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**KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES**

**BIOLOGICAL CONTROL OF ROOT-KNOT
NEMATODE, *Meloidogyne javanica*, BY FUNGI ON
CUCUMBERS AND PEPPERS IN GREENHOUSE**

PSHTIWAN HAMID AHMED AHMED

MASTER THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

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October 2016**

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the degree of Master of Science in**

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HIYAR VE BIBER BITKILERİNDE KÖK-UR NEMATODU, *Meloidogyne javanica*'nın FUNGUSLAR TARAFINDAN SERA KOŞULLARINDA BİYOLOJİK MÜCADELESİ
(YÜKSEK LİSANS TEZİ)

PSHTIWAN HAMİD AHMED AHMED

ÖZET

Bu çalışmanın amacı hıyar ve biber bitkilerinde iki entomopatojen fungus türü (*Beauveria bassiana* ve *Isaria tenuipes*) ile bir kontrol (muamelesiz, nematodlu)'ün *M. javanica* popülasyonları üzerindeki etkileri araştırılmıştır. Çalışma tesadüfî bloklar desenine göre iki nematod seviyesi (bitki başına 1000 ve 2000 yumurta veya J2) ile iki fungus konsantrasyonu (bitki başına 1000 ve 2000 uL) muameleleri halinde beş tekerrürlü olarak kurulmuştur. Farklı nematod inokulasyon düzeylerinin kök urlanma indeksleri üzerinde belirgin etkisi görülmüştür. Çalışmada en yüksek bitki boyu *Beauveria bassiana*'nın uygulandığı saksılarda görülmüş olup, bunlar sırası ile hıyarda 120.04 ± 23.3 cm ve biberde 50.24 ± 7.2 cm olmuştur. Her iki fungusun iki konsantrasyonu da her iki bitkide de nematod gal ve yumurta paketi indekslerini kontrol bitkilerine göre belirgin şekilde düşürdüğü gözlenmiştir ve uygulandığı bitkilerde kök-ur nematodu kontrolü için en iyi sonuçları verebildiği görülmüştür. *Beauveria bassiana* ve *Isaria tenuipes* funguslarının nematodların biyolojik mücadele taktikleri içinde yer alması gerektiği düşünülmektedir.

Anahtar kelimeler: Biber, Biyolojik Mücadele, Hıyar, Entomopatojenik Fungus *Meloidogyne javanica*.

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**BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE, *Meloidogyne javanica*,
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(M.Sc. THESIS)

PSHTIWAN HAMID AHMED AHMED

ABSTRACT

Objective this study was to determine the effects of two entomopathogenic fungi (*Beauveria bassiana* and *Isaria tenuipes*) on root-knot nematode *Meloidogyne javanica* populations on cucumber and pepper in a greenhouse. Two experiments were designed as completely randomized block with two nematode inoculums (1000 and 2000 eggs or J2/plant) and two rates of each of two fungi (1000 and 2000 μ L/plant) with untreated controls. Significant effect of different nematode inoculum levels on root galling index were detected. In the study the greatest plant heights were found in *Beauveria bassiana* treated pots was 120.04 ± 23.3 cm for cucumber and 50.24 ± 7.2 cm for pepper. For both plants varieties, application of both rates of both fungi reduced the number of galls and egg masses significantly as compared to the controls plants and gave the best results for root-knot nematode control. *Beauveria bassiana* and *Isaria tenuipes* are considered two important biological agents that can be implemented into the nematode biological control tactics.

Key words: Biological Control, Cucumber, Entomopathogenic Fungi, *Meloidogyne javanica*, pepper.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	: Percent
°C	: Celsius temperature
μL	: Micro litter
μm	: Micrometer
cm	: Centimeter
EMI	: Egg mass index
g	: Gram
GI	: Galling index
h	: Hour
IPM	: Integrated pest management
J2	: Second stage juveniles
L	: Treatment level
mg/kg	: Milligram per kilogram
mL	: Milliliter
mm	: Millimeter
Mm	: Millimeter
N	: Nematode level
NaOCl	: Sodium hypochlorite
PDA	: Potato Dextrose Agar
RKN	: Root-knot nematode
μL/plant	: Micro litter per plant

1. INTRODUCTION

Among plant-parasitic nematodes, the root-knot nematodes (*Meloidogyne* spp.) are the most economically important group and distributed worldwide as obligate parasites of the roots, tubers and corms of thousands of plant species, resulting in devastating adverse effects on the quality and yield of related crops. Their potential host range encompasses more than 2000 plant species across to world. Among the many genera of nematodes having some economic impact, *Meloidogyne* spp. are responsible for a large part of an annual 100 billion USD loss (Sasser et al., 1987). *Meloidogyne incognita* occurs in every temperate and tropical region, and it is considered possibly one of the most damaging crop pathogen in the world (Blok and Trudgill, 2001). The control of nematodes is often done by the combination of several pest management strategies. Cultural control is widely practiced, but rotation is of limited value for nematodes with a host range as wide as that of *Meloidogyne* spp. (Trudgill, 1997). Chemical control tactics are often applied in RKN management however, the withdrawal of many nematicides and soil fumigants (such as Methyl Bromide) from agricultural production areas raised a need for alternative strategies. Resistant cultivars were proven commercially successful as an alternative in the control of the most damaging species of *Meloidogyne* on tomato and pepper. However occurring virulent biotypes of plant parasitic nematodes challenge these resistant cultivars.

The differential host tests are common ways used for differentiating four common species (*M. hapla*, *M. arenaria*, *M. incognita* and *M. javanica*) of root knot nematodes. The various hosts (pepper, *Mi*-resistant tobacco, cotton, watermelon, tomato and peanut) have a predictable respond to these four species and its races. Host differential tests can be used to assist in species identification as well as diagnosing mixed species (Sasser and Hartman, 1985).

Nutrient and water uptake are substantially reduced because of the damaged on the root system, resulting weak and poor-yielding plants. During infection, RKN establish and maintain an intimate relationship with their host. The nematode hatch as second-stage juveniles and invade the root in the zone of elongation. They migrate intercellular, first to the root apex and then to the vascular cylinder, where permanent feeding sites are

established. The second stage juveniles then undergo three molts to develop into adults. The saccate females remain sedentary, producing large egg masses.

Nematode growth and reproduction depend on the establishment of specialized feeding sites within the host root. Consequently, plant parasitic nematodes usually do not kill the host cells. Instead, they induce a dedifferentiation process that leads to the formation of multinucleated feeding cells, named giant cells. Giant cell formation is the result of repeated nuclear divisions of the initial feeding cell without cytokinesis. Each RKN triggers the development of five to seven giant cells, each containing as many as 100 nuclei, which would go to reduplication. These cells appear to be metabolically active, with a very dense cytoplasm containing several mitochondria, plastids, ribosomes, well-developed Golgi apparatus and smooth endoplasmic reticulum, generally organized in swirls. The central vacuole disappears and many small vacuoles appear. In addition, cell wall ingrowths typical of transfer cells develop. These cell wall ingrowths are thought to enhance solute uptake from the vascular system. Associated with giant cell formation, hyperplasia (increase cells in number) and hypertrophy (increase cells in size) of the surrounding cortical cells lead to the formation of the typical root symptoms that called galls (Williamson and Hussey, 1996). Natural host resistance against *Meloidogyne* spp. were found in several wild plant species and shown to reduce or suppress nematode development and reproduction in host (Roberts, 1995). Some dominant resistance genes were identified and mapped on plant chromosomes, e.g. the tomato Mi, Mi-3 and Mi-9 genes (Yaghoobi et al., 1995, Ammiraju et al., 2003; Kaloshian et al., 1998;), the pepper Me3 gene (Djian-Caporalino et al., 2001) or the peanut Mae and Mag genes (Garcia et al., 1996). One of the best characterized nematode resistance genes is Mi, originally found in the wild species (*Lycopersicon peruvianum*), which confers resistance to several RKN species in tomato. The *Mi* gene were cloned, and shown to belong to the NBS-LRR class of genes which also includes genes conferring resistance to viruses, bacteria and fungi (Hwang et al., 2000; Milligan et al., 1998). Moreover, the first element of the *Mi* resistance signaling pathway was identified recently (Martinez de Ilarduya et al., 2001). All the commercially available tomato cultivars resistant to RKN carry the *Mi* gene, and the emergence of nematode biotypes ‘breaking’ this gene is a serious threat to their future use. Recent investigations on the molecular basis of the plant-RKN interaction were focused on the parthenogenesis species *M. incognita*, *M. arenaria* and *M. javanica*. The characterization of *Meloidogyne* secretions with virulence factors of

nematode responsive plant genes will lead to a better understanding of the molecular events and regulatory mechanisms involved in plant parasitism by RKN. It also should allow the development of target-specific strategies to limit crop damage by these pathogens.

Product secreted by the nematode are assumed to play key roles during in their pathogenicity. Such secreted products could be involved in the invasion of the root tissues or the differentiation of the root cells into specialized feeding cells (Grundler and Wyss, 1994; Hussey, 1989). Such signals may be produced at distance from the differentiating feeding site or after injection of secretory compounds in the plant cell. Electron microscopy observations showed that for feeding, the nematode perforates the cell wall with its stylet. The stylet reaches the plasma membrane of the cells and several feeding tubes are formed, probably issued from the assembly of nematode secreted proteins. The feeding tubes, closely associated with endoplasmic reticulum, may allow the puncturing of nutrients from distal parts of the feeding cell or act as molecular sieves to prevent the obstruction of the stylet (Mims and Hussey, 1991). Several structures of the nematode can be a source of secreted molecules involved in the plant-nematode interaction. The main secretory organs are the amphids, cephalic sensilla and phasmids, which are secretory chemoreceptors, the esophageal glands, the excretory system and the rectum, which produces the gelatinous matrix protecting the eggs from predators and dehydration (Eisenback, 1985). Moreover, the cuticle of the nematode selectively regulates the flow of fluids through the body wall and could be a source of secreted compounds recognized as signal molecules by the plant. Most efforts in analyzing nematode secretions have focused on proteins secreted from amphids and oesophageal glands. Amphids are two sensory organs located bilaterally on the cephalic region of the nematode. They are composed of several cells including nerve cells and secretory cells. Amphids are in close contact with the plant cells that are differentiating in feeding cells. Several glycoproteins secreted by the amphids were isolated, some of them being involved in the perception of signals from the environment (Stewart et al., 1993). The oesophageal glands are specialized cells able to export secretions through the stylet. The activity of the oesophageal glands were shown to vary during parasitism (Hussey and Mims, 1990). Whereas the subventral glands are large and filled with vesicles during the migratory phase of parasitism, the dorsal gland only starts the active production of secretions after sedentarization. While the nematode starts feeding, the subventral glands become less

active. Therefore, subventral and dorsal glands seem to have different roles during parasitism.

Chemical compounds with nematicidal properties were mostly extracted from a range of plants (Chitwood; 1992; 1993; 2002; Ferraz and de Freitas, 2004). Various bio-nematicides of a plant in the nature continue to be evaluated, and are also beginning to work their way on to the markets (Haydock et al., 2006). Some phytochemicals have hostile, suppressive effects on plant-parasitic nematodes, while others are dangerous and toxic (Viaene et al., 2006). Alternative control tactics are required due to the potential removal of chemical nematicides from the market because of toxicity to the human health and environment. Induced resistance, naturally occurring control agents, organic and inorganic soil amendments, biological control, stopping of transgenic plants and their host will be a part of integrated management of plant-parasitic nematodes in future (Oka et al., 2000).

Biological control agents can effect in the regulation of plant parasitic nematode populations, and numerous organisms including fungi, bacteria, viruses, nematodes and other invertebrates have antagonistic activity against plant parasitic nematodes. Various aspects of biological control of nematodes using microbial control agents was already reviewed.

The developmental process of progressing biological agents includes the isolation and identification of microbial agents associated with plant parasitic nematodes especially in suppressive soils (soils that completely suppresses nematode reproduction); examination of their potential ability in controlling nematodes; changing the soil environment in favor of antagonistic agents; understanding the mechanisms of parasitism and pathogenicity; and development of commercial product. Investigating nematode-suppressing soil demonstrated that their controlling activity is due to egg-parasitizing fungi, generalized fungal antagonists, mutualistic fungal endophytes, rhizobacteria and obligate parasitic bacteria. Comprehending the mechanisms of suppressiveness can be useful in plant-parasitic nematode control by helping in manipulating these mechanisms. Lots of natural enemies attack nematodes and decrease their populations, but the number of organism which could be employed for bio-control is restricted. In other words, many soil types all around the world show biological control activity but their effect on nematodes can range from insignificant to complete

suppression. In this section the biological control of plant parasitic nematodes by fungal agents will be emphasized according to recent research progresses.

Numerous species of entomopathogenic fungi from the nature are proven to be good biological agents and are in a practice across to the world. *Beauveria bassiana* is one of fungi which plays the main role in tropical forests (Aung et al., 2008), in Canada (Widden and Parkinson, 1979), and in North of the Arctic Circle. Entomopathogenic fungi such as *Beauveria Bassiana*, *Metarhizium anisopliae*, and *Tolypocladium cylindrosporum* were found in different part of world including Norway (Klingen et al., 2002), and in Finland (Vänninen, 1995). Although a large number of entomopathogenic fungi were detected in Arctic Greenland (Eilenberg et al. 2007), some others fungi belongs to the genus *Lecanicillium*, *Beauveria*, *Conidiobolus* and *Neozygites* were reported on some other Antarctic areas without their host (Bridge et al. 1997).

Fungal biological control is an exciting and rapidly developing research area and there is growing attention in the exploitation of fungi for the control of nematodes. Interaction between nematodes and pathogenic fungi were subjected to widespread mycological studies. Different aspects of fungal biological control of nematodes were classified fungi into three large groups: nematophagous fungi, saprophagous fungi, and endophytic fungi. All nematodes are consisting parasitic and antagonistic fungi as: (i) nematophagous fungi and (ii) endophytic fungi.

2. LITERATURE REVIEW

2.1. History

Cornu first described the name *Anguillula marioni* Cornu for root-knot nematodes, after observing root galls on *Onobrychis sativus* Lam. Subsequently, root-knot nematodes were classified in the genera *Heterodera* or *Anguillula*. In 1884, Müller classified root-knot nematodes as *H. radicola* and in 1887 the type species *Meloidogyne exigua* was described by Göldi. In 1932, Goodey reclassified root-knot nematodes as *H. marioni* (Müller) Goodey. Differences in host responses led Chitwood to redescribe *Meloidogyne exigua* Göldi, *M. incognita* (Kofoid and White, 1919) Chitwood, *M. javanica* (Treub, 1885) Chitwood, *M. arenaria* (Neal, 1889) Chitwood, *M. hapla* Chitwood and *M. incognita acrita* Chitwood as a new species (Christie, 1959). In his description of *M. hapla*, Chitwood cited *Abelia grandiflora* as a host (Bernard and Witte, 1987). Generally, root-knot nematodes are considered to be the third plant parasitic nematode group discovered after *Anguina tritici* and *Heterodera schachtii*. *Meloidogyne* spp. were detected for the first time by Berkeley in 1855 on the galls on the roots of greenhouse-grown cucumbers in England (Hartman and Sasser, 1985).

Chitwood also gave a general description of the genus *Meloidogyne* and differentiated it from the genus *Heterodera* (Hirschmann, 1985). Chitwood's diagnoses of the redescribed and newly described species were based on examinations of morphological features and morphometrics from all life stages of the evaluated species (Eisenback, 1985). These features included the perineal pattern, stylet morphology, and distance from the base of the stylet knobs to the dorsal esophageal gland opening (DEGO) (Christie, 1959). To date, more than 80 *Meloidogyne* spp. have been described (Randig et al., 2002).

2.2. Biology of RKN

Duration and the success of a nematode biology is affected by some factors such as food, water and temperature. These factors determine habitats occupied by nematodes for adaptations and survival. The root-knot nematode life cycle is demonstrated in Figure 2.1. Females lay eggs as a mass into gelatinous matrix composed of a glycoprotein matrix secreted by rectal glands of female. Gelatinous Matrix keeps the eggs together and protects them from unfavorable environmental conditions and predation. It was also demonstrated

that the matrix contain antimicrobial properties (Orion and Kritzman, 1991). The egg masses are usually formed on the surface of galls, although sometimes may also be embedded within the gall tissue. The egg masses are initially soft, sticky and hyaline but becomes firmer and darker later. It is known that only limited analysis of the glycoproteins (Sharon and Spiegel, 1993) of the gelatinous matrix are known.

As of all plant parasitic nematodes, root knot nematodes the egg undergoes to embryogenesis and first molt and the first-stage juvenile occur in the egg. After first juvenile stage, nematode molts second time and hatches as an infective second stage juvenile (J2). Hatch of the J2 from the egg. Nematode hatching is primarily dependent on temperature, sufficient moisture and root diffusate. Usually, J2 hatches during the conditions are favorable for movement and host location. The ability of *Meloidogyne* to survive is enhanced by several physiological and biochemical adaptations, including delayed embryogenesis, quiescence and diapause, and lipid reserves that prolong viability until the J2 finds and infects a host. Since J2 moves very small distances in the soil, it is vulnerable and needs to locate a host as rapidly as possible. J2s are attracted to roots, and there is evidence that susceptible hosts are more attractive as compared to resistant ones.

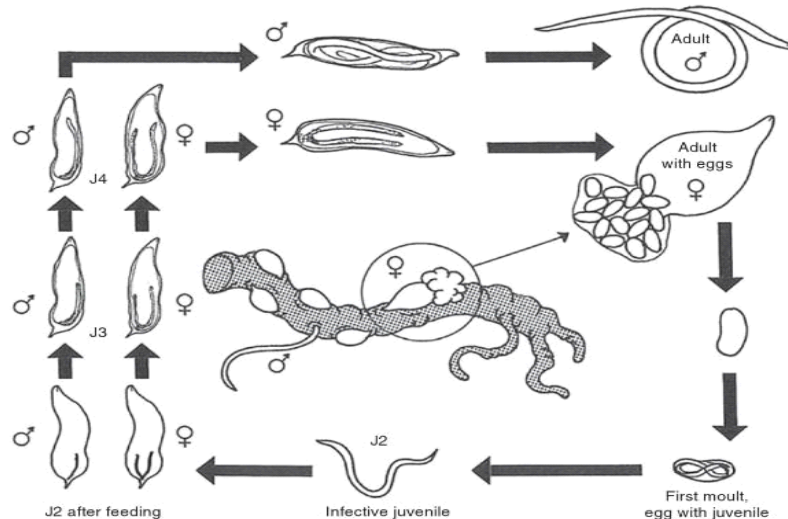


Figure 2. 1. Diagram of the life cycle of the root-knot nematode, *Meloidogyne*. J2: second-stage juvenile; J3: third-stage juvenile; J4: fourth-stage Juvenile. (Karssen and Moans, 2006.)

The J2 start feeding after invading the root, usually on the root tip, and moved through the root to initiate and develop a permanent feeding site. The feeding of the J2 on

xylem and phloem cells induces these cells to differentiate into specialized feeding cells called giant cells. Once a giant cell is initiated, the nematode becomes sedentary and enlarges into a 'sausage' shape. After establishment into the host roots, the J2 molts to the third (J3), then to the fourth-stage juvenile (J4), and finally to the adult stage. The combined time for the J3 and J4 stages is much shorter than for the J2 or the adult, typically 4-6 days. It was reported that J3 and J4 lack a functional stylet, consequently and they are not able to feed. Males, if present, are vermiform and not parasitic. Presence of males in parthenogenetic species may sometimes depend on unfavorable conditions of female development including high population densities and limitation of food supply.

Development and formation of the giant cell is the subject of continuing investigation, facilitated by molecular techniques with the incentive of developing control strategies based on preventing development or formation giant cell. These specialized nursing sites are remarkable for their complexity. They are greatly enlarged from typical phloem and xylem parenchyma, or cortical cells. Volumes of these cells can reach nearly up to 100-fold greater than normal root cells. The giant cells are functionally similar to syncytia induced by other sedentary plant-parasitic nematode females, but are distinct in their development. Like syncytia, they are functional transfer cells, based on morphology (Jones and Northcote, 1972) and because photosynthates pass through the giant cells before being ingested by the nematodes (Bird and Loveys, 1975). Unlike syncytia, each giant cell develops from a single initial cell rather than by coalescence of several adjacent cells. The giant cells are not only multinucleate, but also polyploid, some with eight-fold increase in chromosome number (Huang and Maggenti, 1969; Wiggers et al, 1990). Thus, each giant cell may have up to a 600-fold increase in copy number of each plant gene. Several studies stated that the effects of nematode infection on gene expression, with a variety of genes being up regulated (Gheysen and Jones, 2006) and probably a greater number down regulated. A few studies on gene expression in the giant cells revealed that the mRNA for some genes can be present in giant cells in infected cells as compared to non-infected root cells (Ramsey et al, 2004; He et al, 2005). Such studies demonstrate that the giant cells induced by *Meloidogyne* spp. are unique examples of parasites-host interaction and abnormal development. A recently study by Huang et al (2006) revealed that characterization of parasitism directly depend on some genes of nematodes which are able alter plant growth and development for the benefit of the parasite.

The major nematode dispersal is mostly taken place through infested soil, infested transplants, pests, parasites, pathogens, human activities and land cultivation tools (Lehman et al. 1994). Plant parasitic nematodes, particularly, are able to migrate only limited distances by their own active movement. Studies observed that *Meloidogyne javanica* were able to move horizontally and vertically for 50 cm in 3 days and 75 cm in 9 days, respectively. Another study done with columns containing field soils showed that *M. chitwoodi* moved vertically upwards 45 cm in 4 days and 60 cm in 10 days. The same study also stated that *Meloidogyne hapla* migrated upward 25 cm in 6 days and *M. arenaria* migrated vertically 120 cm to host roots in one month in sandy soils of Florida, USA.

Root knot nematodes have developed very interesting and unusual ways of maintaining populations through asexual reproduction. Reproduction mode of *Meloidogyne* species is called parthenogenesis. In parthenogenic nematodes, males do not occur or may rarely occur. There are three types of parthenogenesis namely apomixis, facultative apomixis and automixis. Firstly, apomixis is the most common type of asexual reproduction and gamete production is taken place through mitosis. In mitotic reproduction, the diploid chromosomes replicate themselves in the oocytes in the ovaries of the females and maintain $2n$ through duplication of the chromosomes. The nucleus splits into two identical daughter nuclei without the exchange of genetic material.

The second type of asexual reproduction, is called facultative apomixes which is also mitotic. Males can be produced but generally only in response to environmental stress. Males of *Meloidogyne* are generally non-functional but in rare instances may be capable of providing genetic exchange to widen gene pool. In some species of plant-parasitic nematodes, including *Meloidogyne*, adverse conditions, including overcrowding, reduction in food sources through plant stress, temperature extremes and host susceptibility, is considered to play a role in change in male development and densities (Triantaphyllou, 1973). Hormonal signals from either the nematodes or the plant may control the genetic switch to males in species in which males occur only under adverse conditions (Papadopoulou and Triantaphyllou, 1982).

The third type of parthenogenesis is called automixis. In this mode, females produce oocytes that divide in half and each daughter oocyte has a haploid (n) number of chromosomes. Through meiotic recombination, one oocyte, called the polar body (n), and

another oocyte, called a daughter cell (n), fuse to form the zygote (2n). This type of reproduction was described for many plant-parasitic nematodes including *Meloidogyne hapla* where males are absent .

2. 3. Identification

Accurate identification of root-knot nematodes is crucial for effective disease control and depends on rapid and accurate classification of the pathogens such root-knot nematodes involved so that appropriate control measures may be taken. In addition, sound decisions regarding quarantine of imported and exported plant material and commodities also demand timely and accurate diagnostics. Plant parasitic nematodes are important factors affecting crop growth and yield in all agricultural production zones. Root-knot nematodes, *Meloidogyne spp.* are the most important throughout the world, especially in the tropics and subtropics where they cause a high reduction in the yield of economic crops especially vegetables (Luc et al. 1990). Severe attack by root-knot nematodes can be prevented by successful identification of many species (Mai, 1985; Netscher and Sikora, 1990).

Meloidogyne spp. can be identified by several methods including morphological, morphometrics, biochemical, molecular and host differential tests. Morphological differences in intra and inter specific species make RKN identification fairly difficult. Most common four species of *Meloidogyne* can be diagnosed by esterase phenotypes (Einsenback, 1982; Hartman and Sasser, 1985). Others methods such as host differential tests can be used particularly to identify the species and races of the same species. Poor morphometric description of nematode species or high similarity between well-known species such as *M. javanica*, *M. incognita*, *M. hapla*, and *M. arenaria* make identification of this group highly difficult (Hartman and Sasser, 1985).

2.3.1 Morphological identification

After their first discovery on the roots of cucumber in an English glasshouse (Berkeley, 1855), the root-knot nematodes were soon recognized as important pathogens on numerous host plants all around the world. Difficulties in identification of root-knot nematodes derive perhaps the results from their morphological variations between the populations within the same species (Levin, 2005). Identification features of root-knot nematodes consists of female stylets, male heads and stylets, and second-stage juvenile (J2)

heads and stylets. However, female perineal patterns became a dominant diagnostic tool of the most of four common *Meloidogyne* species. Perineal pattern presents many benefits that makes worthy diagnostic character. With some minor variations, perineal patterns are considered to be constant between populations and individuals. The perineal patterns of *Meloidogyne javanica* are unique because they contain lateral ridges that divide the dorsal and ventral ridges. It is known that *M. javanica* also sustains a perineal patterns which has a smooth to slightly wavy striae with some of which bending toward the vulval edges. Dorsal arch of *M. javanica* is low and rounded to high and squarish and often contains a whorl in the tail terminal area (Eisenback, 1985).

Females of *Meloidogyne* spp. can be easily recognized by their unique pear shape. The length and width of adult females are approximately 0.44 mm to 1.30 mm in and 0.32 mm to 0.70 respectively. Further, in the most *Meloidogyne* species the body of an adult female is symmetrical with a 'neck' region a little twisted besides, its white body is clearly transparent where the esophageal bulb, excretory pore and stylet, usually are visible. During morphological identification most of the *Meloidogyne* species have characteristic of perineal patterns on the posterior of the adult female. Adult males are usually vermiform with slender bodies rounded posteriorly and tapering interiorly, they have spicules protruding through the cloaca which combine both functions of the anus and sex opening and also have developed stylets, and are unlike females and juvenile stages, females have well-developed esophageal glands for feeding, but males do not feed because are lack with well-developed feeding system (Eisenback et al. 1981). Female of *M. javanica* are pear-shaped with no posterior terminal protuberance. Stylets of adult females measured about 14–18 mm long with basal knobs ovoid and offset.

Besides morphological characters, methods such as biochemical analyses, especially esterase phenotypes of young, egg-laying females, in coincidence with morphological and morphometric examinations, allow for specific, accurate diagnoses (Hussey, 1985).

2.3.2. Isozyme phenotypes

Isozymes phenotypes were applied for determination of routines diagnostic in different species of *Meloidogyne* despite that they are limited to the female adult stage of development. Isozyme electrophoretic profiles, often using esterase and malate

dehydrogenase, have been established for a number of species and can provide a useful, routine diagnostic test, particularly for morphologically variable species like *M. incognita* and *M. javanica*. The adult of female stage suitable stage since it is related with the expression of a specific gene product (Muturi et al. 2003). The process is quick and easy to represent and reference standards generally *M. javanica*, are certain and accurate. It is not difficult for identifying a different *Meloidogyne* spp. which are common in restrictions of diagnostic tests based completely on isozyme phenotypes that includes the capacity loss to utilize from the other development stages (J2 and eggs) and a shortage of many range levels to compare result with it (Molinari et al., 2005; Wesemael et al. 2011). Sometimes, it is complicated to identify and differentiate band sizes between the various species at the time of determinations. In addition to using esterase phenotypes, malate dehydrogenase (Mdh) is also known to be used to separate *M. hapla* from *M. incognita*, *M. arenaria* and *M. javanica*. On the other hand, glutamate dehydrogenase can separate *M. javanica* from *M. incognita*, *M. hapla* from *M. arenaria*. Because of these restrictions, in surveys aimed for identifying *Meloidogyne* spp., the use of isozymes can be applied as an initial step of identification reported by (Esbenshade and Triantaphyllou, 1990; Muturi et al. 2003). Some species, such as *M. arenaria*, show several different profiles, although this may be an indication of the existence of cryptic species. Although isozyme electrophoresis is perhaps the current diagnostic method of choice, with profiles accompanying the description of many new species. However, it is likely that PCR-based methodologies will soon replace esterase phenotypes for many applications with finer resolution, particularly in intraspecific variations of RKN species.

2.4. Management

The entire crop sequence must be considered for complete nematode management strategy. Predictions can be made with acceptable accuracy of the dynamics of target nematodes considered throughout the term of all possible options for a given field. Information on the relationship between the application rates of any control treatments and the mortality of the target nematode should carefully considered. The main goal to control *Meloidogyne* spp. during production is to keep the crop from being attacked. Protecting the crop from the first and secondary infections will be able to result to reach maximum yield of crops. Most current control practices fall into four categories: chemical, cultural, biological

and host plant resistance. These control tactics are either practiced as a single or in combination of more than one to get effective results (Coyne et al. 2006); (Norshie et al. 2011).

2.4.1. Biological control

Biological control is an attractive alternative to chemical control. It offers exciting possibilities for the future management of nematodes. Microbial pathogens, endophytes and antagonists are extremely important in the regulation of plant-parasitic nematode populations, irrespective of agro ecosystems. Biological control of soil-borne pathogens by introduced microorganisms has been studied for over 65 years (Cook and Barker, 1983; Barker, 1987), but during most of that time it was not considered commercially feasible. Bio-control of nematodes was first studied by (Duddington, 1951). The development of biological control agents is also considered an effective alternative for nematode control on vegetables (Van Gundy, 1985; Kerry, 1987; Sikora, 1992). Biological control is considered to encompass control that results from the action of soil microorganisms and the soil microfauna and is mediated through mechanisms such as parasitism, predation, competition and antibiosis (Stirling, 1991).

There are three major types of organisms that are antagonistic to nematodes. Each group differs in its mode of action (a) predators are organisms which actively seek out nematodes and then consume them, (b) parasites are organisms which grow within their host and obtain their nutrition from the host and if they are capable of causing disease in the host they are known as pathogens, (c) the third group of antagonists influence nematode abundance through mechanisms other than predation and parasitism (Stirling, 1991).

Sikora (1992) suggested the term antagonistic potential for all parasites, predators, pathogens, competitors and other organisms in soil that work together to repel, inhibit, or kill plant parasitic nematodes. Antagonists most likely to be receptive to management for the biological control of nematodes are: predacious or trapping fungi; endoparasitic fungi; fungal pathogen/parasites of females, endomycorrhizal and mutualistic fungi; plant-health promoting rhizobacteria and obligate bacterial parasites.

2.4.1.1. Nematophagous fungi

A wide range of fungi have been isolated from both eggs and females of root-knot nematodes (Morgan-Jones and Rodriguez-Kabana, 1988; Stirling and West, 1991). Nematophagous fungi can be classified into two broad groups: the obligate parasites and the facultative parasites. Obligate parasites use their spores to infect nematodes, and can initiate infection either by being ingested and then penetrating the gastrointestinal tract, or by adhering to the nematode cuticle and penetrating directly. The facultative parasites can change their trophic state from saprophytes that grow in the soil and rhizosphere into parasites infecting nematodes, either by way of specialized adhesive spores, or by trapping structures that adhere to migrating nematodes, or through specialized hyphae that develop appressoria that can breach the nematode cuticle or eggshell (Barron, 1977).

2.4.1.2. Nematode-trapping fungi

These fungi known as predatory fungi, consist of a sparse mycelium which has been modified to form organs capable of capturing nematodes (Stirling, 1991). Duddington (1962); Barron (1977) and Gray (1987; 1988) described six different types of traps that have been found in the nematode trapping fungi. A few species of hyphomycetes and two genera in zygomycetes (Stylopaga and Cystopaga) capture nematodes by means of an adhesive that is produced directly on their hyphae. Another trapping structure is the adhesive branch which is produced by a few species of nematode-trapping fungi such as *Monacrosporium cionopagum*. There are two distinct barriers to the infection of root-knot nematodes by fungi, either the cuticle of the J2 within the egg or the eggshell from which the J2 emerges. Various predacious fungi produce erect branches of one to three cells on the hyphae to form single loops or two-dimensional networks. The layer of adhesive material is secreted over the surface of each branch. Further loops are produced on this loop or on the parent hyphae, until a complex three-dimensional, adhesive-covered network of anastomosed loops exists. The adhesive nets are the most common type of trap found in the nematode-trapping fungi and they are found in almost all soils. *Arthrobotrys oligospora* the most frequently found predatory fungus in soil, uses this trapping mechanism. Another type of adhesive trapping device is the adhesive knob. These knobs are one of the most common trapping mechanisms among the hyphomycetes and can be rarely found in basidiomycetes group of fungi.

2.4.1.3. Female and egg parasitic fungi pathogens

The zoosporic pathogens of females are closely related to the zoosporic species which attack vermiform nematodes. *Catenaria auxiliaries* is a widespread fungus in *Heterodera schachtii* in Europe (Tribe, 1977a, b) and has been found in *H. glycines* and *H. avenae* (Crump et al. 1983; Stirling and Kerry, 1983). *Nematophthora gynophila* was found in *H. avenae* infested soils (Kerry and Crump, 1980).

In addition to the zoosporic fungi, a wide range of other fungi have been found in association with nematode females, cysts and eggs. These fungi are facultative parasites such as, *Verticillium spp.*, one of the most important pathogens of root-knot and cyst nematodes. Most species in this genus have been recorded as occurring in cysts and/or eggs of *Globodera*, *Heterodera* and *Meloidogyne* (Morgan-Jones and Rodriguez-Kabana, 1988). *V. chlamydosporium* is the most widely studied species. These fungi form branched mycelial networks which when in close contact with the egg shell, penetrated the egg wall and destroy its contents Lysek, (1978); Morgan-Jones et al. (1983); Lysek and Krajci, (1987); Lopez-Llorca and Duncan, (1988); Morgan-Jones et al. (1983); and Meyer et al. (1990), suggested that *V. chlamydosporium* itself might produce a toxin that affects egg hatch.

Paecilomyces is one of the principal genera in biological control in recent years. Lysek (1976) was the first to observe it in association with nematode eggs. Jatala et al. (1979) found it parasitizing eggs of *M. incognita* and in 1985 and 1986 Jatala revealed that *P. lilacinus* showed promise as a biological control agent against several nematode species. This fungus penetrates the nematode eggs directly by individual hyphae (Morgan-Jones et al. 1984).

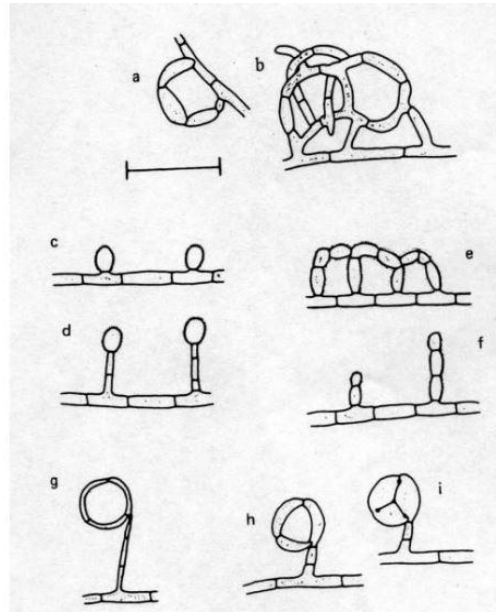


Figure 2.2. Trapping organs of predatory nematophagous fungi: (a) adhesive nets simple and; (b) complex; (c) adhesive spore sessile and; (d) stalked, (e) simple two-dimensional adhesive networks; (f) adhesive knobs and branches; (g) non-constricting rings, (h) constricting rings, open and; (i) closed (Gray, 1988).

Dactylella oviparasitica was the first parasite found in *Meloidogyne* eggs (Stirling and Mankau, 1978a,b). This fungus produces appressoria on the egg surface and then penetrates the eggs through these specialized structures and through enzymatic penetration especially by chitinase production (Stirling and Mankau, 1979).

2.4.1.4. Endophytic fungi

Another group of fungi that was recently used as a biocontrol agent against plant parasitic nematodes especially in root-knot nematodes, are endophytic fungi (Hallmann et al. 1997). These saprophytic fungi colonize healthy plant tissue without causing symptoms. When the colonization is successful it leads to protection of the plant against biotic and/or abiotic stress, these fungi are called mutualistic endophytic fungi (Carroll, 1990). Mousa and Hague (1988) found that in the soybean disease complex between *Fusarium oxysporum* f. sp. *Heterodera glycines* and *M. incognita* active colonization of the giant cells by the fungus resulted in reduced development of juveniles and an increased proportion of males. Hallmann and Sikora (1993) evaluated 200 isolates of endophytic fungi, representing different genera, isolated from tomato roots. Forty isolates were screened for their ability to control *M. incognita* in pot experiments. Hallmann and Sikora (1994, 1995) found a

reduction in gall formation by *M. incognita* between 52 and 75% after application of four endophytic strains of the fungus *Fusarium oxysporum*. They also found that *M. incognita* attraction and penetration of tomato seedlings was significantly reduced following treatment with the culture filtrate of *Fusarium oxysporum*.

2.4.2 Cultural control

All human activities designed to reduce populations of *Meloidogyne* can be characterized as cultural control practices, except for nematicide. These practices include appropriate planting, use of clean tools, cleaning infested plant materials, crop rotation and intercropping. Many of these practical methods are used successfully in different parts of world to manage *Meloidogyne* spp. (Brown et al. 2006). Physical methods such as solarization of the soil and heat treatment before planting can be combines with cultural method for higher effectiveness of control in various *Meloidogyne* species.

2.4.3. Steam heat and solarization

The effectiveness of steam heat and soil solarization in managing root-knot nematodes under glasshouse and field conditions, respectively, is dependent on soil temperature. The lethal soil temperature for fungal or bacterial pathogens occurs at 60-100 °C, whereas 45 °C is considered to be sufficient to control plant-parasitic nematodes (Katan, 1981; Sikora and Fernandez, 2005). Steam sterilization of nurseries and greenhouse soil is done by reaching to 82-93°C at a 15 cm depth for 30 minutes. This temperature is sufficient to kill the nematode and gives better results against root-knot nematodes than those obtainable with applications of systemic nematicides which do not kill root-knot (Lamberti et al. 1976).

Soil solarization is a rather recently developed technique, which shown promise for the control of several soil-borne pathogens and weeds in warm areas. Its effectiveness against nematodes was first demonstrated in Israel and later, attempts to control nematodes by soil solarization were also made in Australia, USA, Italy, and South Africa. In other experiments in Sicily, aimed at the control of soil borne pathogens of pepper in greenhouse culture, yield and fruit size from solarized soil averaged 12.2 and 2.3 times, respectively, over those in controls. Nico et al. (2003) reported that in nursery, solarization of soil up to 40 cm for a period of three weeks was found effective in decreasing nematode eggs. Higher

yield was obtained with soil solarization, alone or in combination with reduced dosages of methyl bromide, and with methyl bromide alone (6 times over control) than with DD (5.4 times the control) (Katan et al., 1976; Katan, 1987). The efficacy of soil solarization is based on the sensitivity of nematodes to relatively high temperatures. Solarization also is a unique method of mulching that integrates pest control, soil and water conservation and increased growth response of crops (Stapleton and DeVay, 1986). The efficacy of soil solarization is based on the sensitivity of nematodes to relatively high temperature greater than 40-50°C. Moreover, temperatures of 36-40°C can be reached at 20-30 cm depth in warm areas. Such temperatures, if prolonged, can be lethal to nematodes or at least may reduce their infectivity because of energy reserve depletion. Soil solarization with clear plastic has been attempted as a means of raising soil temperatures to these lethal levels to control soil-borne diseases (Katan, 1981). This technique however, is only adaptable to regions, for example the Middle East, where sufficient solar energy is available for long periods of time. Solarization also has been shown to have a potential in the subtropical climate of Florida where it reduced root-knot, *Verticillium* wilt and weeds (Overman and Jones, 1986).

In some solarization experiments control of these nematodes was inconsistent (Greco et al. 1985; Barbercheck and Von Broembsen, 1986), in other investigations excellent control was achieved by soil solarization under greenhouse conditions (Cenis, 1984; Cartia et al. 1988; 1989).

Soil solarization reduced significantly important genera of phytoparasitic nematodes: *Meloidogyne*, *Heterodera*, *Globodera*, *Pratylenchus*, *Rotylenchulus*, *Rotylenchus*, *Tylenchulus*, *Tylenchorhynchus*, *Xiphinema*, *Belonolaimus*, *Criconemella*, *Ditylenchus*, *Criconemella* and *Dolichodorus* (Davis and Sorensen, 1976; Siti et al. 1982; Stapleton and DeVay, 1983; Porter and Merriman, 1983; Greco et al. 1985; McSorley and Parrado, 1986; Heald and Robinson, 1987; Stapleton, 1990).

However, phytoparasitic nematodes are more resistant to the effects of solarization than most other plant pests and pathogens. Also, populations of root-knot nematodes must be effectively controlled to a greater soil depth than other targeted organisms. Although soil depths to which phytoparasitic nematodes were controlled by solarization varied, only a few reports examined effects below 30 cm (Stapleton and DeVay, 1983; Stapleton et al., 1987). Porter and Merriman (1983) demonstrated significant reductions of *Meloidogyne javanica*,

Tylenchulus semipenetrans and *Criconebella xenoplax* to a 26 cm depth in microplot in Victoria.

Heald and Robinson (1987) showed that *Rotylenchulus reniformis* was effectively controlled only to a 15 cm depth in Texas. Stapleton and DeVay (1983) however reported more than 95% reduction of *Meloidogyne hapla* juveniles and males at 90 cm soil depth in Australia. Abu-Gharbieh et al. (1987) found that hot season solarization with black polyethylene film, which was then perforated and left in place as cool season mulch for vegetable crops was an effective and economic practice for root-knot control.

2.4.4. Flooding

Flooding is another option can be applied in general in the principal management methods used for plant-parasitic nematodes Land that has lain under water for a continuous period of 3 months or more following either natural or artificial flooding will be free of rootknot nematodes (Bridge, 1987). Such soil becomes almost perfect for use as seedling nursery sites. Thames and Stoner (1953) reported effective root-knot nematode control on two vegetable crops produced after flooding previous rice fields for three months. Sikora (1989) also observed less severe root-knot nematode damage in Philippine vegetable cropping systems based on paddy rice-vegetable rotations when flooding was maintained for at least 4 months than in rotations without paddy rice. IFAS (1989) recommend alternating flooding with drying during the summer vegetable season in Florida for root-knot nematode control.

2.4.5. Soil amendments

The incorporation of organic material into the soil has been shown repeatedly to reduce root-knot nematode densities (Stirling, 1991). He suggested that the mechanism of action may be due to: improvement of soil structure and aggregation resulting in increased aeration and water-holding capacity; to improvement in plant nutrition; to the release of toxic by-products to nematodes of microbial basis or to the enhancement of the growth of organisms able to compete with or destroy nematodes. Reductions in plant parasitic nematode populations in response to applications of organic amendments have been reported (Muller and Gooch, 1982).

Organic amendments were associated with suppression of nematode populations through stimulation of antagonistic soil microflora during their decomposition (Baby and Manibhushanrao 1993).

A number of organic amendments have been used to manage root-knot nematodes. Their use is associated with reduced infection, or survival of nematodes and increased numbers of microbial antagonists of nematodes (Linford et al. 1938; Mankau and Minter, 1962; Mankau, 1968; Sitaramaiah and Singh, 1978).

Plant based soil amendments such as castor, sesame, sorghum-sudangrass, velvet bean and zinnia incorporated into soil or applied as mulch were chosen to control nematodes under greenhouse conditions, because they have been previously used as organic amendments spp. to suppress root-knot nematodes when used in crop rotation (Watson, 1922; 1936; 1945; Watson and Goff, 1937; Lear, 1959; Mankau and Minter, 1962; Mankau, 1968; Mian and Rodriguez Kabana, 1982b; Hung, 1984; Rodriguez-Kabana et al., 1988; Rich et al. 1989; McSorley and Gallaher, 1993; McSorley et al. 1994).

The use of nitrogenous organic matter as a soil amendment is a successful strategy for the management of *Meloidogyne* in other plant parasitic nematodes in vegetables and other root-knot susceptible crops (Mian and Rodriguez-Kabana, 1982a; Rodriguez-Kabana et al. 1990). Oil cakes, sawdust, urea and bagasse also are used in managing root-knot nematodes with some success (Singh and Sitaramaiah, 1966; 1967; Sikora et al. 1973). Culbreath et al., (1985) suggested that when chitin was combined with waste products from the paper industry, root-knot nematodes were reduced.

2.5. Chemical Control

Chemical control was the main tactic for reducing root-knot nematode populations in most moderate-to-high-value crops in intensive production systems throughout much of the 20th century. During the last few decades, root-knot nematode control was based on the use of chemical pesticides applied to the soil or the plant (Duponnois et al. 2001). There are two types of nematicides used in control of root-knot nematodes, fumigants and non-fumigants. Soil fumigation is the most common measure used to achieve economical control in agricultural land (Lamberti, 1979a). The polyphagous nature of the more important

species of root-knot nematodes means that the use of chemicals is likely to be the main method of control.

Minton and Baujard (1990) also reported that chemicals are the major means of controlling nematodes including *M. arenaria*, *M. hapla*, *Pratylenchus brachyurus*, *Belonolaimus longicaudatus* and other nematodes in peanut.

Fumigant nematicides are used to treat soil as drenches, root dips, foliar applications or seed treatments. They can be formulated as gases, volatile liquids, gels, flowables, spray concentrates or granules (Bird, 1987). The gaseous nature of fumigants makes them extremely useful as nematicides. When fumigants are released in the soil, they volatilize and move effectively through soil pore spaces and into soil moisture films containing nematodes. In most cases they are broad spectrum contact nematicides effective against nematode eggs and juveniles and adults (Bird, 1987; Netscher and Sikora, 1990). The fumigants are phytotoxic (Bird, 1987) and must be applied weeks before planting.

Fumigants are generally more effective in controlling root-knot nematodes and in increasing crop yield than the non-fumigant nematicides, because fumigants have a broader spectrum of activity, controlling soil insects, fungal diseases and weeds as well as other plant parasitic nematodes as reported by Netscher and Sikora (1990).

Lamberti (1979b) and Johnson (1985) reported that the fumigant nematicides were highly effective in control of *Meloidogyne* in vegetables. In the past the most commonly used fumigant nematicides were: Methyl Bromide, Chloropicrin, Ethylene Dibromide, 1,2-Dibromo- 3-Chloropropan and Metham Sodium. The efficacy of these fumigants is affected by abiotic factors such as soil texture, moisture, temperature and organic matter (Bird, 1987).

Methyl bromide is generally very effective against most plant parasitic nematodes (Abdalla and Lear, 1975). Excellent control of all root-knot nematode species has been obtained with methyl bromide, which penetrates soil and intact root fragments readily (Khatoom, 1981; Noling, 1989; Abou-Jawadah et al. 2000; Oka et al. 2000). This highly effective fumigant has been removed from the market due to side-effects on the atmosphere and good alternatives have not yet been found for the tropics and subtropics.

The fumigant nematicide Metham Sodium was effective in controlling root-knot nematodes and soil fungi when applied through drip irrigation (Roberts, 1988). On the other hand, Lamberti (1979b) found that Metham Sodium does not give good results against root-knot nematodes even when applied in very high dosages. It is also not as effective at higher soil temperature. The fumigant Ethylene Dibromide gives very efficient control to root-knot nematodes (Lamberti, 1979b). It is not as effective against other pest problems like weeds and fungi.

Hodges and Lear (1973) found that root-knot nematodes are killed by short exposure to 1,2-dibromo-3-chloropropan (DBCP). DBCP was frequently used to control nematodes in established orchards, vine-yards or rose plantings (Raski and Schmitt, 1964; Lownsbery et al. 1968; Johnson et al. 1969). It is no longer on the market.

The non-fumigant granular or liquid nematicide compounds are water soluble (Netscher and Sikora, 1990). The nematicides are not effective against nematode eggs and in most cases do not kill the juveniles or adults at the recommended concentrations used (Netscher and Sikora, 1990).

These granular or liquid non-fumigant systemic nematicides are effective in delaying infection for some weeks. Non-fumigant systemic nematicides are non-phytotoxic and they can be applied effectively by surface and drip irrigation (Overman, 1974; Johnson, 1985; IFAS, 1989). However, they are not as effective as fumigants in increasing yield because they do not have broad spectrum activity (Netscher and Sikora, 1990).

The most common non-fumigant systemic nematicides used to control nematode were: Oxamyl, Fenamiphos, Carbofuran and Aldicarb (Lamberti, 1979b). The systemic action of Aldicarb inhibit root invasion by *M. incognita* juveniles under greenhouse conditions and inhibits egg hatch and juvenile migration and host invasion (Hough and Thomason, 1975; Vovlas and Lamberti, 1976). Vovlas and Lamberti (1976) reported that Carbofuran and Oxamyl prevented root invasion of tomato by *M. incognita* and was very effective against root-knot nematodes for 12 and 25 days. Phenamiphos is a systemic non-fumigant sold in a granular and a liquid formulation.

3. MATERIAL AND METHODS

3.1. Sampling and Source of Root-Knot Nematodes Inoculums

Inoculum of needed nematode were increased on susceptible tomato plants starting from a single nematode egg mass for the species. Inoculum of *M. javanica* was prepared following the method of Hussey and Barker (1973) by extracting nematode eggs from infected roots using a 1.5% NaOCl solution. Infested tomatoes roots collected from the Kahramanmaraş were kept in polyethylene bags in refrigeration at 4 °C, until used. The roots were washed by tap water gently and placed in Erlenmeyer flask. About four weeks later, the nematodes were cultured in a glasshouse on susceptible tomatoes in the growth chamber for 60 days. After this period, eggs of *M. javanica* were collected from galled roots of tomatoes by dissolving the gelatinous matrix with sodium hypochlorite 0.5% and put on a 25 m-pore sieve (Hussey and Barker, 1973). Inoculums of J2/eggs were obtained from a modified Baermann funnel and 1 to 3 day-old inoculum used for the experiment (Rodríguez-Kábana and Pope, 1981).

3.2. Confirmation of Nematodes by Perineal Pattern

Meloidogyne javanica was previously collected from Kahramanmaraş region, however was needed to be confirmed. For this reason, females of the nematodes were dissected by sharp needles and half spear to segregate the adult female under light microscope (Olympus, model SZX16), and they were placed in lactic acid 45% in a petri dish for 1 h, then were placed in a drop of glycerol. The procedure in preparation of nematodes for perineal identification were followed as outlined (Singh, 2009). Under a stereo microscope (Olympus, model BX51) viewing, a slit was made using a lancet in the female on the slides by cutting off the cuticle into half. The posterior region containing of the perineal region was cut accurately, trimmed and brushed by using a fine brush to remove any remaining debris. The cut was processed by a quill and placed on another clean slide in a drop of glycerol. Five to six perineal regions from a single population were put on the same slide and covered with the slip (Figure 3.1.) The perineal patterns of females confirmed that the root-knot nematodes populations used in the study was *Meloidogyne javanica*.



Figure 3. 1. Identification of root-knot nematode females extracted from the infected roots of cucumber.

3.3. Experiments

3.3.1. First experiment

Experiment one and two were conducted in a greenhouse and growth chamber located in Agriculture Faculty of Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey. Two host plants used in the study were a commonly planted cucumber (cultivar Amisos F1) and pepper (E-51 Kobra).

The soil of the pots for green house and growth chamber experiments were consisted 15% organic matter, 25% clay and 60% sand. In both experiments, the pots were 15 cm in diameter and 30 cm in height with a capacity of approximately 2 L.

3.3.1.1. Raising of cucumber and pepper seedlings

Cucumber and pepper seeds were sown in polystyrene trays that were filled with sterilized soil and were put in a growth chamber at 25°C. The trays were watered and fertilized as needed.



Figure 3.2. Raising A) cucumber B) pepper seedlings.

3.3.1.2. Preparing the pots and transplanting the seedlings for the experiments

Two L volume pots filled with 1.8 L of sterilized soil were used for both experiments. A month old cucumber and eight weeks old pepper seedlings were transplanted into the prepared pots (Figure 3.4.).



Figure 3. 3. The transplanting the seedling to soil filled pots in the greenhouse.

3.3.1.3. Manual preparation of potato dextrose agar (PDA)

Potato and Dextrose Agar was prepared manually. For Preparation of potato dextrose agar (dehydrated Potato Infusion and Dextrose), 250 g of potato were washed and peeled off and sliced it into small pieces and put in a 500 ml of distilled water and cooked for 30 minutes in pressure cooker and put in autoclave at 121 °C for 20 minutes. Then, 20 g dextrose and 20 g agar were mixed in a 500 ml of distilled water. The potato extracts collected by fine muslin, and mixed all thoroughly for up to the size of 1lt. Prepared PDA was poured to 15 ml sterilized petri dishes. After solidifying, the petri dishes were stored in room temperature for further use.

3.3.1.4. Transferring fungi (*Beauveria bassiana*, *Isaria tenuipes*) for colonies increase and preparation of spore suspensions

For sterilization a needle was heated by fire and was cooled by dipping it to the edge of PDA petri plate. Later, the reproducing colonies were selected and transferred to PDA filled petri dishes. This procedure were applied to all plates. The plates then, were

sealed with parafilm, labeled and placed in an incubator. The colonies became ready for use in experiment after 15 days at 25 °C (Figure 3.5). After 15 days fourteen ml of distilled water poured into each prepared petri plate and the mycelium was collected with a glass slide and sieved by a fine muslin and the gathered mycelium suspension was kept in sterile Erlenmeyer flasks.

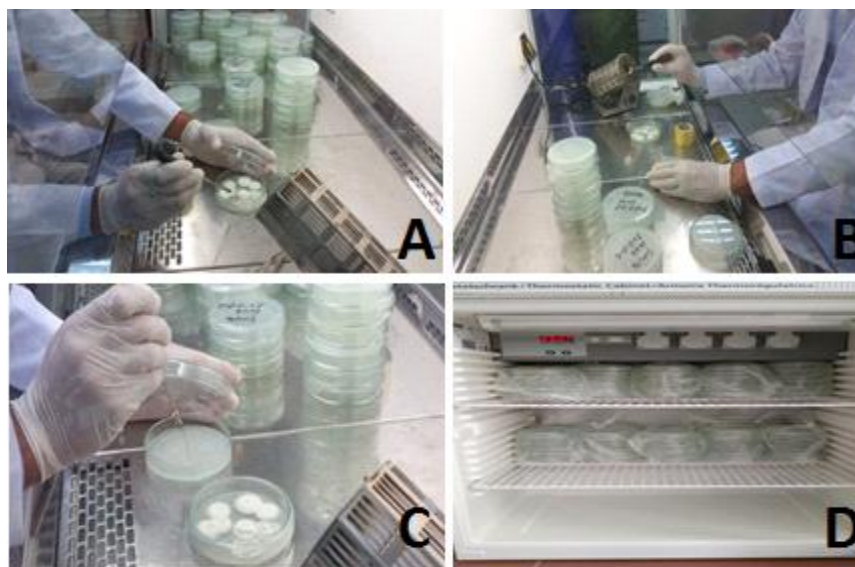


Figure 3. 4. A view of A) sterilizing needle B) selecting a colony that producing spores, C) increasing the number of colony D) incubating petri plats 25° C.

3.3.1.5. Quantifying fungal spores

First the hemocytometer and glass cover were cleaned with ethanol and the glass cover placed over the counting chambers. Each spore counting was performed per a sample of 10 micro liter of spore suspension under the light microscope at 10X magnification with the help of a hemocytometer. The total number of spore found in 4 large squares in the corners and one in the middle (Morris and Nicholls, 1978) (Figure 7).

The quantifying of the number of spore cells/ml in original suspension were performed as follow:

$$\text{Spores/ml (in original)} = (\text{Average no. spores}) \times 2.5 \times 10^5$$

The average number of *Isaria tenuipes* for experiment one was $77 \times (2.5 \times 10^5) = 192.5 \times 10^5$ spores/ml.

The average number of *Beauveria bassiana* for experiment one was $3.2 \times (2.5 \times 10^5) = 83 \times 10^5$ Spores/ml.

The average number of *Isaria tenuipes* for experiment two was $39.8 \times (2.5 \times 10^5) = 99.5 \times 10^5$ Spores/ml.

The average number of *Beauveria bassiana* for experiment two was $25.6 \times (2.5 \times 10^5) = 64 \times 10^5$ Spores/ml.

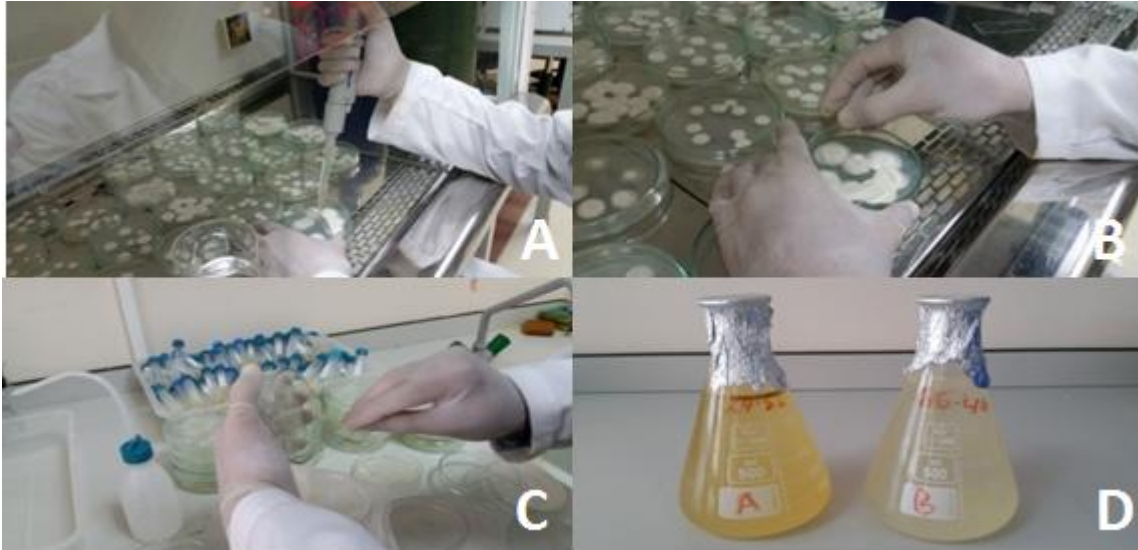


Figure 3. 5. A view of A) 14 ml of distilled water poured into each preparation of petri plate, B) removing the mycelium (fuzzy white) by a glass microscope slide, C) mycelium was collected with a glass slide and sieved by fine muslin; D) gathered mycelium suspension was kept in sterile Erlenmeyer flasks.



Figure 3.6. Quantifying the numbers of spores at a binocular with the hemocytometer.

3.3.1.6. Extraction of root-knot nematode eggs or J2

Plants with galls and egg masses were harvested and nematodes were taken away after 80 days of inoculation with nematodes. Galled cucumber and pepper roots were washed to remove debris. Galled roots were cut off into small pieces of 1-2 cm, and placed in a container. Sodium hypochlorite solution (NaOCl) 0.5% was added and shaken for 20-30 seconds to separate the eggs from the galls. The nematode suspension was sieved through 75 µm mesh covered over 25 µm mesh vents sieve to collect root debris on the top and the eggs or J2 at the bottom. Following these steps, 25 µm sieve containing the egg or J2 was washed freely for few minutes under fresh water to rinse off the remaining of NaOCl. The collected eggs or J2 carefully were kept in a glass beaker in a refrigerator at +4 °C.

For evaluating the amount of inoculums density of eggs or juvenile suspension, the suspension was transferred into a container and mixed firmly. The average of three counts was registered under a stereomicroscope at 40X magnification, each time 5 ml was taken for counting. The total population was estimated by multiplying the average number of three aliquots with the total volume of suspension.

3.3.1.7. Inoculation of the different nematode levels to the pots

Fore-week old cucumber and pepper seedlings were transferred into the 2 L size pots five days after transplanting. Three days later the plants were inoculated with three levels of *M. javanica*. The nematode levels were consisted of: a positive control (without treatment but with both level of nematode but), low (1000 J2/plant eggs soil plant) and high (2000 J2/eggs per /plant). Firstly, 4 holes in a square shape and approximately 2 cm depth on the pot soil surface distended from the roots were formed. The J2/plant eggs suspension were adjusted to 5 ml containing 1000 eggs or J2s and applied to the planted pots by an adjustable pipette. The holes were suddenly after the application and covered with soil (Figure 3.8.).



Figure 3.7. Inoculation of *Meloidogyne javanica* populations in the pots.

3.3.1.8. Applying the different plant extract treatment levels

Three days after inoculations of nematodes, the planted pots were treated by 3 essential application; two entomopathogenic fungi (*Beauveria bassiana* and *Isaria tenuipes*), and one positive control. Mean number of *Isaria tenuipes* spores for both experiments was arranged as $=192.5 \times 10^5$ spores/ml, and for *Beauveria bassiana* was arranged as $= 83 \times 10^5$ spores/ml. Two fungi application level for both experiments were consisted of low (1000 μ L/ plant) and high (2000 μ L/plant).



Figure 3. 8. Application of two different entomopathogenic fungi rates (1000 and 2000 ml/pot).

3.3.1.9. Experiment conditions

The greenhouse maintained a daily average of 14 h 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 equally by drip irrigation with a wall drip tape placed in the center of pots. The plants were fertilized weekly with NPK (20-20-20). Every 10 to 12 days, the plants

were sprayed by soap solution to manage insects (mostly white flies). In second experiment the plants lost their weight and some of cucumbers and peppers have been died because of white flies' damage, but they have been replanted.

3.3.1.10. Plants Harvest

The test plants were harvested about 60 ± 3 days after inoculation. Cucumber and pepper plants were cut off from the pot soil level and the green parts were saved in paper bags separately for weighting as fresh and as dry after the drying procedure. The plants root in the pots however were softly removed and washed gently with the tap water until all debris and soil particles were removed. Finally, the roots were saved in polyethylene bags and kept in a refrigerator at $+4^{\circ}\text{C}$ for further use (Figure 3.10.).

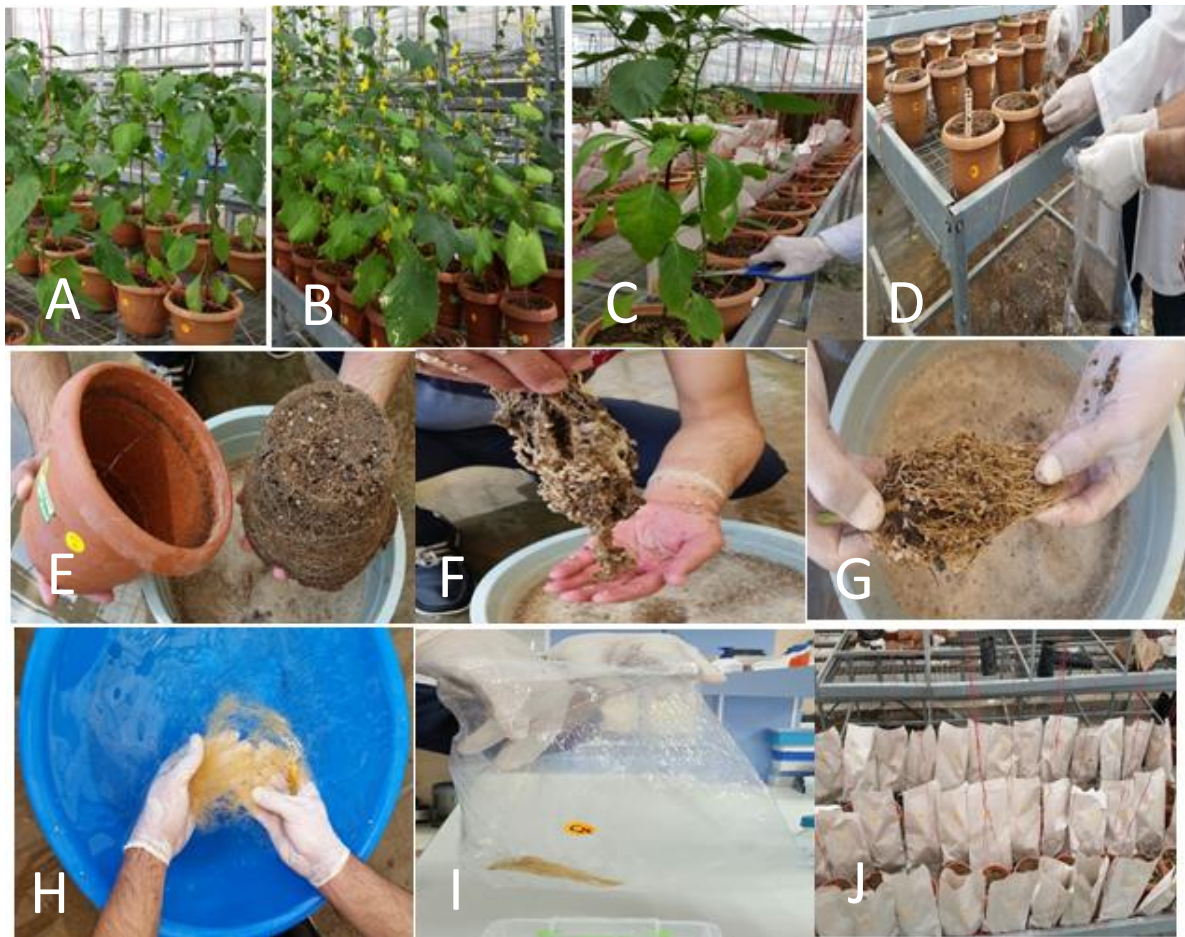


Figure 3.9. Experiment processing steps A) Grown pepper plants, B) Grown cucumber plants, C) Cutting plants above soil level, D) Sampling pot soil, E- F) Removing plant roots from the pots, G) washing the roots off of the soil, H) Harvested clean root I) Placing the roots in polyethylene bags. J) Keeping plants into paper bags for drying

3.3.1.11. Collection of the data of the experiments

Over the course of the each experiment period, from transplanting to the the harvest, plants height were recorded and registered every other week with the total of 5 times. Fresh (as soon as harvested) and dry weights (after application of the roort to a 100 °C temperature for two days in the dryer) were also recorded by an sensitive electronic scale. Root galling were estimated 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). Roots were stained with red food color (Stetina and Young, 2006), 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 roots system (Taylor and Sasser, 1978).



Figure 3.10. Data Collection. A) Recording plant heights, B) Fresh plant parts weighting (Dry and Fresh), C) Fresh plant drying, D) Setting a modified Baermann for nematode extraction from an 80 cc of soil, E) rating root galling, F) Staining the roots, G) Counting the number of egg masses.

3.3.1.12. Statistical analysis

Over the course of the experiment EMI (egg mass index), GI (galling index), plant height, plant fresh (green parts) and dry weight, root fresh weight, and root dry weight were collected and the data was subjected to ANOVA using (SPSS Statistics version 23.0.0 statistical software), and treatment means were separated by dependent variable and Duncan's multiple range test. The experiments were compared using t-test based on $P \leq 0.05$ level.

3.3.2. Experiment two

For the scientific accuracy, the experiment was repeated. For the second repeat cucumber and pepper seedlings were transplanted to the prepared pots on 25th of August, 2015. Pot preparation, nematode inoculation levels, treatment applies, and installation experimental methodology were similar to those of described and explained for first experiment. Cucumber and pepper plants harvest were taken place about 60 ± 3 days, and data of the plant height (over course of the growing period, prior to the harvest), green part fresh, dry fresh weight, root fresh weight, dry root weight, gall and egg mass indices were collecrecorded.

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1. First experiment

In the first experiment it can be noticed that the effect of plant varieties on all the parameters were significant ($P < 0.001$) except root galling index. There was a significant effect of treatment levels (L) on only root galling and egg mas indices ($P < 0.01$) Table 4.1 and 4.2). I was found that the nematode level (N) for all the parameters were non-significant ($P > 0.05$) except for Egg Mass indices ($P < 0.05$). The treatments (T) affected all parameters significantly except for the root fresh weight significantly ($P < 0.01$) (Table 4.1 and 4.2).

For the interactions between the factors, the nematode level and treatment (NxT) interaction sustained a significant effect on all parameters except for for Green part Dry Weight ($P > 0.05$). While for the interaction between nematode level and treatment level (NxL), the effect was non-significant with the Green Dry Weight and Root fresh weight ($P > 0.05$). There was a significant effect of Interaction of treatment and treatment level (TxL) on Root Galling, Egg Mass ($P < 0.001$), and Root Dry Weight ($P < 0.05$). The effect of triple interaction between Nematode level, Treatment, and Treatment level (NxTxL) was not significant for both Root fresh weight and Root dry weight (Table 4.1 and Table 4.2).

Table 4. 1. Analysis of variance four treatments, two treatment levels, two nematode levels of *Meloidogyne javanica* and their interaction on the plant height, green part fresh weight, green part dry weight, root gall index for two plant varieties (cucumbers and pepper) in first experiment.

1 st 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	0.000***	122.836	0.000***	58.690	0.000***	18.716	0.272	1.220
Treatment levels (L)	2	0.915	0.012	0.887	0.020	0.191	1.739	0.000***	40.541
Nematode levels (N)	2	0.912	0.012	0.241	0.625	0.667	0.186	0.168	1.931
Treatment (T)	3	0.000***	16.791	0.000***	14.491	0.000***	7.336	0.016*	3.604
NxT	6	0.000***	13.218	0.002**	6.487	0.190	1.691	0.033*	3.540
NxL	2	0.011*	6.683	0.021*	5.542	0.809	0.059	0.033*	4.682
TxL	6	0.558	0.587	0.195	1.664	0.521	0.656	0.000***	10.040
NxTxL	6	0.000***	14.423	0.001***	7.590	0.041*	3.323	0.000***	10.133

* Significant at ($P \leq 0.05$), ** Highly Significant at ($P \leq 0.01$), *** Very Highly Significant at ($P \leq 0.001$)

Table 4. 2. Analysis of variance four treatments, two treatment levels, two nematode levels of *Meloidogyne javanica* and their interaction on root fresh weight, root dry weight, egg mass index for two plant varieties (cucumbers and pepper) in first experiment.

1 st experiment							
Source	df	Egg mass index	F- value	Root fresh weight (g)	F- value	Root dry weight (g)	F- value
Host Plant (P)	1	0.006**	7.923	0.000***	19.237	0.000***	46.628
Treatment levels (L)	2	0.000***	93.048	0.912	0.012	0.074	3.263
Nematode levels (N)	2	0.016*	5.996	0.473	0.519	0.271	1.229
Treatments (T)	3	0.001***	5.935	0.068	2.455	0.016*	3.614
NxT	6	0.000***	14.586	0.000***	8.584	0.004**	5.842
NxL	2	0.000***	21.551	0.176	1.859	0.008**	7.391
TxL	6	0.000***	18.621	0.319	1.158	0.027*	3.777
NxTxL	6	0.000***	17.652	0.626	0.470	0.316	1.166

* Significant at ($P \leq 0.05$), ** Highly Significant at ($P \leq 0.01$), *** Very Highly Significant at ($P \leq 0.001$)

The data from first experiment indicated that the highest plant height of cucumber was obtained as 115.1 ± 6.08 cm in *Isaria tenuipes* and the lowest was attained as 98.72 ± 25.8 cm for *Beauveria bassiana* treated plants in low nematode and treatment levels. In the same levels, in pepper, the highest plant heights also were obtained in *Isaria tenuipes* treated levels, however the highest root fresh weight were found in *Beauveria bassiana* treated plants (Table 4.3).

Data from low level of nematode inoculums of 1000 egg or J2/plant with high treatment level of 2000 μ L/plant is shown in Table 4.4.

The data from experiment one for cucumber plant host showed that the highest plant height were observed as 120.04 ± 23 cm for *Beauveria bassiana* treated plants, the lowest value was 100.93 ± 11.6 cm for control. The highest green part dry weight was found as 18.98 ± 11.04 g in *Isaria tenuipes* treated cucumbers. In pepper host plant, in the same treatment and nematode inoculum level, the greatest plant height and the greatest green part dry weight were found as 44.6 ± 2.7 and 10.19 ± 1.5 , respectively in the *Isaria tenuipes* treated plants. However, the greatest green part fresh weight was obtained in the *Beauveria bassiana* pots. The data for remaining parameters were varied and relatively low (Table 4.4.).

Data from high level of nematode inoculums 2000 egg or J2/plant with low level of 1000 μ L/plant is shown in Table 4.5.

The data for experiment 1 revealed that, *Isaria tenuipes* treated cucumber plant host sustained the highest plant height, the green fresh weight and root fresh weight as 98.48 ± 11.7 cm, 13.87 ± 1.3 g and 11.18 ± 2.2 g, respectively. Data for galling indices, egg mass indices were low and varied in numbers. On the other hand, the highest plant height, the greatest green part dry weight and the greatest root fresh weight of pepper were recorded as 45.8 ± 5.4 cm, 10.61 ± 2.7 , and 10.91 ± 2.1 g, respectively in *Beauveria bassiana* treated pots. The egg mass indices were revealed significantly low as compared to the controls indicating the effectiveness of both bio control agents. *Beauveria bassiana*, and *Isaria tenuipes* (Table 4.5.).

Data from high level of nematode inoculums 2000 egg or J2/plant with high level of 2000 μ L/plant is shown in Table 4.6.

For cucumber, the highest plant height and the greatest green part dry weight was obtained from *Isaria tenuipes* treated plants as 101.56 ± 15.9 cm and 12.6 ± 3.1 g, respectively. Whereas, in first experiment, in the high level of nematode and treatments, *Beauveria bassiana* treated pepper plants showed the greatest numbers on all parameters, and low numbers for galling and egg mass indices ($P < 0.01$) (Table 4.6). In this case, the egg mass index and galling index numbers were very significantly low and indicating the effectiveness of this agent on the root knot nematodes. The highest green dry weight, was 10.96 ± 1.9 g for *Beauveria bassiana* treated peppers. The highest value for root fresh weight and root dry weight was 12.49 ± 2.5 g and 0.95 ± 0.18 g, respectively in *Beauveria bassiana* treated pepper host at high level treatment (Table 4.6.).

Table 4. 3. The means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, and egg mass indices at 1000 eggs or J2s/plant nematode inoculum level, 1000 μ L/plant, 0 level for each two fungi, and control, respectively, in first experiment (Mean $\pm$ SD)

1 st exp	<u>Cucumber</u>						
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)
<i>Isaria tenuipes</i>	115.10 $\pm$ 6.08a	138.92 $\pm$ 11.2a	13.83 $\pm$ 0.95a	0.00 $\pm$ 0.0c	0.00 $\pm$ 0.0b	12.00 $\pm$ 3.3a	0.602 $\pm$ 0.1a
<i>Beauveria bassiana</i>	98.72 $\pm$ 25.8b	107.30 $\pm$ 5.6b	11.10 $\pm$ 9.2b	1.2 $\pm$ 0.83b	0.00 $\pm$ 0.0b	8.31 $\pm$ 1.6b	0.47 $\pm$ 0.07b
Control	102.04 $\pm$ 35.8a	139.53 $\pm$ 20.4a	13.41 $\pm$ 2.6a	3.60 $\pm$ 0.89a	4.00 $\pm$ 0.707a	10.38 $\pm$ 4.6a	0.61 $\pm$ 0.21a
	<u>Pepper</u>						
<i>Isaria tenuipes</i>	44.60 $\pm$ 2.7a	73.84 $\pm$ 14.9a	10.19 $\pm$ 1.5a	0.75 $\pm$ 0.9b	0.5 $\pm$ 0.57b	10.33 $\pm$ 1.5a	1.01 $\pm$ 0.09a
<i>Beauveria bassiana</i>	42.76 $\pm$ 3.03a	77.25 $\pm$ 11.1a	8.72 $\pm$ 1.2b	0.20 $\pm$ 0.4b	0.4 $\pm$ 0.54b	11.40 $\pm$ 1.8a	0.86 $\pm$ 0.13ab
Control	27.04 $\pm$ 6.2b	39.74 $\pm$ 25.1b	6.10 $\pm$ 1.2c	3.20 $\pm$ 1.3a	4.00 $\pm$ 1.0a	8.79 $\pm$ 4.1b	0.63 $\pm$ 0.9b

Data are means of five replications. Root galling and egg mass indices were based on 0-5 scale, where, 0: no galls/egg masses, 1: 1-2 galls/egg masses, 2: 3-10 galls/egg masses, 3: 11-30 galls/egg masses, 4: 31-100 galls/egg masses, 5: >100 galls (Taylor and Sasser, 1978).

The mean is an average of 5 replications. Means with the same letter within each column are not significantly different according to Duncan's multiple-range test ($P \geq 0.05$). Control = control, with nematodes.

Table 4. 4. The of means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, and egg mass indices at 1000 eggs or J2s/plant nematode inoculum level, 2000 μ L/plant, 0 level for each two fungi, and control, respectively, in first experiment (Mean $\pm$ SD).

1 st exp	<u>Cucumber</u>						
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)
<i>Isaria tenuipes</i>	118.44 $\pm$ 14.5a	141.40 $\pm$ 17.4a	18.98 $\pm$ 11.4a	0.20 $\pm$ 0.44b	0.6 $\pm$ 0.8b	9.41 $\pm$ 1.7b	0.55 $\pm$ 0.7b
<i>Beauveria bassiana</i>	120.04 $\pm$ 23.3a	138.22 $\pm$ 31.3a	13.76 $\pm$ 3.2b	0.20 $\pm$ 0.47b	0.2 $\pm$ 0.45b	8.33 $\pm$ 3.2b	0.56 $\pm$ 0.18b
Control	100.93 $\pm$ 11.6b	128.59 $\pm$ 14.7b	14.14 $\pm$ 2.5b	4.00 $\pm$ 0.0a	4.10 $\pm$ 0.0a	14.14 $\pm$ 2.5a	0.70 $\pm$ 0.09a
	<u>Pepper</u>						
<i>Isaria tenuipes</i>	44.6 $\pm$ 2.7a	73.84 $\pm$ 14.9b	10.19 $\pm$ 1.5a	0.75 $\pm$ 0.957b	0.5 $\pm$ 0.57b	10.33 $\pm$ 1.5b	1.17 $\pm$ 0.1a
<i>Beauveria bassiana</i>	42.76 $\pm$ 3.0b	77.25 $\pm$ 11.1a	8.73 $\pm$ 1.29b	0.2 $\pm$ 0.447b	0.4 $\pm$ 0.54b	11.44 $\pm$ 1.8a	0.86 $\pm$ 0.1b
Control	41.2 $\pm$ 5.2b	68.35 $\pm$ 27.8c	8.19 $\pm$ 3.30b	3.20 $\pm$ 1.3a	4.00 $\pm$ 1.0a	10.54 $\pm$ 5.2b	0.76 $\pm$ 0.3b

Data are means of five replications. Root galling and egg mass indices were based on 0-5 scale, where, 0: no galls/egg masses, 1: 1-2 galls/egg masses, 2: 3-10 galls/egg masses, 3: 11-30 galls/egg masses, 4: 31-100 galls/egg masses, 5: >100 galls (Taylor and Sasser, 1978). The mean is an average of 5 replications. Means with the same letter within each column are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

Control= control, with nematodes

Table 4. 5. The means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, and egg mass indices at 2000 eggs or J2s/plant nematode inoculum level, 1000 μ L/plant, 0 level for each two fungi, and control, respectively, in first experiment (Mean $\pm$ SD).

1 st Experiment		<u>Cucumber</u>					
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)
<i>Isaria tenuipes</i>	98.48 $\pm$ 11.7a	141.87 $\pm$ 1.3a	13.87 $\pm$ 1.3a	1.4 $\pm$ 0.89b	0.40 $\pm$ 0.54b	11.18 $\pm$ 2.2a	0.63 $\pm$ 0.14a
<i>Beauveria bassiana</i>	71.44 $\pm$ 11.5c	108.27 $\pm$ 19.1c	11.15 $\pm$ 2.3b	1.8 $\pm$ 0.83b	0.4 $\pm$ 0.54b	7.15 $\pm$ 1.08ab	0.33 $\pm$ 0.18b
Control	82.16 $\pm$ 15.7b	121.3 $\pm$ 26.8b	11.49 $\pm$ 2.8b	3.50 $\pm$ 0.0a	4.0 $\pm$ 0.0a	6.27 $\pm$ 2.2b	0.39 $\pm$ 0.12b
		<u>Pepper</u>					
<i>Isaria tenuipes</i>	42.24 $\pm$ 1.9b	67.28 $\pm$ 6.1b	8.54 $\pm$ 1.4b	0.6 $\pm$ 0.54b	0.6 $\pm$ 0.89b	9.84 $\pm$ 2.04b	0.81 $\pm$ 0.17b
<i>Beauveria bassian</i>	45.8 $\pm$ 5.4a	81.03 $\pm$ 13.9ab	10.61 $\pm$ 2.7a	0.6 $\pm$ 0.89b	0.8 $\pm$ 0.83b	10.91 $\pm$ 2.1ab	0.86 $\pm$ 0.19b
Control	44.18 $\pm$ 8.9a	89.75 $\pm$ 13.6a	10.23 $\pm$ 1.2a	4.00 $\pm$ 0.51a	4.40 $\pm$ 0.51a	12.15 $\pm$ 2.1a	0.91 $\pm$ 0.14a

Data are means of five replications. Root galling and egg mass indices were based on 0-5 scale, where, 0: no galls/egg masses, 1: 1-2 galls/egg masses, 2: 3-10 galls/egg masses, 3: 11-30 galls/egg masses, 4: 31-100 galls/egg masses, 5: >100 galls (Taylor and Sasser, 1978). The mean is an average of 5 replications. Means with the same letter within each column are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

Control= control, with nematodes.

Table 4. 6 The means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, and egg mass indices at 2000 eggs or J2s/plant nematode inoculum level, 2000 μ L/plant treatment level and control, respectively, in first experiment (Mean $\pm$ SD).

1 st Experiment		<u>Cucumber</u>					
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)
<i>Isaria tenuipes</i>	101.56 $\pm$ 15.9a	111.91 $\pm$ 48.8b	12.6 $\pm$ 3.1a	0.6 $\pm$ 0.89b	0.4 $\pm$ 0.54a	6.52 $\pm$ 3.01a	0.43 $\pm$ 0.15a
<i>Beauveria bassiana</i>	64.52 $\pm$ 8.8c	93.96 $\pm$ 20.8c	9.39 $\pm$ 1.8b	1.2 $\pm$ 1.09a	0.4 $\pm$ 0.54a	4.39 $\pm$ 0.7b	0.32 $\pm$ 0.03b
Control	82.16 $\pm$ 15.7b	121.3 $\pm$ 26.8a	11.49 $\pm$ 2.8ab	4.13 $\pm$ 1.0c	4.40 $\pm$ 0.4b	6.27 $\pm$ 2.2a	0.39 $\pm$ 0.12ab
		<u>Pepper</u>					
<i>Isaria tenuipes</i>	38.12 $\pm$ 5.8b	88.96 $\pm$ 16.7a	9.5 $\pm$ 2.3ab	0.20 $\pm$ 0.44b	0.40 $\pm$ 0.54b	11.81 $\pm$ 2.03a	0.87 $\pm$ 0.09ab
<i>Beauveria bassiana</i>	50.24 $\pm$ 7.2a	90.53 $\pm$ 11.5a	10.96 $\pm$ 1.9a	0.60 $\pm$ 0.54b	0.40 $\pm$ 0.54b	12.49 $\pm$ 2.5a	0.95 $\pm$ 0.18a
Control	41.2 $\pm$ 5.2ab	68.35 $\pm$ 27.8b	8.19 $\pm$ 3.3b	4.30 $\pm$ 5.0a	3.85 $\pm$ 4.4a	10.54 $\pm$ 5.2b	0.76 $\pm$ 0.32b

Data are means of five replications. Root galling and egg mass indices were based on 0-5 scale, where, 0: no galls/egg masses, 1: 1-2 galls/egg masses, 2: 3-10 galls/egg masses, 3: 11-30 galls/egg masses, 4: 31-100 galls/egg masses, 5: >100 (Taylor and Sasser, 1978).

The mean is an average of 5 replications. Means with the same letter within each column are not significantly different according to Duncan's multiple-range test ($P\leq 0.05$).

Control= control, with nematodes.

4.1.2. Second experiment

The second experiment revealed all the parameters were affected differently. It is seen that the effect of treatment level (L) on Green part fresh weight, Root fresh weight, and Root gall index and egg mass index ($P>0.05$) (Table 4.7). The nematode level (N) had a significant effect on only root gall index. Plant height, green part fresh weight root dry weight were affected by the treatment (T) significantly ($P>0.05$).

The interactions of the sources, we can notice that the nematode level and treatment (NxT) interaction was significant on the Green dry weight ($P<0.05$), Root Gall ($P<0.01$), and root dry weight. While the interaction of nematode level and treatment level (NxL), affected only the plant height significantly ($P<0.01$). The interaction of treatment and treatment level (TxL) affected the plant height, root galling and egg mass indices ($P<0.001$).

The effect of three interaction of Nematode level, Treatment, and Treatment level (NxTxL) was significant ($P>0.05$) on plant height, root galling, and egg mass indices ($P<0.001$) (Table 4.7 and 4.8).

Table 4. 7 Analysis of variance four treatments, two treatment levels, two nematode levels of *Meloidogyne javanica* and their interaction on the plant height, green part fresh weight, green part dry weight, root gall index for two plant varieties (cucumbers and pepper) in second experiment.

2 nd Experiment									
Source	df	Plant height	F- value	Green part fresh	F-value	Green part dry	F-value	Root galling	F- value
		(cm)		weight (g)		weight (g)		index	
Plant (P)	1	0.000***	191.719	0.000***	12.971	0.420	0.657	0.000***	27.324
Treatment levels (L)	2	0.149	2.117	0.004**	8.856	0.223	1.505	0.000***	27.546
Nematode levels (N)	2	0.484	0.489	0.852	0.035	0.161	1.995	0.022*	5.443
Treatment (T)	3	0.000***	7.182	0.006**	4.422	0.129	1.940	0.386	1.024
NxT	6	0.000***	15.703	0.000***	11.327	0.036*	3.450	0.005**	5.584
NxL	2	0.000***	19.413	0.401	0.712	0.345	0.901	0.497	0.465
TxL	6	0.003**	6.315	0.459	0.784	0.720	0.330	0.000***	10.589
NxTxL	6	0.006**	5.486	0.246	1.426	0.170	1.809	0.000***	14.842

* Significant at ($P \leq 0.05$), ** Highly Significant at ($P \leq 0.01$), *** Very Highly Significant at ($P \leq 0.001$)

Table 4. 8. Analysis of variance four treatments, two treatment levels, two nematode levels of *Meloidogyne javanica* and their interaction on root fresh weight, root dry weight, egg mass index for two plant varieties (cucumbers and pepper) in second experiment.

2 nd Experiment							
Source	df	Egg mass Index	F- value	Root fresh weight (g)	F- value	Root dry weight (g)	F- value
Plant (P)	1	0.217	1.546	0.618	0.250	0.000***	32.188
Treatment Level (L)	2	0.000***	20.961	0.300	1.086	0.151	2.096
Inoculums levels (N)	2	0.217	1.546	0.771	0.085	0.659	0.196
Treatment (T)	3	0.898	0.197	0.796	0.340	0.005**	4.535
NxT	6	0.460	0.784	0.179	1.752	0.000***	16.314
NxL	2	0.316	1.042	0.816	0.055	0.615	0.255
TxL	6	0.000***	10.762	0.756	0.281	0.528	0.644
NxTxL	6	0.001***	7.896	0.432	0.846	0.947	0.055

* Significant at ($P \leq 0.05$), ** Highly Significant at ($P \leq 0.01$), *** Very Highly Significant at ($P \leq 0.001$)

Data from low level of nematode inoculums 1000 egg or J2/plant with 1000 μ L/plant treatment is shown in Table 4.9.

The data for autumn experiment 2 for cucumber plant host, the highest value for height of plant was 64.72 ± 12.4 cm for control, and the lowest was 52.95 ± 4.4 cm for *Isaria tenuipes* treated plants. The highest green part dry weight was 5.73 ± 1.6 g for *Beauveria bassiana*, and the lowest was 4.53 ± 0.9 g for the control. The greatest root fresh weight was found in *Isaria tenuipes* treated plants as 6.5 ± 2.7 g in cucumber. The significantly lowest gall indices and egg mass indices were found in in cucumber both fungi compared to the controls (with nematodes) indicating the good results (Table 4.9).

Isaria tenuipes treated pepper plants sustained the greatest plant heights, the green fresh weight, the green part dry weight and the root fresh weight as 41.25 ± 3.5 cm, 55.68 ± 7.9 g 7.81 ± 1.0 g and 6.77 ± 1.7 g, respectively. *Beauveria bassiana*, and *Isaria tenuipes* sustained the lowest galling indices as 0.00 ± 0.0 (both) and egg mass indices 0.80 ± 0.23 and 0.25 ± 0.5 , respectively.

Data from low level of nematode inoculums 1000 egg or J2/plant with high level of 2000 μ L/plant is shown in Table 4.10.

For cucumber the highest value for height of plant was 73.36 ± 4.1 cm for *Beauveria bassiana*, and the lowest value was 62.8 ± 8.2 cm for control plants. The root dry weight numbers were relatively low. The highest galling index was found rightly in controls and the lowest were detected in both *Beauveria bassiana* and *Isaria tenuipes* agents as 1.4 ± 0.89 and 3.4 ± 1.80 , respectively. The data for egg mass indices for both fungi were also promising (0.6 ± 0.54 and 1.2 ± 0.44) as compared to the control (3.42 ± 1.5).

The data for pepper at low nematode inoculum level and high treatment level were varied. *Isaria tenuipes* treated pepper plant showed the greatest numbers of 35.36 ± 5.8 cm, 58.88 ± 11.0 g, 6.64 ± 1.15 g and 6.84 ± 2.3 g for plant height, green part fresh weight, the green part, dry weight and root fresh weight, respectively. The gall index was 0.00 ± 0.0 for bot fungi as compared to control of 3.25 ± 0.5 . The egg mass indices for *Isaria tenuipes* and *Beauveria bassiana* were 0.6 ± 0.894 , and 0.6 ± 0.548 , respectively as compared to the control of 3.42 ± 1.5 (Table 4.10).

Data from high level of nematode inoculums 2000egg orJ2/plant with low level of 1000 μ L/plant is shown in Table 4.11.

The data of experiment 2 at the high level of nematode and low level of treatments for cucumber plant host were relatively stronger than those of pepper. The control sustained the greater values of most parameters in this part of experiment for both hosts. In cucumber, the galling index for *Isaria tenuipes* and *Beauveria bassiana* were 2.2 ± 1.9 and 2.4 ± 1.5 , respectively as compared to control of 3.22 ± 0.8 .

The data for pepper host recorded was as follows; the highest value for the height of the plant was 37.24 ± 2.2 cm for *Isaria tenuipes*, and the lowest was 33.2 ± 4.2 cm for the control. The highest value for green fresh weight was 57.39 ± 9.8 g for *Beauveria bassiana*, and the lowest value was for *Isaria tenuipes* 46.43 ± 7.3 g. The highest and the lowest green dry weight were 7.46 ± 1.8 g and 5.73 ± 0.53 g for *Beauveria bassiana*, and *Isaria tenuipes*, respectively. The gall indices were 0.40 ± 0.548 and 0 for *Isaria tenuipes* and *Beauveria bassiana* as compared to a control of 3.20 ± 0.42 . The egg mass indices were 1.2 ± 1.3 and 0.60 ± 0.89 for *Beauveria bassiana* and *Isaria tenuipes*, respectively as compared to a control of 4.2 ± 0.51 (Table 4.11).

Data from high nematode inoculums level of 2000 egg or J2/plant with high level of 2000 μ L/plant is shown in Table 4.12.

There was not many differences in the data of cucumber in this part of the study. The most notable of the data occurred in the galling and egg mass indices. The galling indices in pepper for *Beauveria bassiana* and *Isaria tenuipes* were 2.4 ± 1.6 and 0.4 ± 0.54 as compared to the control of 3.3 ± 0.5 . The data of egg mass indices for *Beauveria bassiana* and *Isaria tenuipes* were 1.4 ± 1.5 and 0 as compared to the control of 4.16 ± 0.5 .

The highest numbers of 34.2 ± 2.3 cm, 53.16 ± 8.6 g $6.6.12 \pm 0.8$ g 7.02 ± 0.8 g and 0.54 ± 0.1 g were found for the plant height, green fresh weight, green part dry weight, root fresh weight and root dry weight, respectively in *Beauveria bassiana* treated pepper plants. The gall indices for *Isaria tenuipes* and *Beauveria bassiana* were 0.6 ± 0.548 and 0, respectively as compared to control of 4.4 ± 0.25 . The egg mass indices for *Isaria tenuipes* and *Beauveria bassiana* were 0.2 ± 0.44 , and 0.6 ± 0.55 , respectively as compared to control treatment of 3.22 ± 0.5 in the study (Table 4.12.).

Table 4. 9. The means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, and egg mass indices at 1000 eggs or J2s/plant nematode inoculum level, 1000 μ L, 0 level for each two fungi, and control, respectively, in second experiment. (Mean $\pm$ SD)

2 nd Experiment							
Treatments	<u>Cucumber</u>						
	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)
<i>Isaria tenuipes</i>	52.95 $\pm$ 4.4b	52.53 $\pm$ 11.9a	5.51 $\pm$ 0.99a	2.00 $\pm$ 1.8c	1.00 $\pm$ 0.816c	6.5 $\pm$ 2.7a	0.33 $\pm$ 0.13a
<i>Beauveria bassiana</i>	62.96 $\pm$ 6.4ab	48.46 $\pm$ 14.9b	5.73 $\pm$ 1.6a	3.00 $\pm$ 1.4b	1.6 $\pm$ 1.5b	5.12 $\pm$ 2.6ab	0.31 $\pm$ 0.09a
Control	64.72 $\pm$ 12.4a	29.47 $\pm$ 16.4c	4.53 $\pm$ 0.9b	4.20 $\pm$ 0.83a	2.80 $\pm$ 0.83a	4.12 $\pm$ 2.4b	0.212 $\pm$ 0.09b
	<u>Pepper</u>						
	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)
<i>Isaria tenuipes</i>	41.25 $\pm$ 3.5a	55.68 $\pm$ 7.9a	7.81 $\pm$ 1.00a	0.00 $\pm$ 0.0b	0.25 $\pm$ 0.5c	6.77 $\pm$ 1.7b	0.50 $\pm$ 0.087a
<i>Beauveria bassiana</i>	38.68 $\pm$ 4.4ab	50.00 $\pm$ 7.03b	6.25 $\pm$ 0.9ab	0.00 $\pm$ 0.0b	0.80 $\pm$ 0.23b	17.58 $\pm$ 23.9a	0.51 $\pm$ 0.09a
Control	31.20 $\pm$ 2.76b	49.15 $\pm$ 8.8b	5.42 $\pm$ 0.57b	4.20 $\pm$ 0.83a	3.40 $\pm$ 1.14a	6.27 $\pm$ 0.97b	0.41 $\pm$ 0.04b

Data are means of five replications. Root galling and egg mass indices were based on 0-5 scale, where, 0: no galls/egg masses, 1: 1-2 galls/egg masses, 2: 3-10 galls/egg masses, 3: 11-30 galls/egg masses, 4: 31-100 galls/egg masses, 5: >100 galls (Taylor and Sasser, 1978).

The mean is an average of 5 replications. Means with the same letter within each column are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

Control = control, with nematodes

Table 4. 10. The means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, and egg mass indices at 1000 eggs or J2s/plant nematode inoculum level, 2000 μ L, 0 level for each two fungi, and control, respectively, in second experiment (Mean $\pm$ SD).

2 nd Experiment		<u>Cucumber</u>					
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)
<i>Isaria tenuipes</i>	62.84 $\pm$ 10.9b	33.13 $\pm$ 13.3c	5.13 $\pm$ 1.1a	1.4 $\pm$ 0.89c	0.6 $\pm$ 0.54c	3.86 $\pm$ 1.14c	0.21 $\pm$ 0.08b
<i>Beauveria bassiana</i>	73.36 $\pm$ 4.1a	42.93 $\pm$ 8.2b	5.36 $\pm$ 0.5a	3.4 $\pm$ 1.8b	1.2 $\pm$ 0.44b	5.38 $\pm$ 2.5b	0.28 $\pm$ 0.07ab
Control	62.8 $\pm$ 8.2b	56.86 $\pm$ 20.5a	5.83 $\pm$ 1.8a	4.42 $\pm$ 0.5a	4.20 $\pm$ 0.83a	7.19 $\pm$ 4.5a	0.35 $\pm$ 0.15a
		<u>Pepper</u>					
<i>Isaria tenuipes</i>	35.36 $\pm$ 5.8a	58.88 $\pm$ 11.0a	6.64 $\pm$ 1.15a	0.00 $\pm$ 0.0b	0.6 $\pm$ 0.89b	6.84 $\pm$ 2.3a	0.48 $\pm$ 0.12a
<i>Beauveria bassiana</i>	34.16 $\pm$ 5.2a	44.9 $\pm$ 7.7b	5.5 $\pm$ 0.70ab	0.00 $\pm$ 0.0b	0.6 $\pm$ 0.54b	6.33 $\pm$ 1.7a	0.46 $\pm$ 0.11a
Control	31.08 $\pm$ 2.7b	34.31 $\pm$ 3.5c	4.4 $\pm$ 0.25b	3.25 $\pm$ 0.5a	3.42 $\pm$ 1.5a	5.45 $\pm$ 1.3a	0.34 $\pm$ 0.04b

Data are means of five replications. Root galling and egg mass indices were based on 0-5 scale, where, 0: no galls/egg masses, 1: 1-2 galls/egg masses, 2: 3-10 galls/egg masses, 3: 11-30 galls/egg masses, 4: 31-100 galls/egg masses, 5: >100 (Taylor and Sasser, 1978).

The mean is an average of 5 replications. Means with the same letter within each column are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

Control = control, with nematodes.

Table 4. 11. The means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, and egg mass indices at 2000 eggs or J2s/plant nematode inoculum level, 1000 μ L, 0 level for each two fungi, and control, respectively, in second experiment (Mean $\pm$ SD).

2 nd Experiment							
Treatments	<u>Cucumber</u>						
	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)
<i>Isaria tenuipes</i>	59.56 $\pm$ 8.8ab	44.72 $\pm$ 18.2b	5.71 $\pm$ 1.5a	2.20 $\pm$ 1.9b	1.0 $\pm$ 0.707b	6.2 $\pm$ 1.4b	0.34 $\pm$ 0.17a
<i>Beauveria bassiana</i>	56.4 $\pm$ 5.7b	24.6 $\pm$ 8.5c	4.95 $\pm$ 1.0b	2.4 $\pm$ 1.5b	1.25 $\pm$ 0.83b	3.7 $\pm$ 1.4c	0.22 $\pm$ 0.05b
Control	70.88 $\pm$ 4.04a	57.49 $\pm$ 2.1a	5.98 $\pm$ 1.6a	3.22 $\pm$ 0.8a	3.8 $\pm$ 1.0a	8.23 $\pm$ 3.7a	0.39 $\pm$ 0.13a
Treatments	<u>Pepper</u>						
	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)
<i>Isaria tenuipes</i>	37.24 $\pm$ 2.2a	46.43 $\pm$ 7.3b	5.73 $\pm$ 0.53b	0.4 $\pm$ 0.54b	0.6 $\pm$ 0.89c	5.65 $\pm$ 1.1b	0.44 $\pm$ 0.05b
<i>Beauveria bassiana</i>	35.76 $\pm$ 3.8b	57.39 $\pm$ 9.8a	7.46 $\pm$ 1.8a	0.00 $\pm$ 0.0b	1.2 $\pm$ 1.3b	7.26 $\pm$ 0.68a	0.52 $\pm$ 0.054a
Control	33.2 $\pm$ 4.2c	48.49 $\pm$ 8.5b	6.83 $\pm$ 0.9ab	3.20 $\pm$ 0.42a	4.2 $\pm$ 0.51a	6.32 $\pm$ 1.3ab	0.46 $\pm$ 0.11b

Data are means of five replications. Root galling and egg mass indices were based on 0-5 scale, where, 0: no galls/egg masses, 1: 1-2 galls/egg masses, 2: 3-10 galls/egg masses, 3: 11-30 galls/egg masses, 4: 31-100 galls/egg masses, 5: >100 galls (Taylor and Sasser, 1978). The mean is an average of 5 replications. Means with the same letter within each column are not significantly different according to Duncan's multiple-range test ($P \geq 0.05$).

Control = control, with nematodes.

Table 4. 12. The means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, and egg mass indices at 2000 eggs or J2s/plant nematode inoculum level, 2000 μ L, 0 level for each two fungi, and control, respectively, in second experiment (Mean $\pm$ SD).

2 nd Experiment							
<u>Cucumber</u>							
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)
<i>Isaria tenuipes</i>	67.0 $\pm$ 11.4b	29.42 $\pm$ 17.5b	5.88 $\pm$ 1.08a	0.4 $\pm$ 0.54c	0.00 $\pm$ 0.0c	2.83 $\pm$ 0.98c	0.17 $\pm$ 0.07b
<i>Beauveria bassiana</i>	64.12 $\pm$ 6.6c	31.45 $\pm$ 10.3ab	4.72 $\pm$ 0.5b	2.4 $\pm$ 1.6b	1.4 $\pm$ 1.5b	4.06 $\pm$ 1.3b	0.23 $\pm$ 0.06ab
Control	70.88 $\pm$ 4.04a	57.49 $\pm$ 19.1a	5.98 $\pm$ 1.6a	3.33 $\pm$ 0.5a	4.16 $\pm$ 0.5a	8.23 $\pm$ 3.75a	0.39 $\pm$ 0.13a
<u>Pepper</u>							
<i>Isaria tenuipes</i>	32.2 $\pm$ 5.7b	43.82 $\pm$ 5.7b	5.85 $\pm$ 0.96b	0.4 $\pm$ 0.54b	0.2 $\pm$ 0.44b	5.62 $\pm$ 1.3b	0.38 $\pm$ 0.06b
<i>Beauveria bassiana</i>	34.2 $\pm$ 2.3a	53.16 $\pm$ 8.6a	6.6 $\pm$ 0.8a	0.00 $\pm$ 0.0b	0.6 $\pm$ 0.54b	7.02 $\pm$ 0.8a	0.54 $\pm$ 0.09a
Control	31.08 $\pm$ 2.7b	34.3 $\pm$ 3.5c	4.4 $\pm$ 0.25c	3.22 $\pm$ 0.5a	4.61 $\pm$ 0.5a	5.45 $\pm$ 1.3b	0.34 $\pm$ 0.04b

Data are means of five replications. Root galling and egg mass indices were based on 0-5 scale, where, 0: no galls/egg masses, 1: 1-2 galls/egg masses, 2: 3-10 galls/egg masses, 3: 11-30 galls/egg masses, 4: 31-100 galls/egg masses, 5: >100 galls (Taylor and Sasser, 1978).

The mean is an average of 5 replications. Means with the same letter within each column are not significantly different according to Duncan's multiple-range test ($P \geq 0.05$).

Control = control, with nematodes.

4.1.3 Comparison of two experiment

The data for the spring season (experiment1) compared with the data for autumn season (experiment2) has shown a very highly significance difference ($P\text{-Value} \leq 0.001$) between all the parameters using t-test as shown in table 4.9., except for the gall index and egg mass index they were not significant, any differences could be regarded as random.

Table 4. 13 Comparison of two experiments by t-test with all source of variance (Mean $\pm$ SD).

Parameter	Experiment	Mean	Deviation	t-test P-Value	Decision
Plant height	1stexp	68.53	± 33.88	0.000***	VHS
	2 nd exp	49.09	± 16.36		
Green part fresh weight	1 st exp	98.06	± 36.99	0.000***	VHS
	2 nd exp	46.31	± 15.94		
Green part dry weight	1 st exp	10.92	± 4.15	0.000***	VHS
	2 nd exp	5.81	± 1.29		
Root fresh weight	1 st exp	9.99	± 3.29	0.000***	VHS
	2 nd exp	6.43	± 5.75		
Root dry weight	1 st exp	0.68	± 0.26	0.000***	VHS
	2 nd exp	0.38	± 0.15		
Gall index	1 st exp	0.83	± 1.14	0.058	NS
	2 nd exp	1.20	± 1.69		
Egg mass index	1 st exp	0.68	± 1.20	0.165	NS
	2 nd exp	0.90	± 1.11		

4.2. Discussion

The studies reveals that the second stage juveniles (J2) of *Meloidogyne javanica* entered the growing roots and their branches inter and intracellularly. The response of host is appears as hypertrophy and hyperplasia in the root tissue near the nematode feeding site. In response to hypertrophy cells became very large and contained dense and granular cytoplasm. Adjacent to the giant cells, the vascular tissue found to be disturbed. Shape, size and orientation of the vascular elements was so much altered that it had become difficult to trace the normal course of vascular strands. Root-knot nematodes are economically very important and need viable control tactics in order to diminish or minimize the crop loss. The root-knot nematode management tactics can be briefly categorized to genetical (resistance), cultural, physical, chemical and biological controls.

In plant nematology, there is a new term resistance is used to describe the ability of a plant to suppress development or reproduction of the nematode as reported by (Roberts, 2002). For root-knot nematodes, the symptoms can be evaluated with as sufficient ease, accuracy and precision as for some fungal diseases, such as leaf spot and rust. Therefore, the term resistance is also used to describe the capacity of a host to suppress the disease as defined by (Sasser et al., 1984; Roberts, 2002). Gall number and the degree of galling may be used to reflect the ability of a plant to lessen or overcome the attack by the root-knot nematode. However, they do not indicate nematode reproduction directly, while egg mass number, egg mass index and egg number per gram root do. This factor can be used in future studies to know the types of plants resistant to root-knot nematodes and the most resistant plant of the same type.

Biological control of nematodes are performed in several methods and control agents. Within this perspective fungi species can be an effective way of nematode control tactic to prevent second stage juvenile penetrate to host, grow and lay egg masses and form galls formation in host plants. Fungus root colonization apparently decreases the nematode population and the growth of plant suppression applied areas. Biological control is more inconsistent, less effective and slower acting than control normally achieved with chemicals. Although improvements in performance might be expected from more research on individual agents, it seems likely that these limitations are inherent in most biological control agents and that their successful application will depend on integration with other control measures.

The biological control agents for nematodes were extensively reviewed in recent years (Kerry, 1987; Stirling, 1991; Sayre and Walter, 1991; Sikora, 1992). Many scientists identified natural enemies with a range of modes of action similar to those currently studied by plant pathologists for the control of soil-borne diseases.

The most studied example of natural control of a plant-parasitic nematode concerns the decline of populations of the cereal-cyst nematode, *Heterodera avenae*, under monocultures of susceptible cereals in many soils throughout northern Europe (Kerry, 1982). This is an example of an induced suppression in which the nematode increases to damaging population densities in the second and third cereal crops, but usually declines thereafter to infestations of <5 eggs/g soil, which cause little loss of yield in northern European conditions; it is essential that the nematode is abundant in the early years of the monoculture to support the build-up of the microbial parasites as reported by (Kerry, 1988). The decline in nematode populations is mainly caused by two parasitic fungi, *Nematophthora gynophila* and *Verticillium chlamydosporium*, which attack the developing female on the root surface; in suppressive soils 95 to 97 percent of the females and eggs are destroyed as reported by (Kerry, Crump and Mullen, 1982).

Studies demonstrated in several soils that (nematophagous) fungi and bacteria increase under some perennial crops, and under those grown in monocultures, and so may control some nematode pests, including cyst and root-knot nematodes as reported by (Stirling,

1991). Such nematode-suppressive soils have been reported from around the world and include some of the best documented cases of effective biological control of nematode pests.

Culture filtrate of *Beauveria bassiana* in greenhouse conditions, was found by Ting et. al. 2008, that they decrease significantly the production of gall formation, nematode population in the soil, in the roots and egg mass. In our study results it was shown that the plant height of two fungi was higher than those of positive control significantly.

Results shown in our experiment that there was a significant effect of treatments on root galling and egg mass indices as compared to control. This implies that treatments reduced nematode damage to plants significantly. Generally, we can say that both fungi species were effective in reducing nematode damage and reflected the other parameters including plant height and green part fresh weights.

There are some other fungi species that found effective in controlling nematodes. A study by Al-Raddad (1995) revealed that nematophagous fungus, *Paecilomyces lilacinus* was the most effective agent for controlling nematodes which supported our founding in this study. Entomopathogenic fungi was clearly observed for reducing the nematodes such as their gall number and egg masses, causing various response in considering root weight.

Soil application of *P. chlamydosporia* and *P. lilacinus* significantly improved the dry root weight (%6 to %7) over the control group as concluded by Khan et al., (2012). They stated also that the total weight of the fruits/plant was, however, significantly greater with neem leaf treatment (19%), followed by *P. lilacinus* (14.5%), *P. chlamydosporia* (11.4%), *T. harzianum* (9.6%), and Aldicarb (11.8%). In our study, we determined that *Beauveria bassiana* treated both pepper and cucumber maintained greater values than those of the remaining parameters. In our study, we determined that *Beauveria bassiana* treated both pepper (120.04 ± 23.3 cm) (Table 4.4.), and cucumber (50.24 ± 7.2 cm) hosts (Table 4.6). The biggest value for green plant part fresh weight (48.46 ± 14.9 g) and the green part dry weight (5.73 ± 1.6 g) was found in *Beauveria bassiana* treated cucumbers (Table 4.9).

Trichoderma viride and *Beauveria bassiana* showed significantly in plant height and in reducing number of root knot nematodes (Pant and Pandey, 2014). Their results aligned with ours, based on the effectiveness of *Beauveria bassiana* followed by *Isaria tenuipes*.

5. CONCLUSIONS

Because of being associated with the environmental problems, use of nematicides led great controversy. Recently, great attention is given to alternative management tactics, such as cultural tactics, plant resistance and biological control of nematodes with microbial agents. In pest management and natural environment, the major conservation of biological control strategy, is focused through studies of entomopathogenic fungi. The control the pests was mainly done through the usage of natural enemies. In order to control the root-knot nematode, many studies are focusing on using biological control as a safety for environment and health of humans, also to prevent harmful effects to the environment. Both bio-pesticides and antagonistic microorganisms can be highly effective against root-knot nematodes when applied properly and timely.

In our study we determined that there was decrease of root galling and egg mass indices which increased the plant height, green fresh and dry weight, root fresh and dry weight in cucumber and pepper in the first experiment, due to the lower effect of nematode on the above parameters in (Table 4.9). Whereas in the second experiment the parameter values were lower significantly due to the change of time and the effect of nematode in the second season. Furthermore, significant differences in the gall index and egg mass, actually reduced in both parameters concludes that the plants were stronger in spring compared with autumn that increased the average of gall index and egg mass as compared to the controls.

This concludes that in the first experiment, reducing egg mass indices in both cucumber and pepper hosts in both low and high inoculums was affected highly by the *Beauveria bassiana* and *Isaria tenuipes*. While the plant height, green fresh and dry weight, root fresh and dry weight in cucumber and pepper were increased by *Beauveria bassiana*, the gall and egg indices were also reduced by *Isaria tenuipes* treated plants.

We can rightly conclude that both *Beauveria bassiana* and *Isaria tenuipes* used in this study are good bio-agents for controlling root-knot nematodes. Therefore it is highly recommended for farmers others to adopt these two fungi to include into the nematode management tactics to prevent crop loses.

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Bachelor's	University of Sulaimaniya College of Agriculture Plant production Department	2008-2009
Master Degree	KSU, Graduate School of Natural and Applied Science, Bioengineering and Sciences	2015-2016

LANGUAGE SKILLS

Kurdish , Arabic and English