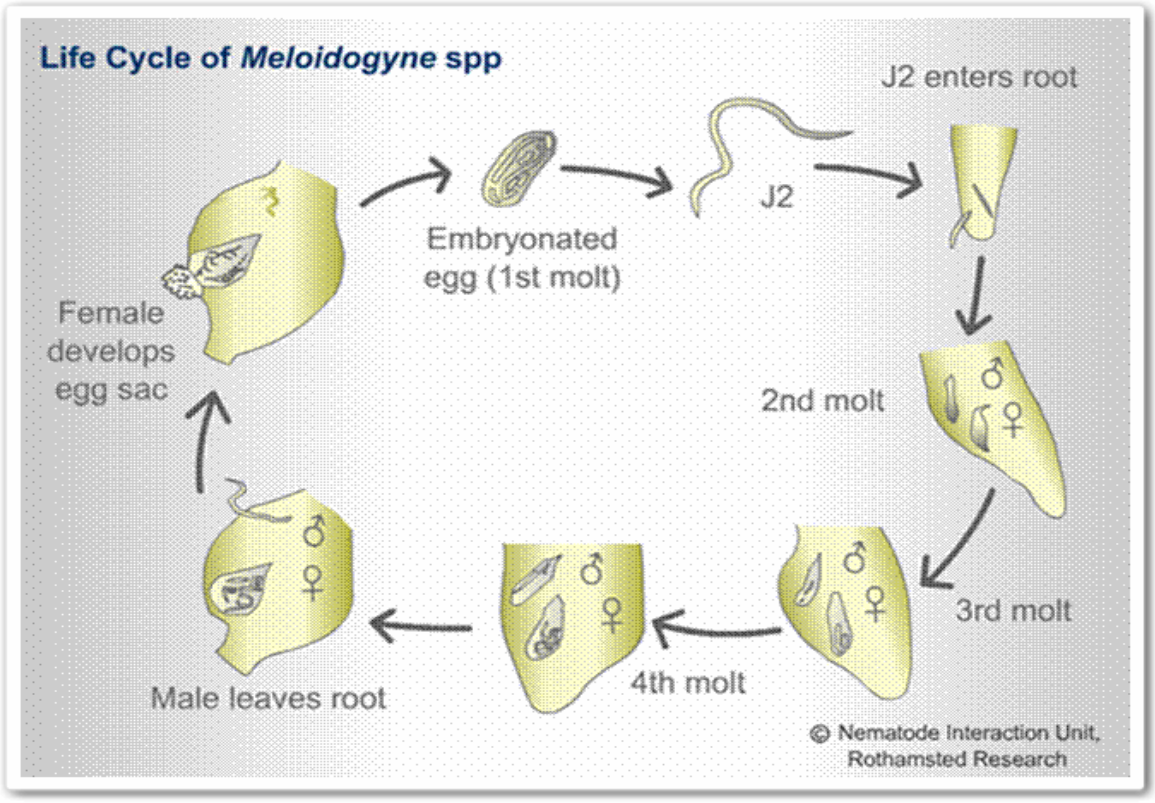

Figure 2.1. Root-knot nematodes life cycle (Rothamsted Research Center, UK).

2.1.2. Movement of *Meloidogyne* species

The majority of *Meloidogyne* species which are distributed across various potato fields have been introduced into the fields as a result of movement of infected potato planting materials both locally and internationally (Wesemael et al., 2011). Infections can also spread across farms when certain stages of *Meloidogyne* species such as eggs and the J2s are moved from one place to the other through adhering to the surfaces of farm

implements or soles of animals and human beings or through running water. Wind has also been found to be a cause in the transmission of the egg stages of *Meloidogyne* species (Jones, 2006). The transferred stages finally develop into subsequent stages therefore facilitating colonization of new niches.

2.1.3. Reproduction of *Meloidogyne* species

Meloidogyne genus is associated with three forms of reproduction; mitotic parthenogenesis (apomixis), meiotic parthenogenesis (automixis) and cross fertilization (amphimixis) (Eisenback et al., 1981). Mitotic parthenogenesis is the most common form of reproduction and it is usually exhibited by species such as *M. arenaria*, *M. javanica* and *M. incognita***.** Meiotic parthenogenesis is associated with *M. graminis*, *M. chitwoodi* and *M. fallax* while cross fertilization can be found in species such as *M. megatyla*, *M. microtyla* and *M. carolinensis*. In mitotic parthenogenesis, eggs produced by the females do not undergo meiosis. They therefore end up with the equivalent number of chromosomes such as those present in high somatic cells after attaining maturity. Males may mate with females in a high population but due to chemicals present in the egg cytoplasm the sperm nucleus disintegrates before fusion with the egg nucleus. *Meloidogyne hapla* has a facultative parthenogenesis. In cross fertilization, males mate freely with females in a high population to give rise to a zygote that undergoes further development to form larvae. The female reproductive system is well developed with two ovaries that are each associated with a germinal zone, oviduct, spermatheca and uterus (Eisenback et al., 1981).

2.2. Identification

Accurate identification of *Meloidogyne* species like any other nematode species has been difficult due to several factors. These include; limited number of nematology taxonomists, inadequate funding to carry out research and also training of young scientists, wide host ranges, sexual dimorphisms, polyploidy and overlapping morphological characters (Oliveira et al., 2011). Nevertheless, different approaches have been devised for unproved accurate identification of various nematode species (Blok and Powers, 2009). Identification methods for root-knot nematodes are based on either morphological, biochemical and/or molecular approaches.

2.2.1. Morphological identification

Morphological identification of *Meloidogyne* species is based on direct observations of various stages of *Meloidogyne* species under a stereomicroscope or electron microscope. Distinct morphological characters that are used to distinguish among different *Meloidogyne* species include, the morphology of the adult females, second stage juveniles and males, the stylet shape (usually stomatostylet), body length, perineal patterns, head and tail, excretory pore, dorsal esophageal gland opening, phasmids and the spicule (Eisenback et al., 1981).

Adult females are approximately 0.44 mm to 1.30 mm in length and 0.32 mm to 0.70 mm in width. These females can be easily identified by their distinct pear shape. Furthermore, the body of an adult female in most *Meloidogyne* species is symmetrical with a 'neck' region slightly twisted to the side and its white body is transparent where the stylet, esophageal bulb and excretory pore are usually visible. Perineal patterns are distinct features comprising of the dorsal arch, lateral lines, striae and punctuations which are present on the anal side of the adult female (Eisenback et al., 1981). Most of the *Meloidogyne* species have characteristic perineal patterns on the posterior of the adult female which are used during morphological identification. Adult males are vermiform with slender bodies tapering interiorly and rounded posteriorly. They have developed stylets, conspicuous annules on their cuticle and spicules protruding through the cloaca which combine both functions of the anus and sex opening (Eisenback et al., 1981). Unlike juvenile stages and females with well-developed esophageal glands for feeding, males lack a well-developed feeding system and therefore they do not feed (Eisenback et al., 1981).

2.2.2. Isozyme characteristics

Isozymes are variants of a particular enzyme which differ from one another in terms of their biochemical properties such as their amino acid sequence and substrate requirements. They can be distinguished from each other using biochemical assays. The change in amino acid sequence in isozymes contributes to a significant change in the electric charge thus making it easy to identify them by use of gel electrophoresis. Some of these isozymes include: esterases, α-glycerophosphate dehydrogenase, malate dehydrogenase (Mdh) and glutamate dehydrogenase (Eisenback et al., 1981).

Isozyme characteristics have been used to identify various *Meloidogyne* species (Esbenshade and Triantaphyllou, 1990). The adult female stage is usually the preferred one

since it is associated with the expression of a given gene product (Esbenshade and Triantaphyllou, 1990). However, the adult stage is not readily isolated from the soil as it generally resides in the host. The infective second stage juveniles are usually in large numbers therefore overshadowing the adult female stage. In 1985, Esbenshade and Triantaphyllou used isozyme phenotypes to distinguish *Meloidogyne* species. They reported esterase patterns from 16 *Meloidogyne* species, with the most common phenotypes being A2 and A3 for *M. arenaria*, HI for *M. hapla*, II for *M. incognita* and J3 for *M. javanica.* In 1990, Esbenshade and Triantaphyllou again used isozymes in their survey involving about 300 populations of *Meloidogyne* species originating from 65 countries and different continents. This was a comprehensive survey to have ever been carried out to identify *Meloidogyne* species using isozymes. Later, 18 esterase phenotypes from 111 populations of *Meloidogyne* species were found in Brazil and in other South American countries while in 2004, China recorded, five esterase phenotypes (Xu et al., 2004).

Isozymes continue to be widely used for studies of *Meloidogyne* species despite some of their limitations (Molinari et al., 2005; Wesemael et al., 2011). Enzyme phenotypes are designated, indicating the *Meloidogyne* species that they specify and the number of bands detected. Phenotypes with the same number of bands are differentiated by small letters (Esbenshade and Triantaphyllou, 1990; Muturi et al., 2003). Enzyme patterns are usually compared with a known standard, frequently isozymes from *M. javanica.* Isozymes are used primarily with the female egg-laying stage using single individuals (Esbenshade and Triantaphyllou, 1990). Use of single isozyme phenotypes has been unsuccessful in resolving species identities due to inconsistent size variations between species. This has led to the use of more than one enzyme to resolve this problem. The enzyme malate dehydrogenase (Mdh) has been found to separate *M. hapla* from *M. incognita*, *M. arenaria* and *M. javanica*, whereas glutamate dehydrogenase can separate *M. incognita* from *M. javanica. M. arenaria* and *M. hapla* (Esbenshade and Triantaphyllou, 1990; Muturi et al., 2003). In surveys targeting *Meloidogyne* species, isozymes can be used as a convenient preliminary stage in species identification. Remarkably many useful esterase patterns are still being discovered, but to determine their specificity and sensitivity, other additional identification methods such as morphological and molecular should be employed.

2.3. Management and control

Management has the objective of minimizing economic losses, and includes the whole system of care and treatment of crop pests, while control refers to specific acts designed to reduce the numbers of nematodes (Hooper and Evans, 1993).

2.3.1. Cultural management

In crop rotations, susceptible crops are rotated with immune or resistant crops. Possible crop rotations for the control of root-knot nematodes are limited due to the wide host range of some species. Grasses have been effective in reducing populations of *M. hapla*, *M. arenaria*, *M. incognita* and *M. javanica* (Netscher and Taylor, 1979). Barley can be used in rotations to reduce *M. hapla* infections (Belair, 1996). Nijs et al., (2004) gave an overview on the host status of various crops for *M. chitwoodi* and *M. fallax* resulting in very few options for crop rotations. Marigolds have proven to be successful against *Meloidogyne* spp, both in greenhouse and field conditions (Ploeg, 1999; Ljani et al., 2000). Their effect against *Pratylenchus* spp. makes them an important option if both nematode genera are present (Pudasaini et al., 2006).

The population of root-knot nematodes decreases markedly during winter and under fallow (Pinkerton et al., 1991; Nolingand Becker, 1994). However, European policy no longer supports fallow periods.

Many weeds are host for *Meloidogyne* (Thomas et al., 2005; Kutywayo and Been, 2006) therefore, adequate weed control is required in crop rotations and fallow periods.

A major limitation to control nematodes by disrupting the continuity of food resources is that this strategy does not fit some intensive agricultural practices and farmers prefer to grow crops that are economically more rewarding (Van der Putten et al., 2006). Crop rotations are often historically inherited and new crops require major investments in machinery and cultural practices. Also the absence of a market for the new crops can limit the introduction of new crop rotations.

Manipulating planting or harvest dates can reduce damage caused by nematodes (Hooper and Evans, 1993), but is not generally practiced as planting and harvest depend strongly on climatologically conditions and the market demands.

2.3.2. Physical control

Heat treatments of planting material (e.g. bulbs) can be an important tool to avoid spreading of nematodes. Steaming of soil is expensive and usually only applied in glasshouses for high value crops and for compost. It is not always effective due to the spreading of nematodes in deeper soil layers (Karssen and Moens, 2006) and, therefore, is generally only effective in shallow soils. Soil solarization requires longer periods of bright sunshine and is only adaptable to regions where sufficient solar energy is available for long periods of time.

2.3.3. Biological control

Nematophagous fungi and bacteria have been the subject of many studies on nematode control (Kerry, 1987). Kiewnick and Sikora (2006) demonstrated that a single pre-plant application of the fungus *Paecilomyces lilacinus* strain 251 could control *M. incognita* on tomato. This fungus is commercialized in Germany, applied as dispersible granules for application in water. Another fungus, *Pochonia chlamydosporia*, provided control of root-knot nematodes on vegetable crops in tropical soils, but results in Europe have been less satisfactory (Tzortzakakis and Petsas, 2003; Viaene et al., 2006). However, one-time application of *P. chlamydosporia* was able to slow down the build-up of *M. javanica* for at least 5-7 months in tomato and lettuce rotations in a glasshouse (Van Damme et al., 2005). *Arbuscular mycorrhizal* fungi (AMF) are endophytic fungi that grow within plant tissues without causing disease and can play a protective role against parasitic nematodes. Establishment of AMF in olive plants significantly reduced severity of root galling as well as reproduction of *M. incognita* and *M. javanica* (Castillo et al., 2006).

*Pasteuria penetrans*is a bacterial parasite of root-knot nematodes and can reduce their numbers significantly in some cropping systems (Trudgill et al., 2000). The effectiveness of *P. penetrans* strongly depends on the endospore concentrations and is manifest at the level of root penetration by J2 and the loss of nematode fecundity (Kariuki et al., 2006). However, the high multiplication of root-knot nematodes on many vegetables does not allow the *P. penetrans* population to keep up numerically with host (nematode) abundance (van der Putten et al., 2006).

Biological control agents will generally provide too little control to be effective alone and their successful use in sustainable management strategies will depend on their integration with other control measures (Viaene et al., 2006).

2.3.4. Resistance

Plant resistance is probably the most environmentally safe method to control root-knot nematodes. Resistance against *Meloidogyne* spp. has been reported in many food crops (Cook and Starr, 2006) but it is not often used. The most important example is the resistance against *M. arenaria*, *M. incognita* and *M. javanica* in Mi-gene bearing tomato cultivars which are widely used. However, resistant breaking populations of *M. incognita* and *M. javanica* have been reported in Greece and Spain (Ornat et al., 2001; Tzortzakakis et al., 2007) and this might reduce current use. Resistance against *M. arenaria*, *M. incognita* and *M. javanica* was reported in prunes rootstocks in France and Spain (Fernandez et al., 1994; Pinochet et al., 1996). Resistance against *M. javanica* was also found in peach and plum rootstocks from Spain, France and Italy (Pinochet et al., 1999). Several *Mi* resistance genes against *M. arenaria*, *M. incognita* and *M. javanica* were found in pepper (Djian-Caporalino et al., 2007). Resistance for *M. hapla* (Chen and Roberts, 2003) and *M. naasi* (Cook et al., 1999) was reported in common bean and ryegrasses, respectively. Promising results have been obtained from several wild tuber-bearing *Solanum* species for resistance against *M. chitwoodi*, *M. hapla* and *M. fallax* (Janssen et al., 1996; Brown et al., 2006).

2.3.5. Chemical control

The increasing concern about pesticide residues in the food chain, risks to human health and the adverse impact on the environment has reduced the use of nematicides and resulted in the ban of methyl bromide. Nevertheless, approximately 48,000 ton active substances are used annually in Western Europe (Haydock et al., 2006). Nematicides are reliable and fast working and can give good economic returns on high-value crops. They may be essential for producing nematode-free export crops. However, in general nematicides do not eliminate the populations of plant-parasitic nematodes and therefore final nematode densities may be too high for a profitable crop to be grown in the following season without further phytosanitary measures being taken place (Hague and Gowen, 1987).

2.3.5.1. Bionematicide

Alternative nematode control methods are required because of the probable removal of nematicides from the market because of the increasing anxiety of possible effects on human health and the environment. Biological control, organic and inorganic

soil amendments, naturally occurring control agents, induced resistance, interruption of host recognition and transgenic plants will be a part of integrated management of plant-parasitic nematodes in the near future (Yuji Oka et al., 2000).

There are plenty numbers of studies about essential oils extracted from plants used as a control tactics. One of these studies has been conducted by Yuji Oka et al., in 2000. Essential oils extracted from 27 spices and odorous plants were used in lab and in a pot trial. Twelve of the twenty-seven essential oils reduced %80 of juveniles of the root-knot nematode, (*Meloidogyne javanica*) at a concentration of 1,000 mL/liter. At this concentration most of these oils also prevented nematode hatching. Essential oils of *Foeniculum vulgare*, *Carum carvi*, *Mentha rotundifolia* and *Mentha spicata* showed the highest nematicidal activity among the in lab tested oils. These oils and those from *Origanum syriacum*, *Origanum vulgare* and *Coridothymus capitatus* mixed in sandy soil at concentrations of 100 and 200 mg/kg reduced the root galling of cucumber seedlings in the pot experiment. The main components of these essential oils were tested for their nematicidal activity. *Carvacrol*, *thymol*, *t-anethole* and (+)-*carvone* frost the juveniles and prevented hatching at >125 μL/liter in lab. Most of these components mixed in sandy soil at concentrations of 75 and 150 mg/kg reduced root galling of cucumber seedlings. In three liters pot trial, nematicidal activity of the essential oils and their components was confirmed at 200 and 150 mg/kg, respectively. The results suggest that the essential oils and their main components may serve as nematicides (Yuji Oka et al., 2000).

In the vitro and in growth chamber results suggest that the essential oil of *Chrysanthemum coronarium* and organic amendments from *Asteraceae* species may serve as nematicides (Perez et al., 2003). The essential oil of *C. coronarium* flower (head of flower) showed strong nematicidal activity in vitro and in growth chamber experiments. Essential oil concentrations of 2, 4, 8 and 16 μL mL−1, significantly reduced hatching of eggs and J2 survival and reproduction rate of *Meloidogyne artiellia* in vitro, with the lowest values occurring at 16 μL mL−1. In pot experiment with chickpea cv. PV 61, essential oil concentrations of 10–40 μL per 500 cm^3 soil, applied on sterile cotton pellets, also significantly reduced the nematode's reproduction rate. The biological processes of mortality and hatching/reproduction were adequately described by the monomolecular and expanded negative exponential models, respectively. Effectiveness of soil amendment with either flowers, leaves, roots or seeds of *C. coronarium*, or flowers from several species of *Asteraceae* (*Chrysanthemum segetum*, *Calendula maritima*, *Calendula officinalis* and

Calendula suffruticosa) at 5 g per 500 cm^3 soil was tested for suppression of *M. artiellia* and growth of chickpea cv. PV 61 under growth-chamber conditions. In these tests, flowers of all five *Asteraceae* species and various parts of *C. coronarium* significantly reduced reproduction rates of *M. artiellia*, by 83.0–95.9%, with the minimum rates occurring in infected chickpea plants amended with flowers of *C. officinalis* and *C. suffruticosa*.

In a study by Adegbite and Adesiyan, (2005) eggs were uncovered to concentrations of root extracts of Siam weed [*Chromolaena odorata* (L.) King and Robinson], Neem (*Azadirachta indica*), Castor bean (*Ricinus communis* L.) and Lemon grass (*Cymbopogon citratus*). This study showed that one hundred percent concentration of root extracts of Siam weed and Neem exhibited 100% inhibition of egg hatch and juveniles mortality. On the other hand, 100% concentration of root extracts of Castor bean and Lemon grass exhibited 93 and 95% inhibition of egg hatch and 62.1 and 75% juvenile mortality respectively. Egg inhibition and juvenile mortality decreased with an increase in the dilution of all the extracts. Similarly with an increase in exposure time, juvenile mortality was also increased (Adegbite and Adesiyan, 2005).

Plant extracts of *Inula viscosa*, a widely distributed perennial plant, were tested for their effectiveness in control of *M. javanica* in laboratory, growth chamber, micro plot, and field experiments. Emulsifiable concentrate formulations of the pastes killed *M. javanica* juveniles in sand at a concentration of 0.01% (paste, w/w) or greater ratio reduced the galling index of cucumber seedlings as well as the galling index and numbers of nematode eggs on tomato plants in growth chamber experiments. In micro plot experiments, the hexane-extract formulation at 26 g paste/m^2 reduced nematode infection on tomato plants. In a field experiment, a reduction of 40% in root galling index by one of two formulations was observed on lettuce plants. It has been elaborated that these plant extracts have a potential to be used as a natural nematicide, although the formulations need improvement (Yuji Oka et al., 2006).

Cold aqueous extracts (20% w/v, 100 ml aliquots) of pre-and post-flowering whole plants, root and stem parts of *Tagetes erecta* were tested for their ability to control *M. incognita* in infested soil (10 kg) in pots planted with susceptible *Lycopersicon esculentum*. Plant height and leaf number were significantly greater in *T. erecta* treated *L. esculentum* than plants grown in untreated infested soils. Whole *T. erecta* plant extracts were more active than stem extracts although both were more effective than root extracts, and extracts from 40-day old plants were more efficacious than those from 70-day old

plants. Root galling indices of *L. esculentum* treated with *T. erecta* plant extracts were significantly lower than untreated checks and comparable with carbofuran-treated plants (Natarajan et al., 2006).

Twenty-seven samples of various plant components (leaves, fruits, and stems) were taken from 21 trees and herbal species in 19 genera from Gezira locality, Sudan. Methanol or hexane extracts of the 27 samples were sorted for nematicidal activity against second-stage juveniles of *M. incognita* in the laboratory. Five plant extracts showed highly promising mortality rates of 95–99% after 72 hr of exposure, which were statistically different from the other extracts. These extracts were from *Dinbera retroflexa* (leaves), *Cucumismelo* var. agrestis (fruits), *Eucalyptus microtheca* (leaves), *Acacia nilotica* (pods), and *Chenopodium album* (leaves). Six extracts derived from the leaves of *Solenostemma argel*, *Aristolochia bracteolate* and *Ziziphus spina*-christi and the seeds of *Aregimone mexicana*, *Datura stramonium* and *Azadirachta indica* produced relatively high mortality rates of 80-94% after 72 hr of exposure. The five most nematicidal plant extracts listed above were extra screened against similar stage juveniles of the nematode species using only 50 ppm for 24, 48, and 72 hr. Three plant extracts, C. *melo*var*agrestis* (fruits), *A. nilotica* (fruits), and *C. album* (leaves), showed 41, 42 and 45% mortality rates, respectively (Gamal Abdalla Elbadri et al., 2008).

The extracts of fresh peels of lemon, orange, and grapefruit exhibited significant nematostatic effect against J2 of *M. incognita* after 48 h treatment. The nemticidal activity was extremely low in all the extracts of fresh peels but was greatly enhanced in the extracts of stored purified peels with 90.8 %, 93.5 %, and 85.0 % mortality of nematodes for lemon, orange, and grapefruit, respectively. The data indicated the possibility of essential oils from the citrus peels might have released in the extracts during storage of the purified peels. The egg hatch inhibition of the extracts from stored purified peels was 85.7 %, 91.0 %, and 78.3 % for lemon, orange and grapefruit, respectively. The reversibility tests detected that the effect of extracts on the hatch of eggs was not permanent. The hatching was partially renewed after the removal of the extracts but was still significantly lower than the control. The infection of *M. incognita* J2 on mung bean roots was significantly inhibited by the extracts of the refrigerator-stored purified peels of lemon, orange and grapefruit. The findings supply an alternative to chemical nematicides for organic farming and help the disposal of citrus juice processing waste as well as the fallen fruits in the orchards in the typhoon season (Bie Yun Tsai, 2008).

The impact of plant extracts of *Eucalyptus* (*E. chamadulonsis*), garlic (*Allium sativium*), marigold (*tagetes erecta*) and neem (*Azadirachta indica*) and essential oils were tested on the reduce population of root-knot nematode *M. incognita* under greenhouse and field conditions neem extract and essential oils treatments were more effective in reducing population of the *M. incognita* in soil and root gall index compared to other treatments. In field experiments, the maximum protection of tomato plant against root-knot nematode was gained by application of neem and essential oil treatments, 44.2 and 32.6%, respectively (Elyousr et al., 2009).

Nine herbal powders were tested against root-knot nematode, *M. incognita* under greenhouse conditions. The herbal powders were collected from Gezira State, Sudan. Herbal powders were used without extraction to extend the application by farmers. Most of the herbal powders were effective in controlling *M. incognita* in the soil compared to the control. Some treatments (e.g., *Acacia nilotica* (L.), *Argemone mexicana* L. and *Azadirachta indica*) had statistically lower Root Galling Index than the control. The number of juveniles per 100 g soil was lower in soil amended with *Dinbera retroflexa*, *Azadirachta indica*, *Salvadora persica* (L.) and *Acacia nilotica* than in unamended soil. The results of both root galling index and number of juveniles were not significantly different from the synthetic nematicide used. Herbal powders from *A. indica* and *Acacia nilotica* may be promising in controlling of this pest because they are easily available to farmers in tropical regions (Elbadri et al., 2009).

Another nematicidal activity of plant extracts study was done in laboratory by Djiwanti et al., (2009). In this study, plant extracts from tobacco (*Nicotianatabacum* L), clove (*Syzygiumaromaticum* L), betelvine (*Piper betle* L) and sweet flag (*Acoruscalamus* L) were effective in reducing the number of the nematodes. Experiments revealed that the total number of live nematodes on roots of pepper plants treated with sawdust of the clove bud was 7% of that of the controls and did not differ significantly from that of plants treated with the recommended synthetic pesticide carbofuran. The application of clove buds as a botanical pesticide for future use against nematodes is highly promising since clove is the sixth major plant grown on Bangka Island, and the market value of clove has decreased sharply over the past years (Djiwanti et al., 2009).

Plant extracts of six different medicinal plants *Adhatoda vesica*, *Plumeria rubra*, *Mussenda glabra*, *Mellia azedarach*, *Xylosoma longifolia* and *Andrographis panniculata* were tested against egg and J2 of *M. incognita* in terms of percentage of mortality and rate

of inhibitory action in egg hatching. Through these six extracts leaves extracts of *A. panniulata* was found to be most effective in both larval mortality and egg hatching followed by *M. azedarach* oil extracts. Although their effect on egg and juveniles of *M. incognita* differ, these six extracts were found to be effective and can be used for the control of root knot nematode, *M. incognita* (Joymati, 2009).

Nematicidal activity extracted from five different plant essential oils (rosemary, thyme, mint, garlic, and sesame) against root-knot nematodes, *M. incognita* race 2. The experiment was with three nematode inoculums densities (0, 1000 and 2000 J2 \plant)and three essential oil volumes (0, 50 and 150 µL\plant) replicated 6 times. There were no significant differences between nematode inoculums density and essential oil volumes used. But all oil treatments suppressed nematode population and resulted an increase in root mass tissue. Compared with control, among the essential oils, thyme (2.82±0.47%) and garlic (5.53±1.68%) treatment reduce root galling significantly and produced the lowest percent of galls on the plants. However, rosemary, mint and sesame treatments were less effective in reducing root galling. Compared with control, thyme (2.46±0.17) and garlic (2.50±0.22) yielded also the lowest egg masses. Among five plants essential oils, application of a rate of 50 µL\plant of thyme or garlic in tomato production areas could give the best results in root nematode control and suggested that it could be an alternative to the current nematode control methods (Cetintas and Yarba, 2010).

Essential oils of *Ocimum gratissimum*, *Azadirachta indica*, *Vernonia amygdalina* and *Moringa oleifera* were evaluated for their nematicidal activity on pathogenicity of *M. incognita* race 2 and on the growth and yield of cowpea. Eggs and juveniles of *M. incognita* were exposed to the extracts from leaves of these indigenous plants for ten days in a completely randomized design with four replicates. Data on egg hatch inhibition and juvenile mortality were recorded daily. Three cowpea cultivars were inoculated with *M. incognita* and later soaked with the botanical extracts at rate of 10,000 mg/kg and 20,000 mg/kg per pots. Egg hatch inhibition ranged from 40% - 63.7% in the extracts compared to the control with 0%. Juvenile mortality in extracts was from 82% - 93.8% compared to the control of 25%. Grain yield of plants treated with *V. amygdalina* at 10,000 mg/kg and 20,000 mg/kg; and 20,000 mg/kg of *A. indica*, *O. gratissimum and M. oleifera* were significantly higher than in the untreated plants. These plants also had nematode reproductive factors comparable to the uninoculated control. This study therefore shows that low to moderate concentrations of these indigenous botanicals extracts are effective in

reducing the pathogenicity of the root-knot nematode and is accompanied by a yield increase in cowpea (Claudius-Cole et al., 2010).

A greenhouse experiment was conducted to control RKN (*M. javanica*) on tomato with aqueous extracts of marigold (*Tagetes erecta*) leaves and flowers, castor beans (*Ricinus communis*) and garlic (*Allium sativum*). The plant material used in this study was dried and crushed and diluted with water at a rate of 25g/100mL. Four-week old seedlings were transplanted in twenty micro plots arranged in a randomized complete block design with five treatments and four replicates, inoculated each plant by 5000 J2 of nematodes. The botanicals were soaked around each plant. Nemacur and non-amended plots served as control. Results of the study showed that tomato is susceptible to RKN infestation and the application of botanicals significantly ($P < 0.001$) controls RKN by reducing galling and reproduction (Tibugari et al., 2012).

The objective of this research is to determine the effects of different levels of plant extracts (0,100, 250 µm per pot) on various nematode inoculation (0, 1000, 2000 J2 or/and eggs per plant/pot) on pepper and tomatoes. During and at the end of the study the necessary data will be taken to evaluate the damage caused by nematodes.

3. MATERIAL AND METHODS

3.1. Sampling and Source of Root-Knot Nematodes Inoculums

Source of nematodes have been taken from infested tomato roots from vegetable farms of Kahramanmaraş, Turkey. Infected roots have been washed softly by tap water and placed in an Erlenmeyer flask. Four week old tomato and pepper seedlings were inoculated and the pots were placed in the growth chamber for 60 days.

3.2. Identification

3.2.1. Morphological characterization

3.2.1.1. Perineal pattern

Root tissues were dissected with a pair of sharp needles and half spear to remove adult females under light microscope (Olympus, model SZX16). The whole females were placed in 45% lactic acid in a Petri dish for 1 hr. Then, the females were removed from the lactic acid and placed in a drop of glycerol. The procedure for perineal patterns of RKN's are followed as outlined by Hartman and Sasser (1985). Slide was placed under a stereo microscope (Olympus, model BX51) and while viewing, an incision was made using a scalpel in the middle of the female to cut the cuticle into half equatorially. The posterior region consisting of the perineal pattern was carefully cut off and trimmed. The perineal pattern section was brushed gently using a fine pointed quill pick to remove any attached debris. The perineal pattern was manipulated using a quill pick and placed on another clean slide with a drop of glycerin. Three to four perineal patterns from a single population were positioned on the slide with the outer side uppermost and a glass cover slide was applied (Figure 3.1, 3.2, 3.3).

Figure 3.1. Extraction of root-knot nematode females from the infected roots.

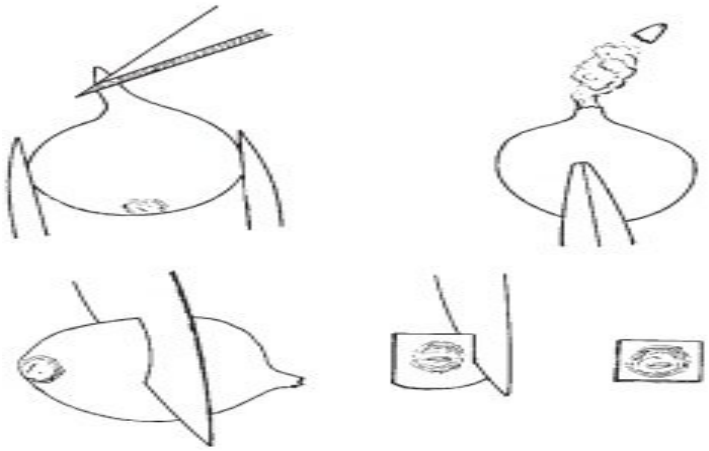

Figure 3.2. Preparation of the perineal patterns of extracted females for viewing on microscope (Hartman and Sasser, 1985).

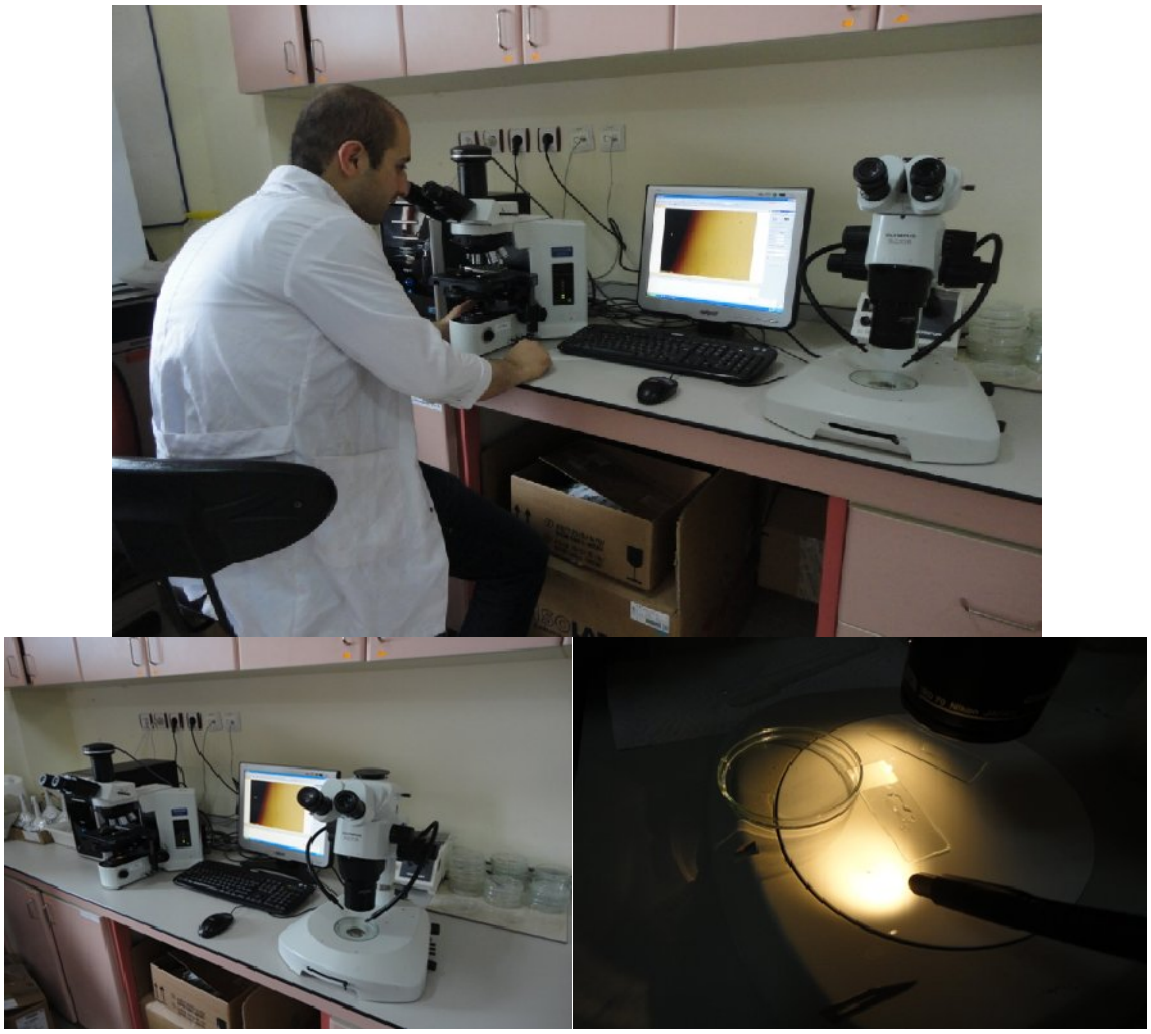

Figure 3.3. Preparation and viewing perineal patterns with the help of Olympus, model BX51.

3.2.2. Isozymes electrophoresis (polyacrylamide gel electrophoresis) (PAGE)

Polyacrylamide gel electrophoresis has been widely used for identification of species isozymes. Esterase, malate dehydrogenase, and α-glycerophospate dehydrogenase give a strong indication of being useful in the identification of the four most common root-knot nematodes; *M. incognita*, *M. arenaria*, *M. hapla* and *M. javanica*.

3.2.2.1. Sample preparation for page (polyacrylamide gel electrophoresis)

The samples were taken from infected root of tomatoes and pepper after 60(±5) days of inoculation. The roots were washed and examined for galling. An adequate number (approx. 66) of young and white milk in color females were extracted and each specimen was placed in a small test tube and stored in a freezer at -20˚C until they were used.

3.2.2.2. Preparation of acrylamide running gel solution

For the running gel, 2.7 mL of bis-acrylamide (100 mL distill water, 29.2 g acrylamide, 0.8 g N,N'-Methylenebis-acrylamide), 2.5 mL gel buffer at pH 8.8 (100 mL of distillated water and 18.15 g of Tris-base were mixed by shaking at the temperatures below 22˚C. pH of the solution was adjusted to 8.8 by adding Hydrochloric acid to decrease or Potassium hydroxide to increase it. Running solution was prepared by 4.8 mL of distillated water, 50 µL of APS (1000 µl of dH2O, 0.1 g of ammonium persulfate), and 5 µL of N,N,N,N-Tetramethylethylenediamine (TEMED) were quickly mixed and immediately poured into the cassette formed by the two glass plates sandwiched over sealing spacers. The cassette was filled up to 2/3 of the height of the slides, and stirred gently (extra attention was paid to not introduce air bubbles, as oxygenation may cause depleted polymerization). The space that was left on the top was filled with N-Butanol solution (%50 N-Butanol, %50 dH2O).It was waited for about 40 minutes until the running gel was completely polymerized. Once polymerization was completed, N-Butanol solution was disposed and the space was washed. Filter paper was used gently to remove water residues (Cetintas et al., 2007) (Figure 3.4).

Figure 3.4. Some steps of preparation of running gels for PAGE.

3.2.2.3. Preparation of the stacking gel

For the Stacking gel, 1.3 mL of acrylamide bis, 2.5 mL gel buffer with pH 6.8 (100 mL dH_2O and 6 g Tris-base were mixed at the room temperature and pH of the solution was adjusted to 6.8.The mixture was stirred gently enough without introducing any air bubbles. The stacking gel was added to the separating gel. A blunt-ended plastic comp was inserted for creating the sample application wells by avoiding the introduction of air bubbles. The gel was allowed to be polymerized completely at room temperature for 25-30 minutes. After polymerization of gels, the comb was removed carefully. The two glass plates were taken off from sealing, and were placed into the clamping frame placed in the tank.

3.2.2.4. Electrode buffer (1X)

For the electrode buffer solution, 1000 mL of dH_2O, 30.3 g of Tris-base and 144 g of glycine were mixed and shaken for 5-10 minutes to create 10X buffer. In order to make 1X buffer, the solutions were prepared by adding 360 mL of dH_2O to 40 mL of 10X buffer. The prepared 1X electrode buffer then was poured into the tank up to line level.

3.2.2.5. Preparation of sample solutions

The specimens were extracted as described above. The samples were placed into the small tubes with 5 µL of dH_2O, 5 µL of sample buffer (5.55 mL of dH_2O, 1.25 mL of gel buffer at pH 6.8, 3 mL of glycerol, and 5 µg of Bromophenol blue). Then, they were loaded to the wells by using a micropipette with long tips. Each gel contained 10 wells. The standard *M. javanica* was placed into wells number 1 and 10. The remaining 8 wells were loaded with the unknown female nematodes specimens.

3.2.2.6. Electrophoresis power supplies

A Bio-Rad mini-PROTEIN II (Bio-RAD, Singapore) electrophoresis unit was used and the voltage was maintained at 80 volts for the first 15 minutes and at 200 volts for the remaining separation running period (Figure 3.5).

3.2.2.7. Preparation of specific staining solution

For staining solution, 0.1 g of α Naphthalene acetate, 5 mL of acetone and 5 mL of dH2O were mixed thoroughly. Then, 6 mL of this solution was mixed with 200 mL of potassium phosphate with pH 6, 720 mL of X_1 (X_1=1000 mL dH_2O+50 mL of monobasic

solution), 180 mL of X_2 (500 mL dH_2O + 25 mL of dibasic solution), and 0.2 g of RR salt in an Erlenmeyer flask and stirred until it was homogenous. The gels were removed from the glass plates and placed in a staining solution, which was kept covered by aluminum foil, for 40 minutes. After staining, the gels were visualized for esterase phenotype bands followed the method of Harris and Hopkinson (1976) (Figure 3.5).

3.2.2.8. Gel protection solution

A solution containing 40 mL of ethanol, 20 mL of glycerol and 140 mL of dH_2O were prepared to protect the ready gels longer. Gels were placed in the solution and covered by aluminum foil for further examinations.

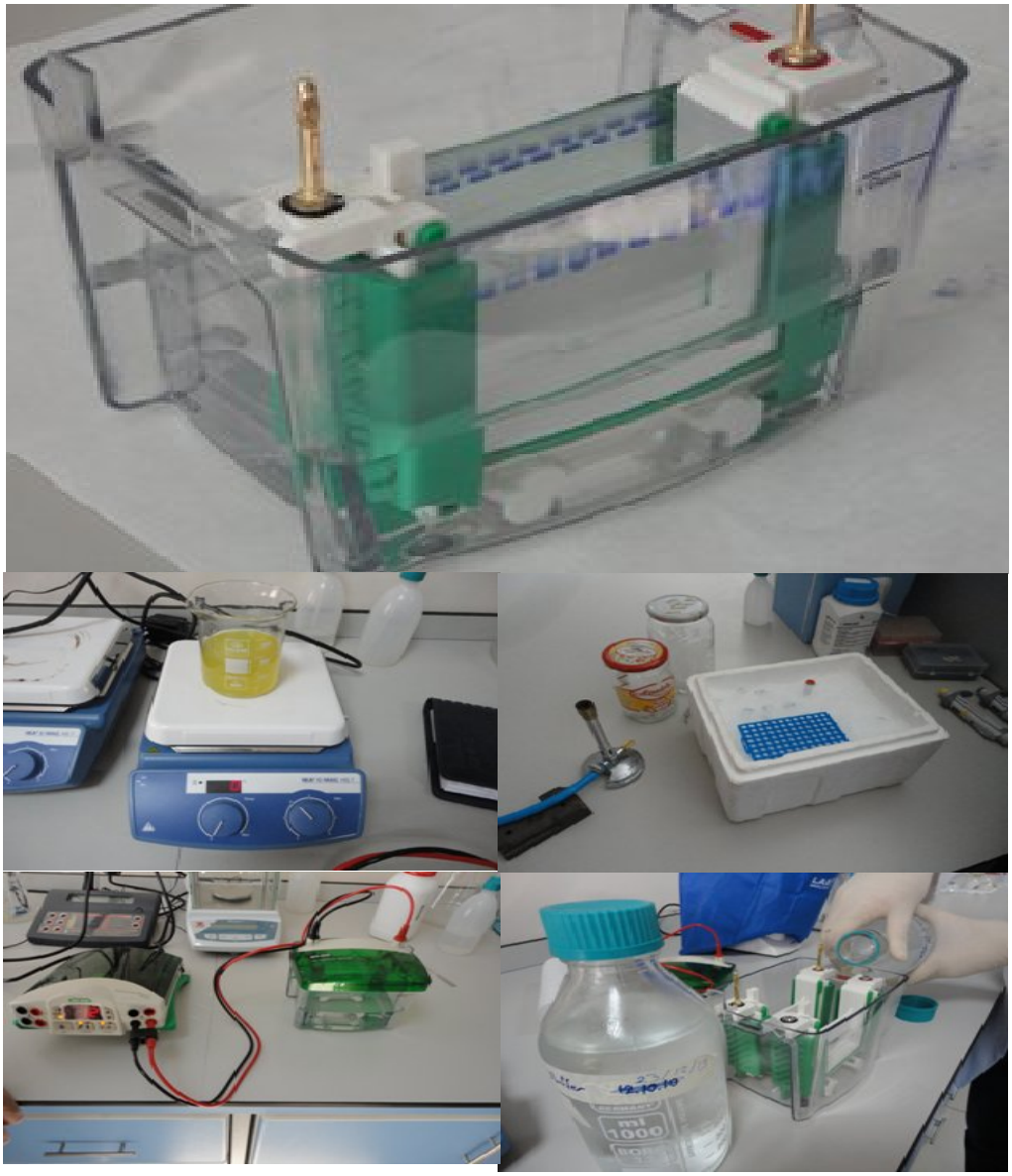

Figure 3.5. Laying the Electro Tank, Sample, Staining Solution, and Power Supply.

3.3. Experiments

3.3.1. Trail one

The experiments were conducted in a glass house and a growth chamber belonging to the Agriculture Faculty of Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey. Two plant types that were used in this study were a commonly grown Tomato cultivar and a pepper.

After the preparation of needed seedlings, and nematode inoculums, the soil for the pots was arranged and the experiment was set up on the 3th of April 2013.

Soil for green house and growth chamber pots experiments were arranged with a ratio of 60% sand, 25% clay and 15% organic matter. The pots size was 15 cm in diameter and 30 cm in height with the capacity of approximately 2 litters.

3.3.1.1. Breeding of tomato and pepper seedlings

The tomato and pepper seeds were planted in polystyrene seedling trays filled with sterilized soil. One week after germination, the most uniform and healthy-looking seedlings were selected and were taken care of by watering them daily and fertilizing them weekly in growth chamber at 26˚C temperature.

3.3.1.2. Filling the pots and transplanting the seedlings

Two liter sized pots were filled with 1.8L of the sterilized soil. The provision for suitable drainage in each of the pot was essential to prevent water logging or stagnation of water. Four-week old tomato and ten-week old peppers seedlings were transplanted into the pots (Figure 3.6).

Figure 3.6. A view of the transplanting the seedling to soil filled pots in the greenhouse.

3.3.1.3. Extraction of root-knot nematode eggs/J2 inoculums

Twelve weeks old root-knot nematodes infected seedlings of tomato and pepper plants were cleaned of debris gently by washing with stream water. The roots were cut into 2 cm small pieces and shaken manually for 2-3 minutes in a beaker (one liter), containing 500 mL of 0.5% sodium hypochlorite (NaOCl) solution to dissolve the gelatinous matrix and to release the eggs from the egg masses. The suspension was quickly passed through 75 µm sieve nested over 25 µm sieve to collect root fragments on the top sieve and freed eggs on the bottom one. Then, 25 µm sieves with freed egg was quickly passed under a stream of fresh water to remove the NaOCl residual. The eggs were washed for several times. Then they were collected in a beaker. This process was repeated twice in order to maximize the number of collected eggs (Hussey and barker, 1973).

3.3.1.4. Counting eggs/J2of root-knot nematode

To estimate the inoculums density of eggs/juveniles (J2's) suspension was transferred into a beaker and mixed vigorously blowing with pipette. The numbers of eggs or J2's were estimated in 5 mL aliquots in a counting dish under a stereomicroscope at 10X magnification. The total population was estimated by multiplying the mean of three aliquots with total volume. When the nematodes were in higher concentrations then the suspension was diluted by adding the required amount of water.

3.3.1.5. Application of different nematode inoculums levels

One week after transplanting the four weeks old tomatoes and pepper seedlings into the pots, they were inoculated with three levels of root-knot nematodes, *M. incognita*. The levels were consisted of control (0 J2/eggs soil plant), low (1000 J2/eggs soil plant) and high (2000 J2/eggs per plant/pot). Four holes were formed in a square shape approximately 2 cm distended from the roots. The J2/eggs suspension were adjusted for each 5mL containing 1000 eggs/J2s and applied to the planted pots by a pipette and the holes were covered with its soil (Figure 3.7).

Figure 3.7. Applying the nematode inoculums with different levels of eggs/J2s to the pots.

3.3.1.6. Applying of plant extract with different treatment levels

One-week after inoculations of nematodes, the planted pots were treated by five essential oils extracts from five different plants sources including Onion (*Allium cepa*), QL Agri 35 (*Quillaja saponaria*), Bay tree (*Laurus nobilis*), Eucalyptus (*Eucalyptus* sp), and Mustard (*Brassica* sp). Source of plants, plant parts and extraction method of oils are shown in (Table 3.1). Five essential oil application rates were consisted of control (0 µL\plant), low (100 µL\ plant) and high (250 µL\plant).The essential oils were mixed with a cup of water and added. The pots were covered by using aluminum foil for 48 hr (Figure 3.9).

Figure 3.8. Plants used in the experiments; a) Bay tree (*Laurus nobilis*),b) Eucalyptus (*Eucalyptus* sp), c) Mustard (*Brassica* sp).d) Onion (*Allium cepa*), e) QL Agri 35 (*Quillaja saponaria*), (Anonymous, 2014)

Table 3.1. The source and extraction methods of plant essential oils used in the study.

Source of plant	Scientific name	Plant parts	Extraction method
Onion	*Allium cepa*	Bulb	Steam distillation
Bay tree	*Laurus nobilis*	Leaves	Steam distillation
Eucalyptus	*Eucalyptus sp*	Leaves	Steam distillation
Mustard	*Brassica sp*	Seeds	Steam distillation
QL Agri 35	*Quillaja saponaria*	Root	Press-Maceration

Figure 3.9. Application of three different levels (0 (control), 150 and 250µL/ pot) of plant extracts (treatment) into the pots.

3.3.1.7. Plant harvest

The test plants were harvested (60±2) days after inoculation. The plants were cut off at the ground level discarded and the green parts were put in paper bags individually. Soil samples were taken from the pots by using spatula and placed in polyethylene bags. The plastic pots were pressed and loosen. In order to ensure an easy removal of the plants from the soil, the sides of pots were pressed to cause the soil loosen. The soil was then removed from the roots by gently shaking the plants. Then the roots were shaken in a bucket filled with water to remove all particles of soil from the root. The roots were stored in polyethylene bags in a refrigerator at 4 ˚C for further analyses (Figure 3.10).

Figure 3.10. Some processing steps; a) Harvesting plants, b)Collecting plant sample, c) Placing plants to carton bags, d)Sampling soil, e) and f) Removing plant roots from the pots, g) Cleaning the roots from soil, h) Washing roots gently.

3.3.1.8. Data collection

Plants heights were recorded biweekly with help of measuring tape starting from transplanting date of seedlings to the harvest. Fresh and dry weights of plant were recorded with the help of an electronic scale (Pioneer, China).

At harvest, plants were removed from pots and root systems were washed individually as mentioned above. Root galling were assessed using the scale of 0-5; where

0=no galls, 1=1-2 galls, 2=3-10 galls, 3=11-30 galls, 4=31-100 galls, and 5= >100 galls per root system (Taylor and Sasser, 1978).

Root systems were stained with food coloring (red) (Thies et al., 2002), and egg mass indices were assessed using a 0-5 scale, where 0=no egg masses, 1=1-2 egg masses, 2=3-10 egg masses, 3=11-30 egg masses, 4=31-100 egg masses, and 5= >100 egg mass per root system (Taylor and Sasser, 1978). Fresh and dry weight of the plant tissue and root systems were determined as mentioned above (green parts).

An 80 cm^3 soil sample from each pot was assayed to determine the number of J2 of *M. incognita* by using modified Baermann technique (Whitehead and Hemming, 1965). After (13±1) days, the extracted samples were taken and sieved (25µm) placed in a dish and counted under stereomicroscope. Data obtained were used for host status rating with the quantitative method. The reproduction factor (RF) = final population (Pf)/initial population (Pi) (good host RF≥1, poor host 0.1 <RF> 1, non-host RF ≤ 0.1) (Sasser et al., 1984) (Figure 3.11).

Figure 3.11. Data Collection. a) Fresh plant parts drying, b) Fresh plant parts weighting (Dry and Fresh), c) Recording plant heights, c) Setting a modified Baermann for nematode extraction from an 80 cc of soil, e) Counting the number of egg masses, f) Rating root galling.

3.3.1.9. Experiment conditions

The greenhouse conditions consisted of a daily average of 14 hr of light cycle, 26±4 ˚C daytime and 20±4 ˚C nighttime temperature. Depending on the timely moisture of the soil, all pots were watered evenly via a single twin-wall drip tape placed in the center of pots. The plants were fertilized weekly with (Fulvix %5 (%50 organic matter, %2.5 N, %0.5 organic N, %4 K_2O)). Every 10 to 12 days, the plants were applied soap solution to manage insects (mostly white fly) population.

3.3.1.10. Experimental designs

The plant glass greenhouse experiment was classified as a randomized complete block design (RCBD) with five treatments and replicated four times.

3.3.1.11. Statistical analysis

Data from nematode EMI (egg mass index), GI (galling index) and Rf (final nematode population from pots), plant height, plant fresh weight, plant dry weight, root fresh weight, and root dry weight were subjected to ANOVA using (SPSS Statistics version 20.0.0 statistical software), and treatment means were separated by dependent variable and the experiments were compared using t-test. Differences ($P \leq 0.05$) were considered statistically significant.

3.3.2. Trail two

The experiment was repeated and tomato and pepper seedlings were transplanted at 23thAugust, 2013. Site preparation, nematode inoculation levels, treatment applies, and installation experimental methodology were the same as those described for first one. After (60±2) days, tomato and pepper plants were harvested, plant height, green part fresh, dry weight, root system fresh, dry weight, gall and egg mass indices, and reproduction factor (Rf) were determined as described in the first repeat above.

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1. Identification

4.1.1.1. Perineal pattern

The observation of the morphology of perineal pattern taken from single females from tomato showed that the morphological character is typically matches with *M. incognita* based on (Eisenback et al., 1985) (Figure 4.1). *Meloidogyne incognita* perineal pattern was oval to rounded, typically with high, squared, dorsal arch, striae usually wavy, lateral field absent or weakly demarcated by forked striae (Figure 4.2).

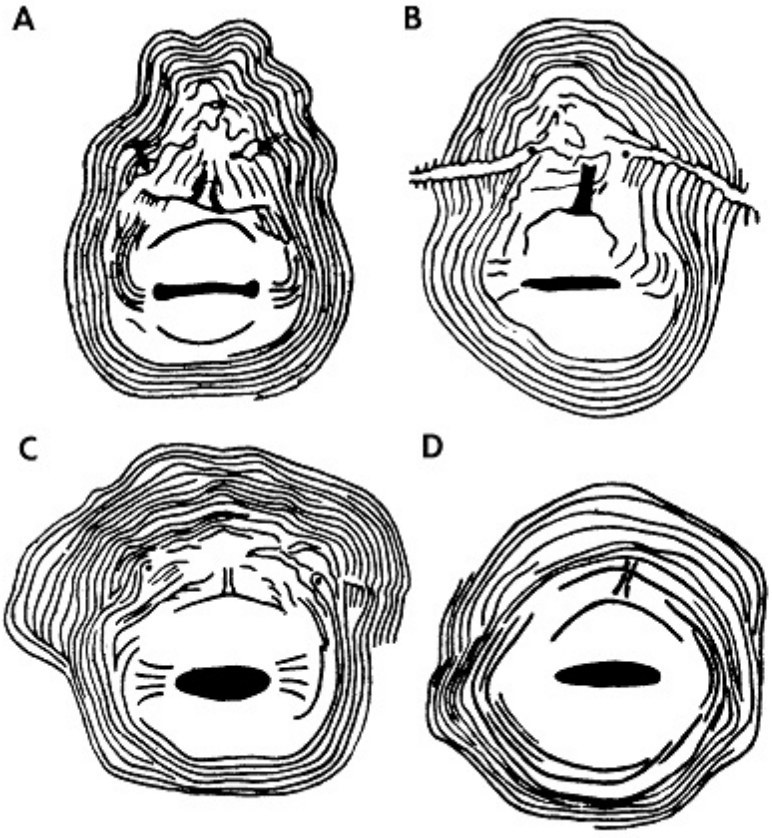

Figure 4.1. Drawings of perineal patterns taken from the original description, a) *Meloidogyne incognita*, b) *M. javanica*. c) *M. arenaria*. d) *M. halpa*. (Eisenback et al.,1985).

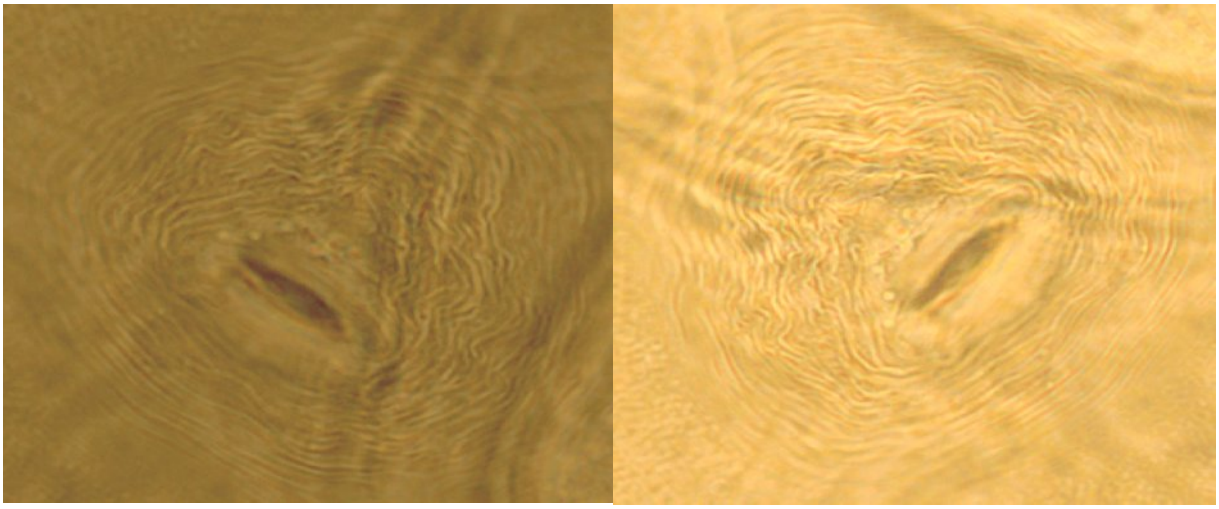

Figure 4.2. Perineal pattern of *Meloidogyne incognita* derived from a single egg mass isolate on tomato grown in a growth chamber.

4.1.1.2. Isozyme

Polyacrylamide gel electrophoresis was run to ensure the nematode species used in the experiment. Results showed that all females taken from plant roots in experiment had unique esterase isozyme bands of *M. incognita* compared to the standard *M. javanica* (Figure 4.3).

Figure 4.3. Esterase phenotypes showing the band belonging to *M. incognita* and standard *M. javanica.*

4.1.2. Experiments

4.1.2.1. Trail one

There was a significantly effect of plant varieties on all parameters (*P≤0.05*) in trial one. There was a significant effect of treatments on plant height and green part dry weights. Also, different inoculums nematode levels influenced significantly the root-galling indices. However, there was not any significant differences between the interaction of nematode level (N) x treatment (T), nematode level (N) x treatment level (L) and nematode level (N) x treatment (T) x treatment level (L) on plant height, green part fresh weight, green part dry weight and root galling (*P≤0.05*) (Table 4.1).

Analysis of variance has shown that egg mass indices were affected significantly by plant varieties and nematode levels. On the other hand, among all variables, root fresh weight was differed by only plant varieties. Additionally, treatments levels and plant varieties affected significantly the root dry weight. Reproduction factor (Rf) was a affected significantly by all variables except for treatment level (L) and Nematode level (N) x treatment Level (L) interaction (*P≤0.05*) (Table 4.2).

Data from low level of nematode inoculums (1000 egg/J2) with low concentration (100µL) is shown in Table 4.3. The plant height was recorded relatively high for Bay tree on tomato (32.25±2.4 cm) and high for Eucalyptus on pepper (30.80±1.9 cm). The green fresh weight was the highest for Bay tree (40.12±11.4 g) and QL Agri 35 (19.17±6.6 g) on tomatoes and pepper, respectively. Green part dry weight was also the highest for Bay tree on tomato (7.37±0.5 g) and the highest for Eucalyptus for pepper (2.40±0.5 g). Among all treatments, the number of galls produced in Bay tree and Eucalyptus (4.75±0.5) were lower than the other treatments on tomato. On pepper, also QL Agri 35 (2.00±1.4) had the lowest of root galling among the remaining treatments. The number of egg masses was recorded the lowest for Onion and Eucalyptus (4.50±0.6) on tomato, and also the lowest on Eucalyptus (3.75±1.0) on pepper. The root fresh weight was recorded the highest for QL Agri (4.90±1.4 g) on tomatoes and the highest for Eucalyptus (0.825±0.3 g) on pepper. The root dry weight sustained the highest for Eucalyptus (3.12±1.6 g) on tomato plant, and the highest for QL Agri 35 (0.75±0.4 g) on pepper. The reproduction factor (Rf) was recorded the lowest on Onion (0.35±0.1) on tomatoes, and the lowest on Onion (0.15±0.2) for pepper (Table 4.3).

Data from low nematode inoculums level with high level of treatment is shown in Table 4.4. The highest plant height was recorded in Eucalyptus for both tomatoes and pepper plant varieties, as 32.60±0.8 cm and 32.35±1.7 cm, respectively. The greatest green plant part fresh weight was observed in Onion for both plants varieties, as 51.45±12.7 g and 19.27±6.1 g, respectively. The green part weight was the greatest in Onion (7.32±0.5 g) for tomato and in Eucalyptus (2.75±0.8 g) for pepper. Root gall indices were obtained very low for Onion (4.25±0.5) on tomato, for Onion and Eucalyptus (2.00±0.0) on pepper. Egg mass index was listed the lowest on Onion 4.00±0.0 and 3.25±1.0, for tomatoes and pepper, respectively. Among all treatments, the greatest root fresh weight was recorded in Eucalyptus for tomatoes (4.82±1.8 g) and pepper plant (0.87±0.2 g).The greatest root dry weight was recorded in Eucalyptus on both plant varieties (3.00±1.5 g, 0.80±0.1 g). Reproduction factor was recorded lowest in Bay tree (0.35±0.1) on tomato, and lowest in Onion (0.25±0.1) on pepper (Table 4.4).

Data from the high level of nematode inoculums with low ratio of treatments is shown in Table 4.5. The greatest plant height was recorded in Eucalyptus treatment for both tomatoes (34.20±2.9 cm) and pepper (29.55±3.4 cm). The greatest weight of green fresh plant part was obtained in Eucalyptus (37.90±10.0 g) on tomato and in QL Agri35 (18.92±6.0 g) on pepper. On the other hand, the greatest weight of green dry part was observed in Eucalyptus for tomatoes and pepper, as 7.17±0.7 g and 2.60±0.6 g, respectively. Galling indices per root system was recorded the lowest in Onion (4.50±0.0) on tomato and was recorded in Eucalyptus (2.00±0.0) on pepper. Egg mass index per root system was recorded very high for all the treatments in tomato, and low in QL Agri 35 (4.00±0.8) treatment on pepper. Among all treatments, the greatest fresh root weight was reported on Onion (4.30±2.5 g) in tomato and on Eucalyptus (1.12±0.4 g) in pepper. However, the greatest dry root weight was recorded in Eucalyptus, 2.75±0.6 g and 1.05±0.3 g, on tomatoes and pepper, respectively. Finally (Rf) was listed the lowest in Eucalyptus for both tomatoes (0.70±0.1) and pepper(0.10±0.1) in the high nematode inoculums level (Table 4.5).

Data from low nematode inoculums level with high ratio treatment is shown in Table 4.6. Among the treatments, Eucalyptus sustained the highest plant height for both plant hosts, tomatoes and pepper, being 34.40±0.6 cm and 29.95±0.6 cm, respectively. Green fresh part weight was recorded as 41.85±12.9 g in Eucalyptus on tomato host plant, and as 20.40±4.7 g in QL Agri 35 on pepper host plant. The greatest green dry plant part

weight was seen in Eucalyptus (7.87±2.2 g) on tomato and in QL Agri 35 (2.80±0.1 g) for pepper. The lowest number of galls per root system was observed in Eucalyptus (4.75±0.5) on tomato and in Bay tree and Eucalyptus (2.50±0.5) on pepper host plant. EMI was listed lowest at Onion (4.75±0.5) on tomato and in Onion and QL Agri 35 (4.50±1.0) on pepper. Root fresh weight was the greatest in Eucalyptus (5.17±2.3 g) among all treatments for tomato, and in Bay tree (1.12±0.6 g) for pepper host plant. Root dry weight was recorded as 3.35±1.0 g in onion on tomato, and 0.87±0.3 g in Bay tree on pepper. The reproduction factor was the lowest in Eucalyptus on both host plants, tomatoes and pepper, being 1.15±0.1 and 0.10±0.1, respectively (Table 4.6).

Data from control (0 egg/J2 per pot) with low level treatment is shown in Table 4.7. The greatest Plant height was recorded in control for both tomatoes and pepper plant hosts 38.92±10.7 cm, 31.26±2.9 cm, respectively. The greatest weight of green fresh plant part was obtained in Bay tree (60.70±2.9 g) on tomato, and in control (29.75±27.7 g) on pepper. Green part dry weight was recorded as 7.45±0.6 g in Bay tree on tomato, and as 4.25±1.3 g in control on pepper. The gall index, egg mass index and reproduction factor were zero (clean). The root fresh weight was recorded the highest in QL Agri 35 treatment with being 4.00±1.3 g in tomatoes and 3.47±1.8 g in pepper. The root dry weight sustained the highest in Eucalyptus being 2.90±1.4 g in tomato and the 1.00±0.4 g in pepper (Table 4.7).

Data from control pots high level treatments is shown in Table 4.8. The plant height was recorded the highest in control for both tomatoes and pepper plant hosts 38.90±10.7 cm, 31.26±3.0 cm, respectively. Green fresh weight part was recorded as 52.50±29.1 g in Onion on tomato, and as 29.75±10.1 g on pepper. The greatest green dry plant part weight was recorded in QL Agri 35 (7.80±0.0 g) on tomato, and (4.25±1.3 g) on pepper. In the control, GI, EMI and Rf data were all 0.0 (clean). The root fresh weight was recorded the highest in QL Agri 35 (3.80±0.0 g) on tomato fallowed by (3.47±1.8 g) pepper. The root dry weight sustained the highest in Bay tree (2.70±0.4 g) on tomato and (1.00±0.4 g) on pepper (Table 4.8).

Table 4.1. Analysis of variance for the effects of four essential oils treatments and their three application rates to three inoculums levels of *Meloidogyne incognita* and their interaction on the plant height, green part fresh weight, green part dry weight, root galling of tomato and pepper. Root galling: 0-5 scale, where, 0: no gall, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls (Taylor and Sasser, 1978) (*P≤0.05*).

1st experiment

Source	df	Plant height (cm)	F- value	Green part fresh weight (g)	F- value	Green part dry weight (g)	F- value	Root galling index	F- value
Host Plant (P)	1	0.00*	25.78	0.00*	112.29	0.00*	260.12	0.00*	199.33
Treatment levels (L)	2	0.00*	7.49	0.22	1.50	0.02*	3.74	0.95	0.050
Nematode levels (N)	2	0.93	0.06	0.12	2.11	0.48	0.73	0.00*	275.02
Treatment (T)	3	0.00*	5.36	0.87	0.23	0.57	0.66	0.89	0.19
NxT	6	0.97	0.21	0.99	0.12	0.86	0.42	0.89	0.38
NxL	2	0.91	0.08	0.85	0.15	0.91	0.09	0.40	0.92
TxL	6	0.43	0.98	0.14	1.61	0.68	0.65	1.00	0.00
NxTxL	6	0.86	0.41	0.75	0.57	0.93	0.29	0.07	1.96

* Significant at (*P≤0.05*)

Table 4.2. Analysis of variance for the effects four essential oils treatments and their three application rates to three inoculums level of *Meloidogyne incognita* and their interaction on the root fresh weight, root dry weight, reproduction factor (Rf) and egg masses of tomato and pepper. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). (P≤0.05). Rf =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al.. 1984)

1st experiment

Source	df	Egg mass index	F- value	Root fresh weight (g)	F- value	Root dry weight (g)	F- value	Rf	F- value
Host Plant (P)	1	0.00*	7.29	0.00*	109.34	0.00*	187.06	0.00*	87.52
Treatment levels (L)	2	0.78	0.24	0.98	0.01	0.00*	5.15	0.27	1.29
Nematode levels (N)	2	0.00*	918.34	0.52	0.64	0.39	0.93	0.00*	28.83
Treatments (T)	3	0.79	0.33	0.87	0.22	0.46	0.85	0.02*	3.38
NxT	6	0.36	1.10	0.94	0.27	0.98	0.17	0.00*	4.32
NxL	2	0.73	0.30	0.54	0.61	0.19	1.66	0.49	0.70
TxL	6	0.29	1.24	0.76	0.55	0.85	0.44	0.00*	3.28
NxTxL	6	0.46	0.94	0.91	0.34	0.85	0.43	0.00*	3.69

* Significant at (*P≤0.05*)

Table 4.3. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 1000 eggs/J2s nematode level with 100µL of treatments level in first experiment in tomatoes and pepper (Mean±SD)

1st exp	Tomato host plant							
Treatments	Plant height (cm)	Green part fresh weight (g)	Green part dry weight (g)	Gall indices	Egg mass indices	Root fresh weight (g)	Root dry weight (g)	Rf
Onion	29.15±0.8	32.75±4.7	7.12±1.0	5.00±0.0	4.50±0.6	4.77±1.9	3.10±1.3	0.35±0.1
Bay tree	32.25±2.4	40.12±11.4	7.37±0.5	4.75±0.5	4.75±0.5	3.47±1.1	2.55±0.6	3.45±0.1
Eucalyptus	32.20±1.5	37.32±6.9	6.05±1.5	4.75±0.5	4.50±0.6	4.10±2.5	3.12±1.6	5.05±0.6
QL Agri 35	30.20±1.4	38.72±14.7	7.32±0.9	5.00±0.0	4.75±0.5	4.90±1.4	2.92±0.9	1.20±0.1
	Pepper host plant							
Onion	29.05±2.8	13.30±4.2	1.90±0.2	3.25±0.5	4.75±0.5	0.75±0.2	0.67±0.1	0.15±0.2
Bay tree	30.25±3.0	15.40±6.4	1.95±0.6	2.25±0.5	4.25±1.0	0.67±0.1	0.57±0.1	0.30±0.0
Eucalyptus	30.80±1.9	18.90±3.9	2.40±0.5	2.75±0.9	3.75±1.0	0.85±0.3	0.72±0.2	0.45±0.2
QL Agri 35	28.60±2.9	19.17±6.6	2.27±0.7	2.00±1.4	4.00±1.1	0.82±0.4	0.75±0.4	0.20±0.1

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984)

Table 4.4. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 1000 eggs/J2s nematode level with 250µL of treatments level in first experiment in tomatoes and pepper (Mean±SD)

1st exp	Tomato host plant							
Treatments	Plant height (cm)	Green part fresh weight (g)	Green part dry weight (g)	Gall indices	Egg mass indices	Root fresh weight (g)	Root dry weight (g)	Rf
Onion	29.95±1.2	51.45±12.7	7.32±0.5	4.25±0.5	4.00±0.0	2.67±0.7	2.35±0.6	2.00±0.2
Bay tree	26.65±1.1	25.85±6.1	5.52±1.0	4.75±0.5	4.25±0.5	3.22±1.1	2.12±0.7	0.35±0.1
Eucalyptus	32.60±0.8	38.95±5.3	6.95±0.4	4.50±0.6	4.25±0.5	4.82±1.8	3.00±1.5	2.65±0.5
QL Agri 35	30.20±2.5	31.80±4.5	6.65±0.3	5.00±0.0	4.50±0.7	3.70±1.8	2.10±0.0	6.10±0.0
	Pepper host plant							
Onion	28.80±4.1	19.27±6.1	2.25±0.5	2.00±0.0	3.25±1.0	0.65±0.3	0.57±0.3	0.25±0.1
Bay tree	30.50±1.9	18.55±5.1	2.25±0.6	2.75±0.5	4.50±1.0	0.80±0.2	0.72±0.2	0.50±0.2
Eucalyptus	32.35±1.7	18.47±9.1	2.75±0.8	2.50±0.6	4.25±0.5	0.87±0.2	0.80±0.1	0.50±0.1
QL Agri 35	28.70±0.4	15.85±0.9	2.05±0.1	2.00±0.0	5.00±0.0	0.80±0.0	0.70±0.0	1.00±0.0

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984)

Table 4.5. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 2000 eggs/J2s nematode level with 100µL of treatments level in first experiment in tomatoes and pepper (Mean±SD)

1st exp	**Tomato host plant**							
Treatments	**Plant height (cm)**	**Green part fresh weight (g)**	**Green part dry weight (g)**	**Gall indices**	**Egg mass indices**	**Root fresh weight (g)**	**Root dry weight (g)**	**Rf**
Onion	29.60±3.4	34.15±14.2	5.52±1.3	4.50±0.6	5.00±0.0	4.30±2.5	1.95±0.5	1.10±0.0
Bay tree	32.20±1.8	33.27±10.9	6.62±1.2	5.00±0.0	5.00±0.0	2.80±0.9	2.07±0.8	2.00±1.6
Eucalyptus	34.20±2.9	37.90±10.0	7.17±0.7	4.75±0.5	5.00±0.0	3.75±1.3	2.75±0.6	0.70±0.1
QL Agri 35	32.10±2.9	29.70±8.3	6.00±1.7	5.00±0.0	5.00±0.0	4.42±0.8	2.67±0.5	2.00±1.4
				Pepper host plant				
Onion	27.00±3.8	14.72±2.2	1.82±0.2	2.25±0.5	5.00±0.0	0.67±0.1	0.65±0.1	0.25±0.1
Bay tree	29.10±1.5	14.02±3.8	2.05±0.4	2.75±0.5	4.75±0.5	0.70±0.2	0.65±0.2	0.30±0.3
Eucalyptus	29.55±3.4	15.92±2.6	2.60±0.6	2.00±0.0	4.75±0.5	1.12±0.4	1.05±0.3	0.10±0.1
QL Agri 35	28.65±2.6	18.92±6.0	2.50±0.3	3.00±0.8	4.00±0.8	0.90±0.3	0.82±0.2	0.15±0.2

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984)

Table 4.6. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 2000 eggs/J2s nematode level with 250µL of treatments level in first experiment in tomatoes and pepper (Mean±SD)

1st exp	Tomato host plant							
Treatments	Plant height (cm)	Green part fresh weight (g)	Green part dry weight (g)	Gall indices	Egg mass indices	Root fresh weight (g)	Root dry weight (g)	Rf
Onion	31.80±2.6	38.40±3.6	7.50±1.1	5.00±0.0	4.75±0.5	5.05±1	3.35±1.0	2.15±0.1
Bay tree	31.50±1.9	29.95±4.1	6.05±0.9	5.00±0.0	5.00±0.0	3.95±1.7	2.67±0.9	1.95±1.8
Eucalyptus	34.40±0.6	41.85±12.9	7.87±2.2	4.75±0.5	5.00±0.0	5.17±2.3	3.32±1.4	1.15±0.1
QL Agri 35	30.20±1.4	31.25±6.8	4.40±0.7	5.00±0.0	5.00±0.0	3.00±1.0	2.30±0.4	4.10±0.0
	Pepper host plant							
Onion	29.30±0.3	17.80±4.6	2.27±0.4	3.50±0.1	4.50±1.0	0.70±0.2	0.60±0.1	0.20±0.0
Bay tree	29.20±3.4	20.32±3.6	2.62±0.5	2.25±0.5	4.75±0.5	1.12±0.6	0.87±0.3	0.35±0.2
Eucalyptus	29.95±0.6	15.07±3.4	2.12±0.2	2.25±0.5	4.75±0.5	0.87±0.4	0.80±0.4	0.10±0.1
QL Agri 35	27.00±2.5	20.40±4.7	2.80±0.1	2.50±0.7	4.50±0.7	1.00±0.4	0.85±0.4	0.40±0.0

* Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls . Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984)

Table 4.7. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 0 eggs/J2s nematode level with 100µL of treatments level in first experiment in tomatoes and pepper (Mean±SD)

1st exp	Tomato host plant							
Treatments	Plant height (cm)	Green part fresh weight (g)	Green part dry weight (g)	Gall indices	Egg mass indices	Root fresh weight (g)	Root dry weight (g)	Rf
Onion	35.10±5.2	46.15±6.6	7.35±0.2	0.0	0.0	2.25±0.1	1.85±0.1	0.0
Bay tree	32.10±4.1	60.70±27.7	7.45±0.6	0.0	0.0	3.20±1.1	1.90±0.4	0.0
Eucalyptus	33.10±1.5	37.10±6.8	7.20±2.5	0.0	0.0	3.90±1.0	2.90±1.4	0.0
QL Agri 35	31.80±2.0	38.75±1.6	7.20±0.8	0.0	0.0	4.00±1.3	2.50±0.3	0.0
Control	38.90±10.7	21.93±15.7	2.95±2.5	0.0	0.0	0.74±0.6	0.48±0.3	0.0
				Pepper host plant				
Onion	24.20±5.1	11.90±6.1	1.70±0.3	0.0	0.0	0.55±0.1	0.45±0.1	0.0
Bay tree	28.30±0.1	10.00±5.5	1.60±0.3	0.0	0.0	0.60±0.1	0.50±0.1	0.0
Eucalyptus	30.80±2.8	18.50±4.2	2.65±0.1	0.0	0.0	0.90±0.1	0.80±0.1	0.0
QL Agri 35	29.90±2.1	26.25±1.3	3.25±0.2	0.0	0.0	0.95±0.1	0.80±0.0	0.0
Control	31.26±2.9	29.75±10.1	4.25±1.3	0.0	0.0	3.47±1.8	1.00±0.4	0.0

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, Root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls . Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984)

Table 4.8. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 0 eggs/J2s nematode level with 250µL of treatments level in first experiment in tomatoes and pepper (Mean±SD)

1st exp	Tomato host plant							
Treatments	Plant height (cm)	Green part fresh weight (g)	Green part dry weight (g)	Gall indices	Egg mass indices	Root fresh weight (g)	Root dry weight (g)	Rf
Onion	33.80±2.3	52.50±29.1	7.10±3.5	0.0	0.0	3.25±0.2	2.05±0.8	0.0
Bay tree	33.00±2.5	36.20±4.2	7.35±1.2	0.0	0.0	3.75±1.3	2.70±0.4	0.0
Eucalyptus	29.50±0.7	47.00±8.5	7.25±1.2	0.0	0.0	3.50±1.4	2.25±0.1	0.0
QL Agri 35	29.80±0.0	37.20±0.0	7.80±0.0	0.0	0.0	3.80±0.0	2.60±0.0	0.0
Control	38.90±10.7	21.93±15.7	2.95±2.5	0.0	0.0	0.74±0.6	0.48±0.3	0.0
	Pepper host plant							
Onion	28.30±4.9	17.60±2.4	2.35±0.5	0.0	0.0	0.55±0.1	0.55±0.1	0.0
Bay tree	27.10±3.0	16.85±0.2	2.00±0.3	0.0	0.0	0.60±0.1	0.55±0.1	0.0
Eucalyptus	30.30±1.0	17.90±4.5	2.40±0.4	0.0	0.0	0.75±0.1	0.65±0.1	0.0
QL Agri 35	29.40±0.0	19.40±0.0	2.20±0.0	0.0	0.0	0.70±0.0	0.60±0.0	0.0
Control	31.26±3.0	29.75±10.1	4.25±1.3	0.0	0.0	3.47±1.8	1.00±0.4	0.0

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, Root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984)

4.1.2.2. Trail two

There was a significantly effect of plant varieties on all parameters (*P≤0.05*) in trial two. There was not significant effect of treatment (T) and treatment level (L) for all parameters. However, different nematode levels (N) affected all parameters significantly. Additionally, there was no any significant influenced between interaction nematode level (N) x treatment (T) for all parameters except for fresh green part weight. No differences observed for remaining interaction source of variance at plant height, green part fresh weight, green part dry weight, and root galling (*P≤0.05*) (Table 4.9).

Analysis of variance has shown that egg mass indices and reproduction factor (Rf) were influenced significantly by plant varieties and nematode levels. However, there were no significant differences among all variables at root fresh weight. On the other hand, there was a significant effect of treatment level (L) on root dry weight. Additionally, there was not any significant differences in treatment and between the interaction of nematode level (N) x treatment (T), nematode level (N) x treatment level (L), treatment (T) x treatment level (L) and nematode level (N) x treatment (T) x treatment level (L) on egg mass, root fresh weight, root dry weight and reproduction factor (Rf) (*P≤0.05*) (Table 4.10).

Data from low nematode inoculums level (1000 egg/J2 per plant) with low level of treatments (100µL) is shown in Table 4.11. The plant height was recorded relatively high for Eucalyptus on tomato (42.55±2.5 cm) and high for Bay tree on pepper (33.55±1.9 cm). The green fresh weight was the highest in QL Agri 35, being 42.87±11.7 g and 35.15±6.7 g for tomatoes and pepper, respectively. Green part dry weight was also the highest in QL Agri 35 as 7.02±0.7 g and 5.07±1.1 g for tomatoes and pepper, respectively. Among all treatments, the number of galls produced in Bay tree and QL Agri 35 (4.5±0.6) were lower than other treatments on tomato. On pepper, Bay tree had the lowest of root galling (2.75±0.9) among the remaining treatments. The number of egg masses was recorded the lowest in Onion (3.75±1.9) on tomato and in Eucalyptus and QL Agri 35 (0.25±0.5) on pepper. The root fresh weight was recorded the highest in Onion (2.32±1.7 g) on tomatoes and in Eucalyptus (1.95±1.2 g) on pepper. The root dry weight sustained the highest in Onion (1.70±0.9 g) on tomato, and in Bay tree (1.60±0.8 g) on pepper. The reproduction factor (Rf) was recorded the lowest in QL Agri 35 (0.45±0.4) on tomatoes, and the lowest in Bay tree, Eucalyptus and QL Agri 35 (0.0±0.0) on pepper (Table 4.11).

Data from low nematode inoculums level with high level of treatment (250µL) is shown in Table 4.12. The highest plant height was recorded in Eucalyptus for both tomatoes and pepper plant hosts (47.50±5.3 cm, 32.55±2 cm) respectively. The greatest green plant part fresh weight was observed in QL Agri 35 (51.87±15.1 g) on tomato and greatest on Onion (36.87±7.4 g) on pepper. The green part dry weight was greatest in QL Agri 35 (7.80±2.1 g) on tomato, and greatest in Bay tree (5.07±0.8 g) on pepper. Root gall indices were obtained very low for Eucalyptus (3.75±1.5) on tomato, for QL Agri 35 (1.25±0.5) on pepper. EMI was recorded the lowest on Eucalyptus (2.50±1.9) for tomato, and lowest in Bay tree (0.25±0.5) on pepper. Among all treatments, the greatest root fresh weight was recorded in Bay tree (1.85±1.2 g) on tomato and greatest on Eucalyptus (1.32±1.6 g) on pepper. The root dry weight was sustained the highest on Bay tree (1.17±0.3 g) on tomato, and highest in Eucalyptus (1.32±0.4 g) on pepper. The reproduction factor was recorded the lowest on Onion (0.10±0.0) on tomato, and lowest on Bay tree and QL Agri35 (0.0±0.0) on pepper (Table 4.12).

Data from high nematode inoculums level (2000 egg/ J2 per plant) with low level of treatment (100µL) is shown in Table 4.13. The greatest plant height was recorded in Eucalyptus (49.25±7.0 cm) on tomato, and greatest in Onion (28.95±1.8 cm) on pepper. The weight of green fresh plant part was obtained in Bay tree for both tomatoes and pepper plant varieties (43.45±3.9 g, 39.67±6.8 g). On the other hand, the greatest weight of green dry part was observed in Bay tree for tomatoes and pepper 7.17±0.5 g, 5.30±0.9 g, respectively. Galling indices per root system was recorded the lowest in Eucalyptus (4.50±1.0) on tomato, and was recorded lowest in Bay tree (1.75±0.5) on pepper. Egg mass index per root system was recorded lowest in Eucalyptus (3.25±2.1) on tomato, and lowest on Bay tree (0.25±0.5) on pepper. Among all treatments, the greatest fresh root weight was reported on Onion (2.27±1.8 g) in tomato, and on Bay tree (2.07±1.1 g) in pepper. However, the greatest root dry weight was recorded in Bay tree (1.65±0.2 g) on tomato, and on QL Agri 35 (1.12±0.4 g) on pepper. Finally Reproduction factor was listed the lowest in Eucalyptus (0.15±0.1) on tomato, and lowest in Bay tree, Eucalyptus (0.0±0.0) on pepper (Table 4.13).

Data from high nematode inoculums level with high level of treatment (250µL) is shown in Table 4.14.The plant height was recorded high for QL Agri 35 (49.60±0.0 cm) on tomato and high for Onion (28.40±4.7 cm) on pepper. Green fresh part weight was recorded as 49.37±32.6 g in Bay tree on tomato host plant, and as 36.05±2.2 g in QL Agri

35 on pepper host plant. Among the treatments, QL Agri 35 sustained the greatest green dry plant part weight for plant hosts, tomatoes and pepper, being 7.13±0.2 g, 5.10±0.2 g, respectively. The lowest number of galls per root system was observed in Bay tree for both tomatoes and pepper plant hosts 4.25±0.5, 1.50±1.3, respectively. EMI was listed lowest at Onion (1.75±1.7) on tomato and in QL Agri 35 (0.25±0.5) on pepper. Root fresh weight was the greatest in Eucalyptus (3.80±3.5 g) among all treatments for tomato, and in Bay tree (1.85±1.5 g) for pepper host plant. Root dry weight was recorded as 2.65±2.2 g in Eucalyptus on tomato, and 1.10±0.3 g in Onion on pepper. The reproduction factor was the lowest in QL Agri 35 (0.06±0.1) on tomato, and in Onion, QL Agri 35 (0.0±0.0) on pepper (Table 4.14).

Data from control (0 egg/ J2 per plant) with low level of treatment (100µL) is shown in Table 4.15. The plant height was recorded relatively high for Onion on tomato (47.70±12.6 cm) and high for QL Agri 35 on pepper (32.00±5.1 cm). The greatest weight of green fresh plant part was obtained in Bay tree (52.85±38.8 g) on tomato and in QL Agri 35 (30.05±4.0 g) on pepper. Green dry part weight was recorded as 7.85±0.5 g in Onion on tomato plant host, and as 4.21±1.4 g in control on pepper host plant. GI, EMI and Rf were recorded nothing (0). The greatest root fresh weight was recorded in Eucalyptus for tomato (2.80±2.5 g), and in control (3.47±1.8 g) on pepper. However, the greatest dry root weight was recorded in Eucalyptus for both tomatoes and pepper plant varieties 2.35±2.2 g, 1.05±0.2 g, respectively (Table 4.15).

Data from control with high level of treatment (250µL) is shown in Table 4.16. The greatest plant height was recorded in Onion (40.20±11.3 cm) on tomato and in QL Agri 35 (34.00±0.0 cm) on pepper. The greatest weight of green fresh plant part was obtained in Onion (40.90±12.7 g) on tomato and in QL Agri 35 (39.30±0.0 g) on pepper. On the other hand, the greatest weight of green dry part was observed in Onion (6.20±2.3 g) on tomato and on QL Agri 35 (5.90±0.0 g) on pepper. GI, EMI and Rf were recorded nothing. Root fresh weight was greatest in Onion (3.45±0.1 g) on tomato, and in control (3.47±1.8 g) on pepper. Root dry weight was listed as 2.40±0.0 g in Bay tree on tomato, and as1.20±0.0 g in QL Agri 35 on pepper (Table 4.16).

Table 4.9. Analysis of variance for the effects four essential oils treatments and their three application rates to three inoculums level of *Meloidogyne incognita* and their interaction on the plant height, green part fresh weight, green part dry weight, root galling of tomato and pepper. Root galling: 0-5 scale, where, 0: no gall, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls (Taylor and Sasser, 1978) (*P≤0.05*)

2nd experiment

Source	df	Plant height (cm)	F- value	Green part fresh weight (g)	F- value	Green part dry weight (g)	F- value	Root galling index	F- value
Plant (P)	1	0.00*	95.47	0.00*	13.89	0.00*	29.37	0.00*	120.64
Treatment levels (L)	2	0.23	1.45	0.57	0.56	0.17	1.76	0.87	0.13
Nematode levels (N)	2	0.01*	4.56	0.00*	5.47	0.00*	6.41	0.00*	154.46
Treatment (T)	3	0.24	1.41	0.18	1.62	0.38	1.02	0.52	0.75
NxT	6	0.08	1.88	0.04*	2.25	0.32	1.17	0.57	0.79
NxL	2	0.20	1.59	0.25	1.39	0.40	0.90	0.87	0.13
TxL	6	0.16	1.54	0.41	1.01	0.30	1.20	0.95	0.26
NxTxL	6	0.07	1.99	0.97	0.20	0.34	1.12	0.96	0.23

* Significant at (*P≤0.05*).

Table 4.10. Analysis of variance for the effects four essential oils treatments and their three application rates to three inoculums level of *Meloidogyne incognita* and their interaction on the root fresh weight, root dry weight, reproduction factor (Rf) and egg masses of tomato and pepper. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). (*P≤0.05*). Rf=Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984)

2nd experiment

Source	df	Egg mass index	F- value	Root fresh weight (g)	F- value	Root dry weight (g)	F- value	Rf	F- value
Plant (P)	1	0.00*	81.67	0.10	2.65	0.08	3.01	0.00	17.05
Treatment ratio (R)	2	0.32	1.14	0.97	0.03	0.02	3.72	0.57	0.54
Inoculums levels (N)	2	0.00*	26.10	0.56	0.57	0.82	0.19	0.03	3.35
Treatment (T)	3	0.99	0.02	0.36	1.05	0.60	0.62	0.82	0.30
NxT	6	0.95	0.24	0.92	0.33	0.65	0.70	0.98	0.17
NxR	2	0.18	1.68	0.49	0.71	0.93	0.07	0.37	0.99
TxR	6	0.94	0.28	0.51	0.87	0.92	0.32	0.93	0.30
NxTxR	6	0.60	0.75	0.95	0.26	0.30	1.21	0.79	0.51

*Significant at (*P≤0.05*)

Table 4.11. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 1000 eggs/J2s nematode level with 100µL of treatments level in second experiment in tomatoes and pepper (Mean±SD)

2nd exp	**Tomato host plant**							
Treatments	**Plant height (cm)**	**Green part fresh weight (g)**	**Green part dry weight (g)**	**Gall indices**	**Egg mass indices**	**Root fresh weight (g)**	**Root dry weight (g)**	**Rf**
Onion	39.50±6.8	41.72±12.9	6.95±2.5	5.00±0.0	3.75±1.9	2.32±1.7	1.70±0.9	0.55±0.1
Bay tree	35.10±0.9	31.72±5.30	5.22±1.8	4.50±0.6	4.00±1.1	1.52±1.1	1.32±1.0	1.00±1.7
Eucalyptus	42.55±2.5	42.55±5.30	6.72±1.1	5.00±0.0	4.00±0.0	1.20±0.4	1.10±0.4	0.95±1.0
QL Agri 35	41.60±8.0	42.87±11.7	7.02±0.7	4.50±0.6	4.25±0.5	1.37±1.3	1.05±0.9	0.45±0.4
				Pepper host plant				
Onion	30.55±1.8	31.85±6.9	4.35±0.7	2.25±0.5	1.00±0.8	1.65±1.3	0.87±0.2	0.05±0.1
Bay tree	33.55±1.9	33.12±6.9	4.90±0.7	2.75±0.9	0.50±0.6	1.67±1.0	1.60±0.8	0.0±0.0
Eucalyptus	27.95±1.4	30.97±2.4	4.27±0.2	1.75±0.9	0.25±0.5	1.95±1.2	1.10±0.5	0.0±0.0
QL Agri 35	27.45±4.3	35.15±6.7	5.07±1.1	2.50±1.0	0.25±0.5	1.47±0.7	1.07±0.2	0.0±0.0

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling: 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls . Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984)

Table 4.12. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 1000 eggs/J2s nematode level with 250µL of treatments level in second experiment in tomatoes and pepper (Mean±SD)

2nd exp	Tomato host plant							
Treatments	**Plant height (cm)**	**Green part fresh weight (g)**	**Green part dry weight (g)**	**Gall indices**	**Egg mass indices**	**Root fresh weight (g)**	**Root dry weight (g)**	**Rf**
Onion	39.15±9.4	36.72±13.3	5.67±2.4	4.50±0.6	3.25±0.9	0.87±0.7	0.77±0.7	0.10±0.0
Bay tree	40.05±8.6	37.10±13.8	5.95±1.4	4.00±0.8	3.25±1.7	1.85±1.2	1.17±0.3	0.25±0.2
Eucalyptus	47.50±5.3	51.75±12.6	7.37±1.6	3.75±1.5	2.50±1.9	1.20±0.2	1.00±0.3	0.20±0.1
QL Agri 35	43.25±15.2	51.87±15.1	7.80±2.1	5.00±0.0	4.50±0.6	1.10±0.3	0.90±0.3	0.30±0.4
				Pepper host plant				
Onion	30.05±3.4	36.87±7.4	4.87±1.1	3.50±1.3	1.00±2.0	1.85±1.0	1.27±0.3	0.10±0.1
Bay tree	31.90±1.6	35.22±4.9	5.07±0.8	2.00±1.4	0.25±0.5	2.87±1.6	1.30±0.3	0.0±0.0
Eucalyptus	32.55±2.0	34.67±4.2	4.90±0.4	3.75±1.5	1.50±1.9	3.47±1.6	1.32±0.4	0.25±0.1
QL Agri 35	27.75±1.3	28.92±2.8	4.47±0.3	1.25±0.5	0.50±0.6	1.92±1.2	1.22±0.6	0.0±0.0

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling: 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984).

Table 4.13. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 2000 eggs/J2s nematode level with 100µL of treatments level in second experiment in tomatoes and pepper (Mean±SD)

2nd exp	**Tomato host plant**							
Treatments	**Plant height (cm)**	**Green part fresh weight (g)**	**Green part dry weight (g)**	**Gall indices**	**Egg mass indices**	**Root fresh weight (g)**	**Root dry weight (g)**	**Rf**
Onion	39.30±3.6	40.35±10.9	6.72±1.5	5.00±0.0	5.00±0.0	2.27±1.8	1.47±0.7	0.32±0.5
Bay tree	37.95±6.3	43.45±3.9	7.17±0.5	5.00±0.0	5.00±0.0	1.67±0.6	1.65±0.2	0.92±1.6
Eucalyptus	49.25±7.0	35.62±5.8	5.37±1.2	4.50±1.0	3.25±2.1	1.55±0.7	1.42±0.7	0.15±0.1
QL Agri 35	36.15±3.9	39.87±8.7	6.52±1.1	5.00±0.0	3.75±1.3	1.52±1.3	1.22±1.1	0.25±0.4
				Pepper host plant				
Onion	28.95±1.8	22.82±4.9	3.45±0.9	2.50±1.3	1.25±2.5	1.17±0.3	1.10±0.3	0.07±0.1
Bay tree	26.25±1.6	39.67±6.8	5.30±0.9	1.75±0.5	0.25±0.5	2.07±1.1	1.05±0.2	0.0±0.0
Eucalyptus	27.20±2.5	27.52±3.4	3.92±0.3	2.25±0.5	1.00±0.8	1.40±0.5	0.95±0.2	0.0±0.0
QL Agri 35	25.45±2.9	33.35±3.7	4.55±0.4	3.00±1.4	1.50±1.9	1.85±1.6	1.12±0.4	0.10±0.1

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls . Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984).

Table 4.14. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 2000 eggs/J2s nematode level with 250µL of treatments level in second experiment in tomatoes and pepper (Mean±SD)

2nd exp	**Tomato host plant**							
Treatments	**Plant height (cm)**	**Green part fresh weight (g)**	**Green part dry weight (g)**	**Gall indices**	**Egg mass indices**	**Root fresh weight (g)**	**Root dry weight (g)**	**Rf**
Onion	32.80±7.3	31.40±9.7	4.35±1.8	4.75±0.5	1.75±1.7	1.65±1.5	1.17±0.7	0.65±1.0
Bay tree	29.65±5.9	49.37±32.6	4.00±1.8	4.25±0.5	2.25±2.2	1.57±2.3	0.75±0.9	0.10±0.2
Eucalyptus	36.65±4.5	31.30±12.1	6.02±3.4	4.75±0.5	3.75±1.9	3.80±3.5	2.65±2.2	0.70±0.6
QL Agri 35	49.60±0.0	43.40±1.3	7.13±0.2	4.33±0.6	2.00±2.0	0.96±0.2	0.86±0.1	0.06±0.1
				Pepper host plant				
Onion	28.40±4.7	33.50±6.7	4.45±0.9	2.50±0.6	0.50±0.6	1.57±0.7	1.10±0.3	0.0±0.0
Bay tree	26.65±2.5	35.90±9.1	4.67±1.3	1.50±1.3	1.25±2.5	1.85±1.5	0.90±0.1	0.05±0.1
Eucalyptus	26.55±2.9	26.97±10.3	3.82±1.4	2.00±0.0	1.50±2.4	1.22±0.4	0.92±0.4	0.05±0.1
QL Agri 35	27.95±0.7	36.05±2.2	5.10±0.2	3.25±0.9	0.25±0.5	1.75±1.3	1.05±0.2	0.0±0.0

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984).

Table 4.15. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 0 eggs/J2s nematode level with 100µL of treatments level in second experiment in tomatoes and pepper (Mean±SD)

2nd exp	Tomato host plant							
Treatments	**Plant height (cm)**	**Green part fresh weight (g)**	**Green part dry weight (g)**	**Gall indices**	**Egg mass indices**	**Root fresh weight (g)**	**Root dry weight (g)**	**Rf**
Onion	47.70±12.6	48.85±2.80	7.85±0.5	0.0	0.0	2.70±0.6	2.20±0.6	0.0
Bay tree	34.40±7.3	52.85±38.8	5.30±2.4	0.0	0.0	2.35±2.3	1.10±0.7	0.0
Eucalyptus	36.10±5.8	34.25±10.8	6.70±3.2	0.0	0.0	2.80±2.5	2.35±2.2	0.0
QL Agri 35	30.10±4.4	26.25±16.0	4.85±4.2	0.0	0.0	2.70±3.1	1.55±1.6	0.0
Control	38.90±10.7	21.93±15.7	2.94±2.5	0.0	0.0	0.65±0.5	0.48±0.3	0.0
			Pepper host plant					
Onion	24.80±5.9	26.00±26	3.70±3.1	0.0	0.0	3.25±3.7	0.85±0.5	0.0
Bay tree	28.10±0.4	18.45±17.5	1.60±0.3	0.0	0.0	1.65±1.3	0.90±0.4	0.0
Eucalyptus	30.80±2.8	18.25±3.9	3.10±0.6	0.0	0.0	2.40±1.9	1.05±0.2	0.0
QL Agri 35	32.00±5.1	30.05±4.0	4.00±0.8	0.0	0.0	1.10±0.1	0.95±0.2	0.0
Control	31.26±2.9	29.75±10.1	4.21±1.4	0.0	0.0	3.47±1.8	1.00±0.4	0.0

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling: 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984).

Table 4.16. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf for 0 eggs/J2s nematode level with 250µL of treatments level in second experiment in tomatoes and pepper (Mean±SD)

2nd exp	Tomato host plant							
Treatments	**Plant height (cm)**	**Green part fresh weight (g)**	**Green part dry weight (g)**	**Gall indices**	**Egg mass indices**	**Root fresh weight (g)**	**Root dry weight (g)**	**Rf**
Onion	40.20±11.3	40.90±12.7	6.20±2.3	0.0	0.0	3.45±0.1	2.15±0.9	0.0
Bay tree	39.20±11.3	29.75±4.9	5.60±1.3	0.0	0.0	2.85±0.1	2.40±0.0	0.0
Eucalyptus	22.10±11.1	25.75±21.6	3.70±3.8	0.0	0.0	1.40±1.5	1.20±1.4	0.0
QL Agri 35	16.00±0.0	3.10±0.0	0.40±0.0	0.0	0.0	0.10±0.0	0.10±0.0	0.0
Control	38.90±10.7	21.93±15.5	2.94±2.5	0.0	0.0	0.65±0.5	0.48±0.3	0.0
	Pepper host plant							
Onion	32.20±0.6	22.25±4.2	3.35±0.9	0.0	0.0	1.25±1.0	0.85±0.3	0.0
Bay tree	30.00±1.1	24.40±10.5	3.05±1.2	0.0	0.0	0.60±0.1	0.55±0.1	0.0
Eucalyptus	28.90±1.0	21.10±9.5	3.10±1.4	0.0	0.0	2.90±3.1	1.00±0.6	0.0
QL Agri 35	34.00±0.0	39.30±0.0	5.90±0.0	0.0	0.0	2.00±0.0	1.20±0.0	0.0
Control	31.26±2.9	29.75±10.8	4.21±1.4	0.0	0.0	3.47±1.8	1.00±0.4	0.0

*Data are means of four replications where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling : 0-5 scale, where, 0: no galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al., 1984).

4.1.3. Comparison of two experiment

Data from t-test was shown in table 4.17. All sources of variances were significant between two experiments except for gall index. Plant height (34.6±8.9 cm) and green part fresh weight (34.9±12.3 g) were both greater at 2nd experiment. Green part dry weight was recorded the highest in the 2nd experiment (4.9±1.8 g). Root fresh weight (2.3±1.9 g) and root dry weight were recorded the highest in 1st experiment (1.6±1.1 g). Both EMI (1.4±1.8) and Rf (0.1±0.2) were low in 2nd experiment (Table 4.17).

Table 4.17. Data are means and t-test between two experiments for all source of variance (Mean±SD).

Parameter	Experiment	Mean	Sig. (2-tailed)	Group
Plant height	1st exp	30.9±4.3	0.000*	b
	2nd exp	34.6±8.9	0.000*	a
Green part fresh weight	1st exp	27.3±13.6	0.000*	b
	2nd exp	34.9±12.3	0.000*	a
Green part dry weight	1st exp	4.4±2.4	0.027*	b
	2nd exp	4.9±1.8	0.024*	a
Root fresh weight	1st exp	2.3±1.9	0.000*	a
	2nd exp	1.7±1.3	0.000*	b
Root dry weight	1st exp	1.6±1.1	0.000*	a
	2nd exp	1.1±0.5	0.000*	b
Gall index	1st exp	2.6±2.0	0.981	a
	2nd exp	2.6±1.9	0.981	a
Egg mass index	1st exp	3.2±2.1	0.000*	a
	2nd exp	1.4±1.8	0.000*	b
Reproduction factor	1st exp	0.8±1.3	0.000*	a
	2nd exp	0.1±0.2	0.000*	b

*Significant at ($P \leq 0.05$).

4.2. Discussion

Sustainable agriculture faces a major and growing challenge from nematodes, especially in intensively cultivated greenhouse production (Liu et al., 2006). The root-knot nematode, M. incognita, a destructive disease of many crops in tropical and subtropical regions has a very wide host rang including crops and weed but not all are equally good at supporting nematode reproduction. Nematicides are usually expensive. They may lead to environmental pollution and their toxic residues may accumulate in edible plant products. Therefore, utilizing essential oils with methods of nematode control will shift the farmer from the concept of control to the concept of management.

The use of various parts of indigenous plants as botanical extracts has become important in disease management in recent years following the environmental hazard caused by chemical control measures (Olowe, 1992; Mangala and Mauria. 2006). Other researchers (Puri, 1999; Oka et al., 2000; Afouda et al., 2008) have reported successes in using various plant extracts in nematode management. Although in our experiment for using all of the plant extraction is the similar result and observed different effects on two species of plant grown tomato and pepper.

Our results showed that the Mustard essential oil was effect to the both plants and wilted the plant for both experiments in each ratio. But Mustard oil (Brassica campestres L.) also contains high concentrations of allylisothiocyanate, which may explain its efficacy in the control of M. incognita and M. javanica (Chitwood, 2002). However, the quantity of nematicidal substances produced differs from one brassica species to another, which could affect the results of studies. Zasada and Ferris (2004) reported differences in concentrations of isothiocyanate among brassica species and observed that the glucosinolates in broccoli were insufficient to control nematodes, in contrast to the results observed for white mustard (Brassica hirta Moench). The same authors attributed the absence of nematode control to the high volatilization of its main component, allylisothiocyanate.

First experiment results showed that there no significant difference between treatment with control which it means that all treatments had a good effect to reduce the nematodes damage, and the ranks from more affect to low affect, the Eucalyptus essential oils for all doses nematodes and treatments was placed one, the second Onion, third QL Agri 35, and last one Bay tree, respectively. Elyousr et al., (2009) reported at the

glasshouse study neem extract and essential oils treatments including Eucalyptus were more effective in reducing population numbers of the M. incognita in soil and root gall index compared to other treatments.

Second experiment results showed that the Bay tree essential oils had more effective than other treatments for both nematode inoculums level with both ratios of treatments, the second QL Agri 35, third Eucalyptus and last one Onion, respectively.

The nematicidal effect of the tested extracts may possibly be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock et al., 1989. Trifone and Atanasov, 2009). The mechanisms of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Konstantopoulou et al., 1994).

When the oximecarbamate was applied on nematodes, delay in the reproduction of nematodes was due to the dislocation of the feeding process (Evans, 1973). So it was concluded when the doses of the bio-products were used, they disrupted the nervous system of the nematodes and dislocated the nematodes from feeding sites and checked the invasion. According to (Kondrollochis, 1972) the feeding process of the nematodes is inhibited which ultimately results in to decrease in reproduction. Regarding the curative effect of bio-products on nematodes it was noted that they were absorbed by the roots of the tomato, pepper and they became able to reduce the development of the nematodes which resulted in the reduction in gall and egg mass. In another hand they reduce the reproduction rate and the activity of J2 in the soil. Akhtar and Mahmood (1994) used the oil cakes and neem extracts and they observed that suppression of root-knot development was greater in pre-infected seedlings than in inoculated after dip treatment. The systemic nematicides absorbed by roots mainly prevent the invasion of nematodes in to the roots and to lesser degree the feeding and development of nematodes that have already invaded the roots (Wright, 1981; Hague and Gowen, 1987). Schoonhoven (1987) observed that after absorption these products are translocated to other parts of plants through xylem and phloem but their effectiveness was reduced. Evans (1973) studied the mode of action of nematicides, he described in detail paralysis, narcosis and detoxification in nematodes. The mode of action of azadirachtin (a neem refined product) is like alkayl halides because

hyperactivities were observed in nematodes exposed to low concentration of neem products (Javed, 2007). These products are absorbed by the plants and when nematodes come in contact with them for feeding so they inhibit or delay their development. Maqbool and Abid (1991) observed the roots dip treatment in leaf extracts of different plants including neems, the nematode damaging effects were masked and treatments improved the plant growth

Sasser et al., (1982) in an experiment of root-knot nematode management in tobacco concluded that avermectin B1 was as effective as ethoprop, fenamiphos, aldicarb, oxamyl and carbofuran. The avermectins would minimize the contamination problem of ground water because they have the quality to bind tightly to soil (Clark et al., 1994).

Mallek et al., (2007) reported that *Allium* spp. residues inhibited germination of weeds and that the effects were closely tied to the concentration. To establish the likelihood of inhibition to tomato growth, we tested different doses of raw garlic straw for their effects not only on *M. incognita* but also on tomato growth in the pot experiment. Comparison of two experiment showed that there was a significant difference between two experiments. This difference maybe because having a different conducting time, fluctuations in temperature, humidity in the greenhouse and the sensitivity of practical work.

It is worth to mentioned that the use of plant extracts or essential oils in the nematodes control has a number of advantages compared with synthetic pesticides, such as the possibility of producing new compounds that pathogens are unable to neutralize, and the fact that they are of lower toxicity, rapidly biodegradable, have wide ranging action and are derived from renewable resources. Therefore, this study indicates the potential of using plant chemical extracts in controlling nematodes and require for additional studies on different plant species, chemical extracts, application times and concentrations.

CONCLUSION

Root-knot nematodes*, Meloidogyne incognita* are polyphagous plant parasites which parasitize different high plant species. Tomatoes and pepper are the most popular and widely used vegetables in the world. The crops have developed into a huge number of cultivated types suitable to different environments, method of production, and food uses**.**

Since root-knot nematodes are of great economic importance, so much attention has been paid to their control. There has been an increase in the intensity of search for efficient, ecologically sound and safe control methods. Among various control measures, identification, exploitation and utilization of plant essential oil is one of the most ecologically safe and economically viable strategies for management of the root-knot nematodes. Bio-nematicides could be a good choice for reducing the losses that nematodes cause and consequently, increase yield as well as fruit quality of crops.

Our study has showed that all plant essential oils such as Eucalyptus, Onion, Bay tree and QL Agri 35 were affected to the root-knot nematode inoculums low and high in the soil and plant, but Mustard showed a great phytotoxicity to all plants for all levels indicating that it was not good choice in nematode management. Additionally, there were no significant differences among treatment levels.

Date from first trail demonstrated that eucalyptus was more effective in reducing egg mass index both plants than other plant essential oils tested. Also, eucalyptus increased the plant height, green dry weight, root fresh weight and root dry weight in both pepper and tomatoes. The green fresh weight was recorded greater and number of juveniles was significantly low on QL Agri 35. Data From the second trail elucidate that eucalyptus was more effective in increasing the plant height, root fresh weight and root dry weight for both tomato and pepper. In another hand, green fresh weight and dry weight was increased ,however, egg mass index was reduced on QL Agri 35. It was evident that eucalyptus essential oil deserves a serious consideration for inclusion into the nematode management tactics.

The plant essential oils have the potential for use in nematode control. Therefore they are recommended to farmers for use bio-nematicide instead of synthetic nematicide due to environmental hazard and safety. However, farmers should apply low volume of plant extraction at the beginning of transplanting of seedlings. Further field or greenhouse studies in different conditions and locations need to be conducted to see the possible

implementation opportunities of these essential oils finding to farmers in vegetable growing areas.

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