NEMATICIDAL EFFICACY OF SOIL FUNGAL ISOLATES AGAINST THE ROOT-KNOT NEMATODE (*Meloidogyne incognita*) OF TOMATO

BY

UMAR, Maishera Safiya

MTech/SLS/2017/6920

DEPARTMENT OF PLANT BIOLOGY

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA

MAY, 2021

ABSTRACT

The root-knot nematode (*Meloidogyne incognita*) is one of the plant parasitic nematodes that causes serious economic damage on tomato plant. Due to loss of effectiveness and environmental hazard of chemical nematicide, the search for new agents of control has become paramount. Therefore, nematicidal efficacy of soil fungal isolates on second stage juvenile of *Meloidogyne incognita* was carried out. Soil samples for the isolation of fungi and nematode were collected from botanical garden and infested soil of tomato farms. Fungal species were isolated using serial dilution method while nematode was extracted using pie-pan method. The *in vitro* antagonistic effects of fungi against *M. incognita* were assessed in potato dextrose broth. *In vivo* was carried out in screen house where healthy and sterilized tomato seeds were sown in a 7 litre plastic buckets containing 5 kg of sterilized loamy soil. The buckets were arranged in a completely randomized block design on a bench with three (3) replicates. After germination of the tomato seeds, two weeks from sowing,each bucket was thinned to one plant. One thousand (1000) juvenile second stage (J2) *M. incognita* were inoculated in four holes round the stem of each plant in a bucket and on the next day 10 ml of fungi culture filtrates was inoculated into the same hole. Four fungal species isolated were; *Aspergillus niger, Aspergillus nidulans, Penicillium chrysogenum* and *Rhizopus stolonifer* while nematode isolated was *M. incognita*. The results of fungicidal activity against *M. incognita* after 24 and 48 hrs were *A. niger* (39.28 and 78.47 %), *A. nidulans* (29.12 and 57.49 %), *P. chrysogenum* (18.59 and 47.48 %) and *R. stolonifer* (8.05 and 26.43 %). In *in vivo* experiment, *A. niger* gave the highest values for average plant height at maturity, number of fruits per plant and number of leaves per plant (46.43 cm, 10.33, and 69.67 respectively) and lowest value for number of galls per plant root (3.67). Lowest values were recorded for *R. stolonifer* in average plant height, number of fruits per plant and number of leaves per plant (26.13 cm, 5.33 and 46.33 respectively) and were the highest value for number of galls per plant roots (16.67). The values recorded for *A. niger* and *R. stolonifer* differed significantly ($P< 0.05$). Disease incidence was highest in the control at harvest after treatment with the value 39.49 % and *A. niger* had the lowest value of 7.18 % at harvest after treatment, the values recorded for the control and *A. niger* differed significantly ($P< 0.05$). The results of this study conclude that these fungal species could serve as biological agent for effective management of root-knot nematodes and bio-fertilizer for the enhancement of plant growth parameters.

TABLE OF CONTENTS

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURE

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Tomato (*Solanum lycopersicum*) is an annual crop belonging to the family solanaceae. It is one of the most important vegetable crops which is being cultivated worldwide. The crop is a rich source of lycopene, used in the cancer treatment especially in prostate cancer (Singh *et al*., 2011). According to the National Cancer Institute, there is enough data to show that people who consume large amount of tomato products have significantly decreased risk of prostate, lung, and stomach cancer (Adam *et al*., 2015). Nigeria is ranked to be the second largest producer of tomato in Africa with an annual production of 17,500,000 metric tons (FAO, 2012). However, large number of phyto-pathogens (sting caused by *Belonolaimus longicaudatus* and stubby-root caused by *Trichodorus* spp.) have been reported to be associated with reduction in tomato productivity. Among them, root-knot nematodes which had been reported to be the major pathogen affecting the crop (Singh *et al*., 2011; Keshari and Gupta, 2015).

Meloidogyne incognita causes root-knot disease in tomato plant by abnormal expansion of root cell forming giant cells (Singh and Patel, 2013). *M. incognita* is one of the key nematodes which is widely distributed (Siddiqui and Shaukat, 2014) and difficult to control (Chitwood, 2016) because of its high reproduction rate, resulting into massive yield loss of tomato fruits ranging from 32 to 40 % (Anwaar and Mckenry, 2012). *M incognita* has also been identified as one of the three most economically damaging genera of plant parasitic nematodes on horticultural and field crops. Obligate parasites of roots of thousands of plant species, including monocotyledonous, dicotyledonous, herbaceous and woody plants. The

genus includes more than 90 species (Moens *et al*., 2015), with some species having several races. *M. incognita* occurs in 23 of 43 crops listed as having plant parasitic nematodes of major importance, ranging from field crop, through pasture and grasses to horticultural, ornamental and vegetable crops (Acquaah, 2012). If root-knot nematodes become established in deep-rooted, perennial crops, control is difficult, and options are limited.

However, fumigant and non-fumigant nematicides have been developed for controlling this nematode over years, but there are increasing concerns over risks of these chemicals on the environment and human health which led to the withdrawal or restriction of some chemicals, for instance methyl bromide, the most effective and widely used soil fumigant has already been banned in developed countries (Nyezepir and Thomas, 2016). The use of some non-fumigant nematicides organophosphates and carbamates based has been restricted depending on the region, crop, and production system, such as integrated production and organic farming. Consequently, the search for naturally occurring compounds with nematicidal activity has been stimulated as an alternative to using existing compounds (Chitwood, 2016). Soil fungi that inhabit the rhizosphere may serve as a source for such compounds since they share the same environment as the nematodes and produce metabolites as a strategy for protecting their habitat from plant-parasitic nematodes or other organisms (Moosavi and Zare, 2012).

These fungi may release compounds that directly kill nematodes, suppress nematode motility, reduce egg hatching and interfere with metabolic processes, through such mechanisms, they could regulate nematode populations (Moosavi and Zare, 2012). According to Kumar *et al*. (2010) who study the culture filtrates of *Aspergillus niger* and were most effective against second stage juvenile followed by *Fusarium moniliforme, F.*

solani, F. oxysporum and *P. debarianum.* Satyandra and Nita. (2010) carried out *in vitro* study to determine the efficacy of indigenous fungi *Acremonium strictum, Aspergillus terreus, A. nidulans, A.niger, Chetomium aubense, Chladosporium oxysporum, Fusarium chlamydosporium, F. dimarum, F. oxysporum, F. solani, Paecilomyces lilacinus, Pochonia chlamydosporia, Trichoderma viride* and *T. harzianum*on egg parasitism, egg hatching, mobility and mortality of root-knot nematode, *M. incognita* in which all tested fungi showed varied effects against the nematode. Kalele *et al*. (2010) studied the bio-control potential of *Paecilomyces lilacinus* on *M. incognita* on tomato under glasshouse conditions.

1.2 Statement of the Research Problem

Despite the production and market value of tomato, several reports indicated that *Meloidogyne incognita* is the major problem in tomato growing areas which results in poor growth, decline in quality and yield of the crop, whereby, a high level of damage can lead to total crop loss (Esheteu *et al*., 2014). Chemical nematicides have been used for nematode control for the last 50 years (Nyezepir and Thomas, 2016). However, the most limiting factors in using synthetic nematicides in developing countries is that they are either too expensive, sub-standard or due to lack of technical knowledge. Excessive use and poor handling of these chemicals can lead to health hazard, nematode resistance and destabilization of the ecosystem of the crop and reduced resistant to other stresses (e.g drought, other diseases) (Huang, 2016). Therefore, there is an increasing requirement for developing non-chemical alternative methods for the management of *M. incognita*.

1.3 Justification for the Study

Tomato is one of the most economically important crops majorly consumed worldwide and worth a tremendous amount of money due to its massive yield (Abdissa *et al*., 2010).

Bio-management of root-knot nematode by *Trichoderma* spp. has been found safe for soil biota, it is a well-known alternative to chemical nematicide specially for the purpose of protecting crops against nematodes (Adam *et al*., 2014).

Paecilomyces lilacinus isolates were comparatively effective in controlling the cyst nematode and their use resulted in a significant increase in tomato yield (Ashoub *et al*., 2018). Soil fungi that were commercially produced such as *Pochonia clamydosporia, Trichoderma longibrachiatum, Paecilomyces lilacinus*, they revealed promising results as effective biological control agents on root-knot nematode in tomato as reported by Dallemole-giaretta (2014).

Fungi release compounds such as oxalic acid and kojic acid that directly kill nematodes, suppress the nematode motility, reduce egg hatching and interfere with metabolic processes and through such mechanism, they could regulate nematode populations (Moosavi and Zare, 2012).

It is therefore important to evaluate a secure alternative like the soil fungi which is harmless to tomato and the environment.

1.4 Aim and Objectives of the Study

The aim of this study was to determine the nematicidal efficacy of soil fungal isolates against the root-knot nematode (*Meloidogyne incognita*) of tomato (*Solanum lycopersicum*).

Objectives of this research are to:

i. isolate and identify the fungi from soil samples collected from the study site.

ii. extract and identify *Meloidogyne incognita* from soil samples collected from tomato vendor in Bosso market Niger State.

iii. to evaluate efficacy of fungal culture against second stage juvenile (J2s) of *Meloidogyne incognita in vitro*.

iv. to find out the impact of these fungal culture on plant growth parameters of tomato plants, root-gall formation and disease incidence *in vivo*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of Tomato

Tomato (*Solanum lycopersicum* L.) belongs to the Family Solanaceae, Order Solanales and Genus *Solanum*. Tomato plants are vines, initially decumbent, typically growing 180 cm (6 ft) or more above the ground if supported, although erect bush varieties have been bred, generally 100 cm (3 ft) tall or shorter (Sato *et al*., 2012).

Tomato plants are dicots and grow as a series of branching stems with a terminal bud at the tip that does the actual growing. When that tip eventually stops growing, whether because of pruning or flowering, lateral buds take over and grow into other fully functional vines (Peet, 2018). Tomato vines are typically pubescent, meaning covered with fine short hairs. These hairs facilitate the vining process, turning into roots wherever the plant is in contact with the ground and moisture, especially if the vines connection to its original root has been damaged or severed (Sato *et al*., 2012).

Most tomato plants have compound leaves, and are called regular leaf plants, but some cultivars have simple leaves known as potato leaf style because of their resemblance to that particular relative of regular leaf plant, there are variations such as rugose leaves which are deeply grooved and variegated, angora leaves which have additional colors where a genetic mutation causes chlorophyll to be excluded from some portions of the leaves Tomaat (Lee *et al*., 2019). The leaves are 10-25 cm (4-10 in) long, odd pinnate with 5-9 leaflets on petioles each leaflet is up to 8 cm (3 in) long with a serrated margine, both the stem and leaves are densely glandular hairy (Asare *et al*., 2017). There flowers, appearing on the

apical meristem have the anthers fused along the edges forming a column surrounding the pistil's style. Flowers in domestic cultivars can be self- fertilizing, they are 1-2 cm (0.4-0.8 inches) across, yellow, with five pointed lobes on the corolla. Although in culinary terms, tomato is regarded as a vegetable, its fruits are classified botanically as berry. As a true fruit, it develops from the ovary of the plant after fertilization, its fresh comprising the pericarp walls. The fruits contain hollow spaces full of seeds and moisture called locular cavities and they vary among the cultivated species, types, cavities and globe- shaped varieties (Munos *et al*., 2011).

2.2 Economic Importance of Tomato

Currently, about 40 % of the domestic market sales of products in Nigeria come from horticultural crops in which tomato is one of the most important crops grown in the country (Donkoh *et al*., 2013). Tomato is cultivated continuously throughout the year. In Nigeria, the record confirmed that approximately 1.8 million metric tons of fresh fruits were produced for domestic consumption, with national demand of about 2-3 million metric tons annually and has demand gap of about 500,000 metric tons (FAO, 2010).

This sector, as a component of agriculture, employs large numbers of people. Other factors that make tomato important so much in this country is that it has a constituent in almost all diets. The fruit is eaten either raw or cooked in the form of soup, jam, ketch-up and salad, among others (Bais *et al*., 2016). It is a good source of vitamins and minerals which help to make a balanced diet for children and pregnant women (Preedy and Waltson, 2019). The leaf is used for medicinal purposes for example to cure ring-worm; a fungal disease that affects the skin (Hassan *et al*., 2010).

2.3 Constraints in Tomato Production

Tomato production is adversely affected by several factors, which include high cost of labour, climatic factors, inadequate marketing, storage systems and diseases (Hwang *et al*., 2014). It has been estimated that an average of over 40 % of the yield of tomato is lost annually due to diseases. Disease pathogens do not only reduce the yield of tomato in terms of quantity, but also reduce quality (Abubakar *et al*., 2016). Tomato is prone to infection by many diseases right from the seedling stage to harvest. Several fungi, insects, viruses, bacteria and nematodes frequently attack tomato plants as well as fruits (FAO, 2012).

Nematodes cause serious problems in all tomato growing areas, majority of the tomato varieties, both cultivated and wild, are parasitized by one or several nematode species (Yang *et al*., 2016). In Nigeria, Osei *et al*. (2011a) who observed higher populations of plant-parasitic nematodes on tomatoes than on Velvet bean (*Mucuna pruriens* L.) and Tree marigold *Tithonia diversifolia* (Hemsl.). Several species of nematodes attack the crop, but the most predominant species is the root-knot nematode (*Meloidogyne* spp.) (Kwara *et al*., 2014).

2.4 Rhizosphere Microorganisms

Productivity of plant community results from interactions of shoots and roots with the environment. One of the more important and least understood biotic zones of interaction is the soil immediately surrounding the roots and influenced by them: the rhizosphere. The great majority of organisms in the rhizosphere belong to the micro-organisms and these include representatives of all the major groups: bacteria, actinomycetes, fungi, protozoa and microalga. In addition, microfauna, such as nematodes and mites, are found at significantly elevated concentrations in the rhizosphere as compared to root-free soil (Xiang *et al*., 2017).

Microorganisms and processes in the rhizosphere profoundly influence plant growth through effects on uptake, storage, and cycling of nutrients, suppression of pathogens and development of soil structure. Protecting the functional biodiversity of rhizosphere organisms through proper management practices is essential to maintaining ecosystem health and resiliency (Benizri *et al*., 2011).

2.5 Nematodes

Nematodes are the most abundant multicellular animals on earth. Numerically, between 80 and 90 % of all multicellular animals on earth may be nematodes (Compant *et al*., 2010; Khan *et al*., 2019). Nematodes can be found in different environments, e.g soil, sea or fresh water, as free-living, parasitic or predacious animals. Their food sources include plants, algae, bacteria, fungi and other nematode species, as well as almost all vertebrates including fishes, and invertebrates like insects and spiders. Large amounts of nematodes are found within the top 10 cm of the soil, although some may be living much deeper (Cao *et al*., 2011).

Nematodes are so abundant and relatively easy to sample they have been much used as indicators of soil conditions (Yeates, 2011; Sohlenius, 2011; Ferris *et al*., 2019). Nematodes have widely differing nutritional behaviors and therefore occupy several trophic levels in soil food webs. They can be grouped according to the type of food they consume, based on the morphology of their mouthparts. The most common groups are plant-parasitic, fungivorous, bacterivorous, omnivorous and predatory nematodes (Bawa *et al*., 2014).

2.5.1 Plant-parasitic nematodes

Plant-parasitic nematodes obligatorily feed on plant tissue. They cause serious damage to many crops worldwide. Their damages have exceeded $10 billion per year in the United States (Mendes *et al*., 2013). Members of this group have a stylet in their mouth and use it to penetrate the plant cells wall to absorb their contents. In addition to their direct effect on plant health, nematodes can play different roles in disease complexes; they act as vectors (e.g for several viruses), wounding agents, host modifiers, resistance breakers and rhizosphere modifiers; they may cause increased root exudation, thereby affecting microbial communities and activity in the rhizosphere (Brussaard *et al*., 2015; Desaeger *et al*., 2019). These nematodes do not all have equal effects on plants. Nematodes that feed shallowly on the root cortex or epidermis (e.g *Helicotylenchus, Paratylenchus*) usually have far less effect on plant productivity and energetics than vascular parasites e.g *Meloidogyne, Heterodera* (Anonymous, 2012). On the other hand, the plant species and their ages affect the infection for example, seedlings are particularly exposed to damage by nematodes because their tissues are more susceptible to attack by parasites and more favorable for nematode development (Von *et al*., 2017).

2.6 Root-knot Nematodes

Root-knot nematodes *Meloidogyne* spp. are plant-parasitic nematodes. About 2000 plants are susceptible to their infection and they cause approximately 5 % of global crop loss (Hussey and Janssen, 2016). Root-knot nematodes infection starts when infective second-stage juveniles (J2s) hatch from eggs in the soil and penetrate plant roots behind the root cap and migrate intercellularly in the cortical tissue to the vascular cylinder and then become sedentary. Then they inject secretions into five to seven undifferentiated procambial cells in the vascular cylinder near the head of the J2 to become multi-nucleate

(Brussaard *et al*., 2015) and to form very specialized feeding cells called giant cells, on which the second juveniles (J2) and later the spherical females feed (Brussaard *et al*., 2015). Furthermore, cell size increases dramatically, walls are remodeled by formation of ingrowths and the cytoplasm becomes dense with an increase in cell organelles (Diedhiou *et al*., 2018). The nourishment from the giant-cells helps the root-knot nematodes complete their life cycle. Females lay eggs into a gelatinous matrix outside the roots surfaces (Bhattacharjee and Dey, 2014).

The infection of the root systems by phyto-parasitic nematodes of the genus *Meloidogyne* is easily recognized by one formation of galls. These galls descend from the development of the giant cells and the females inside the roots. Root-knot nematode damage results in poor growth, a decline in quality and yield of the crop and reduced resistance to other stresses (e.g. drought, other diseases). A high level of root-knot nematode damage can lead to total crop loss. Nematode damaged roots do not utilize water and fertilizers effectively, leading to additional losses. Infection of young plants may be lethal, while infection of mature plants causes decreased yield. *Meloidogyne* species constitute the major nematode problem in developing countries. The most common species is *M. incognita* (Kofoid and White) Chitwood, which causes considerable losses in many crops (Meyer and Roberts, 2014).

Meloidogyne spp. can be managed by cultural practices, resistant cultivars and chemical nematicides, one of the primary means of control for root-knot nematodes. Multiplication of nematodes can also be regulated by soil microorganisms such as Bacteria, Nematophagous, and Mycorrhizal fungi (Miyashita *et al*., 2014).

2.7 Distribution and Economic Importance of Root-knot Nematodes

Root-knot nematodes (RKN) are plant parasites of the genus *Meloidogyne*, family Heteroderidae. Their common name refers to the characteristic galls or root-knots associated with nematode infestation. This genus comprises more than 90 species, with some species having several races (Hunt and Handoo, 2016). It also includes some of the most widespread and economically damaging nematodes worldwide, like *M. incognita, M. javanica, M. arenaria, M. hapla, M. chitwoodi and M. enterolobii*. Root-knot nematodes occur throughout the world with some species being primarily distributed in tropical and sub-tropical climates such as *M. incognita, M. javanica, M. arenaria* while others are well adapted to temperate or cool climates such as *M. hapla, M. chitwoodi* and *M. fallax.* 1000 root-knot nematode (RKN) populations collected in 75 countries, 53 % were identified as *M. incognita*, 30 % as *M. javanica*, 8 % as *M. arenaria*, 8 % as *M. hapla* and 2 % as *M. exigua* or other species (Siddiqui and Shaukat, 2014). However, discovery of *Meloidogyne* species previously unknown will continue to appear as farming practices evolve and our knowledge and expertise extends, especially into resource-poor areas, where information on species identity and distribution is less than optimal. *Meloidogyne* spp. is also a difficult group of nematodes to identify, but as diagnostic techniques improve, our ability to accurately identify species and their distribution improves. (Castillo and Volvas, 2009)

Root-knot nematodes are obligate parasites which cause significant damage on roots of many plant species (Nurul *et al*., 2016). Damage caused by root-knot nematodes is related to their population densities in soils at sowing and their reproduction potential (Heve *et al*., 2015). Perry *et al*. (2019) stated that damage thresholds have been established for several crops, where the average is approximately 0.5-2 juveniles/mL of soil (or from the lower limits of detection, over 1000 individuals/500 cm^3 of soil). Janati *et al*. (2018) reported that

root-knot nematodes are particularly damaging to vegetables in tropical and subtropical countries of the world and cause losses up to 80 % in heavily infested fields.

Many crops grown as vegetables mainly tomato, okra and eggplant are susceptible to root-knot nematodes. Estimates of vegetable crop losses due to root-knot nematodes, mainly *M. incognita* and *M. javanica*, have ranged from 17 to 20 % for eggplant and 24 to 38 % for tomato (Wesemael *et al.*, 2011). According to Burkett *et al*. (2009) damage caused by root-knot nematodes on eggplant, tomato and okra were estimated at 18, 54 and 91 %, respectively.

2.8 Life Cycle of *Meloidogyne* spp.

Mature females of root-knot nematode (RKN) remain with their head in the galled root tissue and deposit up to 1000 eggs into a gelatinous matrix (egg sac or egg mass) that is protruding to the posterior end of the female on the root surface. The gelatinous matrix protects the eggs from dehydration and attack by microorganisms (Belay *et al*., 2016).

Within eggs the embryo rapidly develops to a first-stage juvenile (J1s) that molts within the egg to the infectious second-stage juvenile (J2s). After hatching the J2s migrates through the soil in search of a suitable host which can be the parent plant or a nearby new host. Juveniles invade the root in the zone of elongation. With the help of their stylet they burrow into the root with no obvious damage. From the site of penetration juveniles migrate inter-cellularly towards the root tip where they turn around and move into the differentiating vascular cylinder (Miyashita *et al.,* 2014). Once they have reached the zone of proto-xylem development they initiate a feeding by injecting pharyngeal gland secretions (the saliva) into root cells, which induces nuclear division without cytokinesis (Smant *et al*., 2018).

This process gives rise to large, multinucleate cells causing dramatic physiological changes in the parasitized cells, transforming them into giant cells, which seriously impair plant nutrient and water uptake (Trudgill and Blok, 2014).

Concomitant with giant cell formation, the surrounding plant cells enlarge and divide rapidly, resulting in the formation of a gall (Escober *et al*., 2015). At the site of penetration and gall formation secondary roots can form resulting in extensive formation of lateral roots. In further juvenile development, the metabolic capacity of the feeding site increases funnelling plant resources to the feeding nematode (Escober *et al*., 2015). Within few days of feeding, the J2s grow thick like a sausage and undergo three more molts before transforming into adults. The fourth-stage juvenile (J4s) already distinguishes between male and female. The vermiform males emerge from the root and migrate into the soil. Females keep swelling to become saccate or pear-shaped. At this stage, the female is large enough to be seen with the naked eye when galled root tissue is teased apart. Although sexual propagation does occur in some root-knot nematode (RKN) species, main propagation is by parthogenesis and with egg deposition the cycle is completed, under favourable condition, the life cycle may be completed within 30 days and several generations can develop in one season (Castagnone-Sereno, 2014).

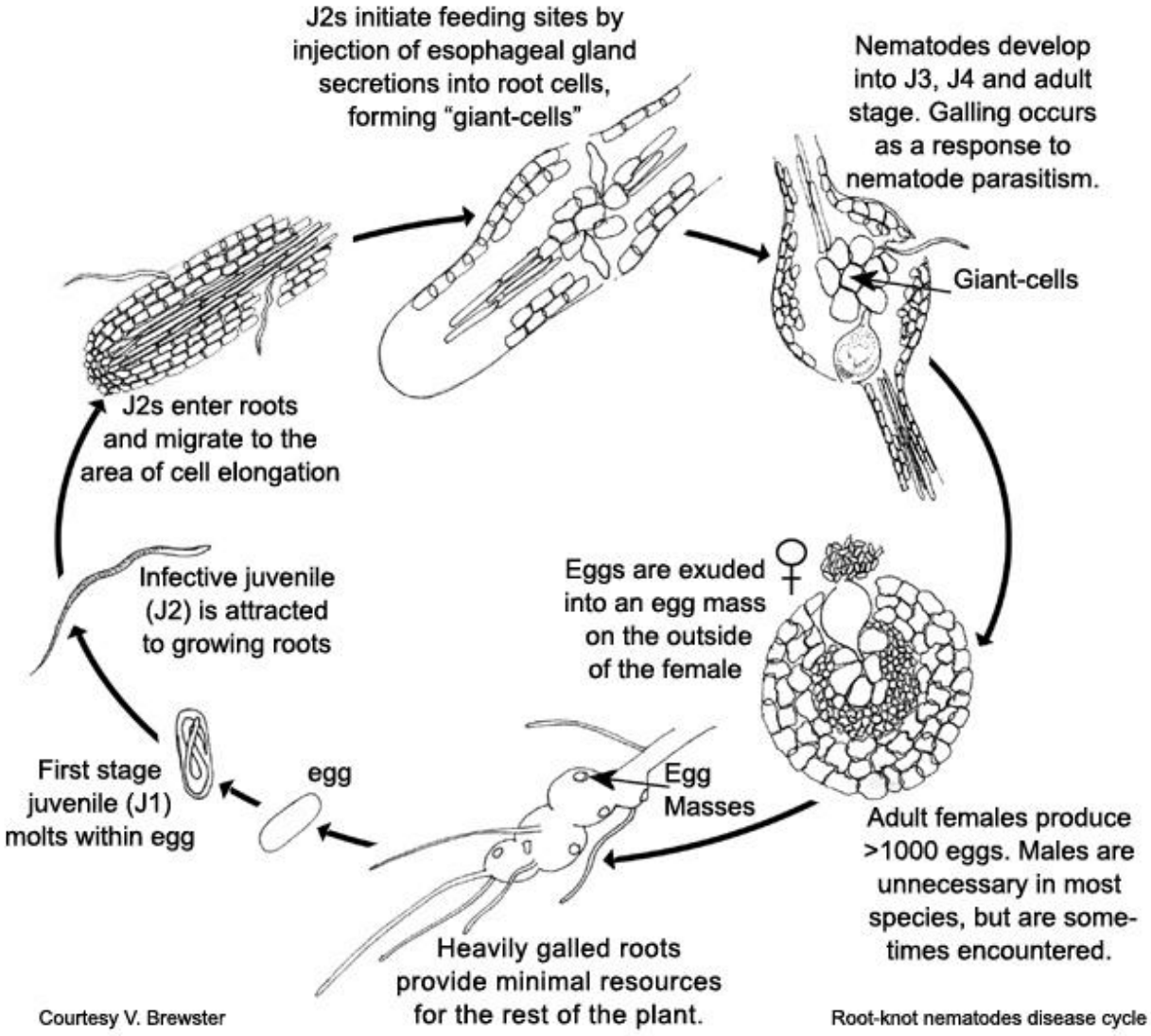


Figure 1: *Meloidogyne* Life Cycle

Source: American Phytopathological Society (2012)

2.9 Means of Survival of Root-knot Nematodes

Root-knot nematodes are obligate parasites, therefore, the absence of suitable host plants for long period ultimately leads to their death (Karssen and Moens, 2015). Survival strategies of root-knot nematodes are dependent on abiotic and biotic conditions. In the absence of susceptible crops, however, they often survive on alternative hosts such as

weeds (Manzanilla *et al*., 2017). In general, favourable condition for plant growth are also favourable for *Meloidogyne* reproduction. Mao *et al*. (2017) found that the optimum moisture levels for hatching of *M. incognita* juveniles from the egg were slightly above field capacity. Survival is mainly influenced by moisture content of the soil and, to a lesser extent, by temperature (Karssen and Moens, 2015). Under adverse condition, where host plants are absent, juveniles will use their energy reserves in the soil as a means of survival (Wang *et al*., 2019). Juveniles and eggs survive period of moisture stress in the state of an hydrobiosis which enables long-term survival in stressful environments (Evans and Perry, 2018). In many genera, the eggs are the survival stage, being protected in a gelatinous matrix (Regina *et al*., 2016). Egg masses collected from dry soil contain empty eggs and an hydrobiotic eggs with second stage juveniles in diapause (Karssen and Moens, 2015). Most nematodes can undergo temporary quiescence in response to environmental stress. The incidence of diapause differs greatly between species of root-knot nematodes and between populations of the same species (Evans and Perry, 2018).

2.10 Reproduction and Development of Root-knot Nematodes

Root-knot nematodes are associated with three types of reproduction, mitotic parthenogenesis (apomixes), meiotic parthenogenesis (automixis) and cross fertilization (amphimixis) (Holterman *et al*., 2009). Mitotic parthenogenesis is the most common type of reproduction and it's usually occurs in *M. incognita, M. javanica and M. arenaria* (Castognone-Sereno, 2014). *M. hapla* undergoes facultative meiotic parthenogenetic reproduction (Agrios, 2015). Phylogenetic analysis have shown that root-knot nematodes can undergo asexual reproduction through obligate mitotic parthenogenesis (Holterman *et al*., 2009).

Females of root-knot nematode species lay eggs in gelatinous masses composed of glycoprotein matrix by the rectal glands. The egg masses are usually found on the surface of galled roots, although they may also be embedded within the gall tissue. The egg mass is initially soft, sticky and hyaline but becomes firmer and dark brown with age (Perry *et al*., 2019).

Most of the root-knot nematodes species produce between 50 and 500 eggs, but a few occasionally produce several thousand eggs per female (Crow and Dunn, 2018). The juveniles progress through three moulting stages while continuing to feed on the cells of their host plants. When mature, the adult females lay eggs to complete the lifecycle (Westerdahl and Becker, 2011). The optimum soil temperature for root-knot nematode development is 25–28°C. Within this range, the nematode requires three to four weeks to complete its lifecycle. Although they may develop at lower temperatures, the development will take more time (Westerdahl and Becker, 2011). According to Crow and Dunn (2018), the length of reproduction cycle varies considerably depending on nematode species, host plant and temperature of the habitat. When soil temperature is high (above 30^{o}C), root-knot nematodes complete their life cycles in about 30-35 days (Charchar and Santo, 2019).

2.11 Damage Symptoms of Root-knot Nematode Species in Tomato Plant

Typical symptoms of root-knot nematode damage can be observed on both above-ground and below-ground plant parts (Noling, 2011). Foliar symptoms of root-knot nematode infestation of roots generally include stunting, premature wilting and slow recovery to improved soil moisture conditions, leaf chlorosis (yellowing) and symptoms characteristic of nitrogen deficiency (Maria *et al*., 2014). An increased rate of ethylene production, thought to be largely responsible for symptom expression in tomato, has been shown to be

closely associated with root-knot nematode root infestation and gall formation (Kim *et al*., 2019).

The most characteristic symptom of the disease which occurs below ground is the presence of galls on the root system (Janati, 2018). Roots develop multiple small galls; these galls often fuse to cause extensive swelling and distortion of the root system (Kennelly, 2014). Gall size may range from a few spherical swellings to extensive areas of elongated, convoluted, tumorous swellings which result from exposure to multiple and repeated infestation (Nico *et al.*, 2010). However, the size and the form of the gall depend on the species involved, number of the nematodes in the tissue, and host and plant age (Crow and Dunn, 2018). In tomato, the roots react to the presence of root-knot nematodes by the formation of large, fleshy galls, whereas in most other vegetables, galls are large and firm (Regina *et al*., 2016). The roots also become much shorter and bushier than those of healthy plants (Kennelly, 2014). In addition, root-knot nematode infestation in young plants often leads to hooking of the tap root due the presence of females on one side of the cortex (Janati, 2018).

2.12 Control Methods of Root-knot Nematodes

Due to the prolific reproduction nature and the difficulty in controlling root-knot nematodes, different management techniques are being employed by farmers to mitigate the problem. The strategies used to control root-knot nematodes in the tropics include: nematicides, crop rotation, soil solarization, flooding, biological control and soil amendments such as the use of manure, plant parts, composts and wood ash (Blacket, 2019).

2.12.1 Use of synthetic nematicides in controlling root-knot nematodes

To prevent huge crop losses, the control of root-knot nematodes is essential for food security. The use of synthetic chemicals (nematicides) has been the most popular among the farmers in developed countries (Greco, 2009) because some nematicides can quickly control nematodes and prevent significant losses (Santhosh *et al*., 2016). Synthetic chemical control methods encompass the use of different inorganic compounds in different formulations to kill or interfere with the growth and reproduction cycle of root-knot nematodes in infested fields (Strajnar and Sirca, 2011). The most common used chemicals are those that act either as contact nematicides (non-volatile) or as fumigants (volatile) (Asif *et al*., 2015).

2.12.2 Use of contact nematicides (non-volatile)

Most of the non-fumigant nematicides currently registered for use in vegetables are applied in the soil, however, some can be applied on the foliar, for example, Vydate L (Buskov *et al*., 2019). These compounds are uniformly incorporated into the soil to enhance contact with the nematodes or in the case of systemics, to facilitate absorption by the plant (Gams and Bisset, 2011). These nematicides are either oximecarbamate or organophosphate in nature (Ardakani, 2012). Common examples of contact nematicides include: aldicarb, cadusafos, calcium hypochlorite, carbofuran, 1,2-dibromo-3-chloropropane (DBCP), ethephon, ethopropos, fensulphothion, formalin, isazophos, nemafos, oxamyl and thionazin (Farahat *et al*., 2010). Applying these types of nematictdes on established crops, already showing severe damage, is useless and may cause residues in the edible parts of the plants. Their use is suggested when nematode soil population densities at planting are from low to medium (Greco, 2009). The nematicides mentioned above were found to be effective on

active stages of the nematodes but not on eggs (Meher *et al*., 2010). Consequently, there can be resurgence of the population from the subsequent generation, thus causing pesticide resistance and total yield loss. Therefore, concern has emerged regarding the use of non-fumigants repeated use of non-fumigant types of nematicide decreases their efficacy (Meher *et al*., 2010).

2.12.3 Use of carbofuran in the management of nematodes

Carbofuran is a broad-spectrum pesticide used in the control of arthropod pests and nematodes (Jada *et al*., 2010). Carbofuran functions through contact and systemic activity controlling soil and foliar insects and nematodes in many crops. It was recommended for the control of rice water weevil in the USA (Jada *et al*., 2010). This was the only insecticide that was effective for the control of the weevil. To date, rice water weevil resistance to carbofuran has not been documented (Jada *et al*., 2010). In a separate research, results indicated that application of carbofuran at planting of groundnut significantly reduced the population of *M. javanica*, compared to the other treatments (Charegani *et al*., 2012). Also, Gitanjali and Bora, (2018) who reported that application of carbufuran increased the multiplication of *Trichoderma viride* in the soil. The combined application of carbofuran and *Pseudomonas fluorescens* or *Trichoderma viride* significantly improved the growth of okra and reduced the population of *M. incognita* in the soil.

2.12.4 Use of fumigants (volatile nematicides)

Fumigant nematicides are formulated as liquids which rapidly volatalize to gas and move through open air spaces in soil. Fumigant nematicides include methyl bromide, methyl iodide, chloropicrin, 1,3 dichloropene, dimethyl dibromide, metam sodium and potassium (Noling, 2011). The use of broad-spectrum fumigants effectively reduces nematode

populations and increases vegetable crop yields, particularly when compared with non-fumigant nematicides (Dias-Arieira *et al*., 2012). Fumigant treatments are most effective in controlling root-knot nematode when residues of the previous crop are either removed or allowed to decompose. When plant materials have not been allowed to decay, treatment may not be effective in decreasing the populations of root-knot nematodes in soil, particularly when they are in egg stage (Hortic, 2017).

Substantial yield may be obtained from crops grown in nematicide-treated soil but, because of the high rate of fecundity and development of root-knot nematodes, populations are rare and totally eliminated by these chemicals (Garcia *et al*., 2018). The general soil sterilant, methyl bromide is extensively used in intensive vegetable production systems to control root-knot nematodes (Hunt and Handoo, 2016). However, this chemical has been banned in several countries because of its negative effect on the environment (Noling, 2012). Generally, the most limiting factor in using synthetic nematicides in developing countries is that they are either too expensive, sub-standard or due to lack of technical knowledge. The excessive use and poor handling of these chemicals can lead to health hazard, nematode resistance and destabilization of the ecosystem (Youdeowei, 2014).

2.12.5 Soil solarization in management of root-knot nematodes

Soil solarization is a non-chemical technique in which transparent polyethylene tarps are laid over moist soil for a six to 12 week period to heat non-cropped soils to temperatures lethal to nematodes and other soil-borne pathogens (Bakr *et al*., 2013). Soil temperatures are magnified by the trapping of incoming solar radiation under the clear, polyethylene panels. To be effective, soils must be wet and maintained at high soil moisture content to increase the susceptibility (thermal sensitivity) of soil-borne pests and thermal conductivity

of soil (Nico *et al*., 2010). Many types of fertilizers and organic amendments can increase the pesticidal effects of solarization when incorporated in soil prior to heating by releasing biotoxic volatile compounds (Bakr *et al*., 2013).

Certain plants have phenolic compounds which when released in the soil can kill or repel root-knot nematodes. These plants could be incorporated into the soil and combined with solarization to effectively control root-knot nematodes. For example, the combination of Broccoli residues and solarization proved effective against root-knot nematodes (Buskov *et al*., 2019). Solarization significantly reduced the number of juveniles in the soil, number of galls, females and egg-masses of root-knot nematodes (Villian *et al*., 2018). A reduction of 78 to 100 % of root-knot nematodes (*M. javanica and M. incognita*) at 15 – 30 cm deep was observed (Buskov *et al*., 2019). Similarly, Bakr, *et al*. (2013) reported that the highest reduction (95 %) in the number of juveniles/250 mL soil compared with the control was recorded in the solarized experiment. However, this method could not be effectively carried out in some parts of the sub-region due to the fact that the period of the rainy season is short (six months) and taking a month from the season would be a total failure for the farmers. In addition, the price of the polythene sheet is expensive and this might not be easily affordable to the farmers.

2.12.6 Use of crop rotation in the management of root-knot nematodes

Crop rotation is a very old practice for reducing root-knot nematode infestations. Many root-knot nematodes can reproduce and survive on only specific crop species. Repeated planting of the same crop in the field without interruption will enable any root-knot nematode species to reproduce successfully (kokalis *et al*., 2013). For crop rotation to be effective, crops unsuitable (non-hosts) for nematode infestation, reproduction and

development must be introduced in the rotation sequence. In some cases, resistant crop varieties have been used within the rotation sequence to minimize the infestation of some root-knot nematode species. In tomato, a single dominant gene has been widely used in plant breeding efforts and varietal development, which has shown resistance to most of the economically important root-knot nematode species (Perry and Moens, 2013). Also, cowpea (*Vigna unguiculata* L.) is well adapted to cultivation as a cover crop in the tropics, and many cowpea cultivars are poor hosts to root-knot nematodes (Kokalis *et al*., 2013).

The roots of marigolds exude chemicals that kill root-knot nematodes (Wang *et al*., 2019). The cultivation of selected marigold varieties in the rotation consistently resulted in a yield increase of 50 % and lowered root-galling by 30 % of the subsequently grown tomato (Wang *et al*., 2019). Groundnut can also be used in rotation with susceptible tomato varieties. According to Keshari (2015) revealed that groundnut is considered to be a good trap crop for *Meloidogyne* spp.

Crop rotation requires careful planning but, majority of the farmers in the sub-region need the necessary knowledge to carefully plan the cropping sequence in the field to avoid excessive build-up of nematodes. In addition, rotations used for nematode management are being altered drastically as a result of the increasing need for food, feed, fibre and more recently, biofuel. Demand and higher prices are affecting crop selection, which then changes the structure of traditional cropping systems and as a result, nematode densities in the soil (Sikora and Fernandez, 2016).

2.12.7 Biological control of root-knot nematodes

Soil microbial communities play an important role in plant health and disease suppression. Beneficial microbes in the microbial communities could promote soil ecosystem health that contributes to suppression of plant pathogens (Sharon *et al*., 2011). Sikora and Fernandez (2016) who indicated that tropical soils are rich in beneficial microbes and the biological control potential of the resident microbial fauna and flora is under-exploited. Fungi are one of the most commonly used biological control agents. More than 30 genera and 80 species of fungi are known to parasitize root-knot nematodes (Sun *et al*., 2011). Some fungi use mycelial traps or sticky spores to capture nematodes, for example, *Aspergillus* spp., *Arthrobotrys* spp. and *Monacrosporium* spp. Other fungi parasitize eggs and root-knot nematode females, for example, *Pochonia chlamydosporia* (Goddard), *Paecilomyces lilacinus* (Thom.) and *Trichoderma* spp. (Mitkowski and Abawi, 2011).

Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from soil, decaying wood, and other forms of plant organic matter. They are, for the most part, classified as imperfect fungi, in that case their sexual state is unknown (Lee *et al*., 2012). Rapid growth rate in culture and the production of numerous spores (conidia) that have varying shades of green characterize fungi in this genus. The reverse side of colonies is often un-coloured, buff, yellow, amber, or yellow-green, and many species produce large quantities of thick-walled spores (chlamydospores) in submerged mycelium (Sun *et al*., 2014). The potential of *Trichoderma* species as bio-control agents of plant diseases was first recognized in the early 1930s (Lee *et al*., 2012). This was confirmed by Lee *et al*. (2012) who stated that different species of *Trichoderma* have been widely used as antagonistic fungal agents against several pathogens as well as plant growth enhancers.

Faster metabolic rates, anti-microbial metabolites, and physiological conformation are key factors which primarily contribute to antagonism of these fungi (Lee *et al.*, 2012). Dababat and Sikora (2010) reported that results of field experiments showed a reduction of *M. incognita* population by 36-40 % after application of several isolates of *T. viride* (Pers) and *T. koningii* (Oudem). All the *Trichoderma* strains had the ability to colonize *M. incognita* in separated eggs and juvenile experiments (Dababat and Sikora, 2010).

Trichoderma. viride is one of the most commonly reported and widely distributed of all soil fungi. Many physiological, antifungal, and insecticidal activities have been attributed to this species (Domsch *et al*., 2010). *T. viride* represents widely studied fungi that show antagonistic activity towards soil-borne fungal pathogens and nematodes (Domsch *et al*., 2010). The fungi control pathogens such as nematodes by colonizing the plant roots and providing a physical barrier for the plant against the nematodes, and also enhance the plants root growth and nutrient absorption (Senthilkumar and Ramakrishnan, 2011). Crawford and Clardy (2011) who reported that nematicidal activity of *Trichoderma viride* may be due to the eggs and larvae being infected through the increase in chitinase and protease activity. As chitin is a major component of egg shell of nematodes, *T. viride* penetrates the eggs, leading to the reduction in population. According to Hussain and Zouhar (2017) who studied direct parasitism of eggs through increase in extra cellular chitinase activity as indicated by egg infection capability and inducing plant defense mechanism, leading to systemic resistance, are the two possible mechanisms for the suppression of nematodes. However, Belay *et al*. (2016) who reported that various mechanisms have been suggested for the biocontrol activity of *T. viride* against plant parasitic nematodes like antibiosis, parasitism and enzymatic hydrolysis. Enzymes such as chitinase, glucanases and proteases

seem to be very important in the parasitism process of the nematode eggs by *T. viride* (Dinesh and Maheshwari, 2013).

According to Jang *et al*. (2016) who evaluated the *in-vitro* experiment of *Aspergillus niger* at various concentrations showed nematicidal activity against second juvenile stage (J2s) and inhibited egg hatching. Its effects on killing J2s and suppressing egg hatching increased in a dose-dependent manner. The J2s mortality rate was 25.3 % at 1.25 % culture filtrate and >90 % at 2.5–20 % culture filtrate at 1 day after treatment. Complete inhibition of egg hatching was observed at 5–20 % culture filtrate after 7 days of exposure as compared to the control. The rate of inhibition of egg hatching was reduced to 77.5 % and 14.0 % at 2.5 % and 1.25 % culture filtrate, respectively.

Khan and Kounsar (2018) who studied the effect of fungal bio-agents on the growth of mungbean (*Vigna radiata*). Root nodulation and root-knot disease caused by *Meloidogyne incognita* was tested in field microplots. The nematode caused significant decreases in plant growth and root nodulation. Application of fungal bio-agents controlled nematode pathogenesis leading to a decrease of 4.8-26.5, 7.2-30.1 and 11.8-27.6 % in the number of galls, egg masses/root system and J2/kg soil. Fungal bio-agents synergized root nodulation, leading to 21.0-57.8 % increase in the number of functional nodules on nematode infected roots. Mukhtar *et al*. (2012) who reported that *Trichoderma* spp. individually or in combination with caster or neem cake was able to parasitize the egg masses and ultimately reduced the galling and nematode population of *M. incognita*. Application of *Trichoderma* spp. starts penetrating the larval cuticle through hyphae and also the nematodes eggs through some enzymatic activity which dissolves the chitin layer which is supposed to be protective layer of the nematodes against many pathogenic agents inhabiting the soil. Also,

Asaduzzaman, *et al*. (2010) who demonstrated that *Trichoderma* spp. reduced gall index and nematode reproduction which advocates the high potential to control root-knot nematode, *M. incognita*. *T. harzianum* can alter the physiology of roots including the root exudates. According to the results of Abd Al-Fattah *et al*. (2010) who noticed the highest level of nematode mortality was obtained by *Trichoderma harzianum,* which produced nematode mortality of 38 % compared with 14.3 % of water control. Also in the *in vitro* experiment, exposing the nematode to culture filtrate of *Trichoderma* at a concentration of 90 % significantly decreased mobility of *M. incognita* J2s after 24 hours when compared to the water control. Mortality after testing recovery of inactive nematodes in tap water was still high and ranged between 14.3- 38.3 % in the treatments compared to 3 % and 9.7 % in the water and media controls, respectively.

Muhammad *et al*. (2019) stated that under *in vitro* conditions, culture filtrate of *Pochonia chlamydosporia* was active against the root-knot nematode, *M incognita* in egg hatch inhibition and killing juveniles at a maximum of 58.17 and 76.3 % respectively whereas the control (potato dextrose broth PDB) did not produce any negative impact on egg hatch inhibition and mortality of juveniles. Also, in the *in-vivo* experiment of tomato plant, Muhammad *et al*. (2019) noticed significant increase in plant height (cm) (37.6 %), number of galls/plant (22.1 %), number of fruits (34.6 %) and number of flowers (51.0 %) as compared to control. The biocontrol effect against different pathogens in the presence of *Penicillium chrysogenum* is reported in a wide range of plants and pathogens providing evidence for the effect of *P. chrysogenum* on nematode infection. Dry mycelium enhanced plant growth and reduced root galls to protect cucumber and tomato plants against *M. javanica* (Devran *et al*., 2011). *P. chrysogenum* used alone and in the combination of two

could boost plant growth and reduce reproduction of *M. incognita* in tomato (Liu *et al*., 2011). Yao *et al*. (2014) who also reported that *P. chrysogenum* is one of the most important fungi to control *M. incognita* in tomato.

Mascarin and Junior (2012) reported that *Trichoderma harzianum* efficiently decrease the population of *M. incognita* in cucumber and enhanced plant growth under greenhouse conditions. *Fusarium oxysporum* showed repelling effect towards *M. incognita* in tomato as well as promoted plant growth (Selim, 2010; Terra *et al*., 2018). Devran *et al*. (2011) reported that dry mycelium enhanced plant growth and reduced root galls to protect cucumber and tomato against *Meloidogyne javanica.* Heve *et al*. (2015) reported that the rhizosphere and rhizoplane of root-knot nematode infested tomato revealed consistent association of *Acremonium strictum* and other fungal bioagents, *Aspergillus niger, Paecilomyces lilacinus, Rhizoctonia solani* and *Trichoderma harzianum* isolated from egg-masses of *M. incognita*, identified, maintained and were investigated through *in vitro* trails for their potentiality against *M. incognita*.

The effect of culture filtrate of *Alternaria humicola*, *Aspergillus niger*, *Curvularia lunata*, *Sclerotium rolfsii, Trichoderma lignorum* and *T. viride* obtained from rhizosphere of tomato cv. Marglobe raised from seed coated with oilcakes, on the mortality and larval hatching of *Meloidogyne incognita* was studied (Singh *et al*., 2011). The culture filtrates of all the fungi tested proved to be nematoxic and inhibited the hatching of larvae of *M. incognita* presumably because of the presence of higher amount of phenols. *Aspergillus niger, A. nidulans, Cladosporium oxysporum, Fusarium oxysporum, F. dimerum* and *Paecilomyces lilacinus* were tested *in vitro* for their potentiality against *M. incognita* (Weimen *et al*., 2019). All these, *A. niger, A. nidulans* and *F. dimerum* were found toxic to

nematode larvae. However, *A. niger, A. nidulans* and *P. lilacinus* showed inhibition of egg hatching. *C. oxysporum* and *P. lilacinus* exhibited a very high egg parasitizing capacity and larval mortality of *M. incognita*.

Sikandar *et al*. (2019) revealed that pathogenic level of root-knot nematode was also observed in cucumber that the number of galls and egg masses was increased with increase in inoculums level while seeds treated with *Penicillium chrysogenum* significantly reduced compared to control. It also inhibited the development and rate of reproduction of nematodes in the roots. Cucumber is susceptible and an excellent host for multiplication of *M. incognita* but, seeds treated with *P. chrysogenum* significantly reduced the infection of nematodes and galls at a different level of inoculums also enhanced biomass. Sharma (2018) tested the efficacy of culture filtrate of eight biocontrol agents such as *Paecilomyces lilacinus, Pochonia chlamydosporia, Trichoderma harzianum*, *Trichoderma viride*, *Latisariaar valis, Gliocladium virens* and *Bacillus subtilis* along with nematicide, against root-knot nematode affecting mulberry under *in vitro* condition. Results revealed that culture filtrate of *T. harzianum*, *P. chlamydosporia* at 100 % concentration showed high larval mortality by the end of maximum exposure period.

Effect of six fungal filtrates, *Aspergillus niger*, *A. flavus*, *A. ochrachus*, *A. nidulans, Trichoderma harzianum* and *T. viride* were studied under laboratory conditions against *Meloidogyne incognita* larvae. All these fungal filtrates significantly affected nematode mortality after 24 hrs. There was a degree of antagonism between the different tested fungal filtrates on larvae percentages kill. Also they proved that the fresh fungal filtrates, without storage were the most effective on percentage kill of *Meloidogyne incognita* larvae (Nagwa *et al*., 2019). Culture filtrates of *Aspergillus niger, Pythium debarianum, Fusarium*

oxysporum, *F. solani* and *F. moniliforme* were studied *in vitro* to see their influence on mortality of second stage juveniles (J2s) of the root-knot nematode, *Meloidogyne incognita*. Culture filtrates of *A. niger* were most effective against second stage juveniles followed by *F. moniliforme, F. solani, F. oxysporum* and *P. debarianum*. However, this effect varied according to the type of growth media, filtrate dilutions and time of exposure (Basyony, 2018). Kumar *et al*. (2010) conducted *in vitro* experiment to assess the nemato-toxic effect of some opportunistic fungi isolated from the rhizosphere on *Meloidogyne incognita*. Among the fungi *Paecilomyces lilacinus* recorded 72.76 % egg mass parasitization followed by *P. lilacinus* (71.0 %). The fungi which were found effective against egg masses were further evaluated on J2s. In case of juveniles, *P. lilacinus* isolate caused 93 % and *Pochonia chlamydosporium* 84 % mortality in concentration of fungal filtrate after 72 hrs exposure period.

Kalele *et al*. (2010) studied the bio-control potential of *P. lilacinus* on *M. incognita* on tomato under glasshouse conditions and discovered that the soil application of *P. lilacinus* at 4 g/3 kg soil was found to be highly effective against *M. incognita*. It recorded the lowest nematode population and increased plant growth such as shoot length (65.5 %), root length (84.3 %), shoot weight (127.5 %) and yield (195.2 %).

Ashraf and Khan (2017) studied the effect of culture filtrates of four opportunistic fungi, *Paecilomyces lilacinus, Cladosporium oxysporum, Trichoderma virens* and *Talaromyces flavus* on the egg hatching and larval mortality of *M. incognita*. Among them, *P. lilacinus* was most effective recording 100 % mortality of juveniles even in 10 times diluted culture filtrate after 96 hours of incubation followed by *Cladosporium oxysporum* whose performance was the same with *Paecilomyces lilacinus*. Culture filtrate of *Pseudomonas*

fluorescens was found toxic to juveniles of *Meloidogyne incognita, Heterodera cajani* and *Rotylenchulus reniformis* (Sankaranarayanan *et al*., 2016). At 48 hrs, there was 95 % mortality of *R. reniformis*, 94 % mortality of *M. incognita* and 85 % mortality of *H. cajani*. As the exposure time increased, there was an increase in the mortality of juveniles. Dababat *et al*. (2011) tested *Trichoderma harzianum* and *Trichoderma viride* for their capacity to reduce the incidence of the root knot nematode *Meloidogyne incognita* on tomato. *In vitro* studies demonstrated that all tested isolates were effective in causing nematode mortality compared with the control.

Ganaie and Ahmadkhan (2010) conducted an experiment to assess the nemato-toxic effect of some opportunistic fungi on *M. incognita*, *in vitro*. Among the fungi, *P. lilacinus* (PIT_1) recorded 72.76 % egg mass parasitization followed by *P. lilacinus* (PIT_2) 71.0 %. In case of juveniles, *P. lilacinus* (PIT_1) caused 93 % mortality in undiluted fungal filtrate at 72 hrs exposure period. Ashoub *et al*. (2018) conducted laboratory experiment to evaluate the ability of some fungi species. A proportional relation was found between mortality percentage and tested fungal culture filtrate concentrations at different exposure times. *Trichoderma viride* was the most effective species followed by *Aspergillus niger* and *Rhizoctonia* spp. in parasitizing nematode eggs.

A study was carried out *in vitro* to determine the efficacy of indigenous fungi *Acremonium strictum, Aspergillus terreus, A. nidulans, A.niger, Chetomium aubense, Chladosporium oxysporum, Fusarium chlamydosporium, F. dimarum, F. oxysporum, F. solani, Paecilomyces lilacinus, Pochonia chlamydosporia, Trichoderma viride* and *T. harzianum*on egg parasitism, egg hatching, mobility and mortality of root-knot nematode, *M. incognita* (Satyandra and Nita, 2010). All tested fungi showed varied effects against the nematodes.

Culture filtrates of *A. strictum* was very effective against the nematode in regards to egg parasitism (53 %), egg hatching inhibition (86 %) and mortality (68 %) compared to controls. *A. terreus* did not show egg parasitism but was found to be highly toxic against second stage juveniles (J2s) causing high mortality (around 68 %). Dababat *et al.* (2011) tested *Trichoderma harzianum* and *Trichoderma viride* for their capacity to reduce the incidence of the root-knot nematode *Meloidogyne incognita* on tomato. *Trichoderma* slightly reduced nematode damage to tomato *in vivo*. The isolates could not be isolated from the endorhizal but, were successfully re-isolated from the rhizosphere 45 days after fungal inoculation.

Kumar and Jain (2010) under take a screen house study for evaluating some fungal and bacterial antagonists as seed dressing treatment against root-knot nematode, *Meloidogyne incognita* infecting okra. The seeds of okra variety were treated with *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescens* each at 10 g/kg seed and carbosulfan 25 (DS) at 3 % (w/w) was also included as treated check. Growth parameters of okra plants were better and *M. incognita* populations were reduced in all the treatments compared to inoculated control. Raveendra *et al*. (2011) conducted an experiment to test the efficacy of organic amendment (neem), bio-control agent (*Trichoderma viride*), trap crop (*Tagetes patula*) and chemicals (carbofuran and phorate) as individual treatments and in combinations on the growth of tobacco plants and on the development and multiplication of root-knot nematode *M. incognita*. The bio-control agent, *T. viride* significantly increased the plant growth with respect to plant height, shoot weight, number of leaves, root length and root weight of tobacco plant infested with *M. incognita*. It was concluded that the improved plant growth was obtained in combination treatments than in individual

treatments, which might be due to the additive and interactive effect of the treatment on tobacco plants.

Xalxo *et al*. (2013) isolated twenty-seven different fungal isolates belonging to various genera from different rhizosphere soil samples by using serial dilution method where the most frequent was *Aspergillus* accounting 44.4 % of the total isolates with *Aspergillus niger* being the frequent and abundant species. The second most common genera were *Trichoderma* followed by *Penicillium, Rhizopus* and *Fusarium*. Xalxo *et al*. (2013) also tested the *in vitro* experiment of those fungal isolate against *Meloidogyne incognita* and the results were achieved and successful against *M. incognita*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

This research work was carried out in the laboratory and Screen House of the Department of Plant Biology, Federal University of Technology Minna, Nigeria.

3.2 Collection of Materials

Tomato seeds were obtained from tomato vendor in Bosso market Minna, in Niger State, Nigeria. Soil, free from plant debris and pebbles, was collected in a sterile polythene bag from Biological Garden of the Department of Plant Biology, Federal University of Technology Minna, Nigeria and transported to Plant Biology Laboratory. Soil samples, free from hard particles and infested with root-knot diseases (by examining these features: wilted plant, yellowing leaves, root galls on tomato plants) were collected in a sterile polythene bags from Fegyi Awodo tomato farm in Minna, Niger State and transported to the laboratory in the department of plant biology.

3.3 Preparation of Potato Dextrose Agar (PDA)

Two hundred grams (200 g) of peeled irish potato were washed and boiled for 20 minutes in 1000 mL of sterile distilled water. The supernatant was drained into one Litre flask and made up to 1000 mL with sterile distilled water. Twenty grams (20 g) of agar powder and 20 g of glucose powder was weighed and added into the 1000 mL flask containing the drained supernatant. The mouth of the conical flask was corked with cotton wool wrapped in foil paper. The mixture was sterilized at 121^{o}C for 15 minutes in an autoclave. After sterilization, the mixture was allowed to cool down for 10 minutes, then, 0.5 mL drop of

100 % concentration of chloramphenicol was added into each Petri plates that was dispensed to prevent bacterial growth (Adebola *et al*., 2014).

3.4 Isolation and Identification of Fungi from Soil

Serial dilution was performed for the isolation of fungi as follows: One Gram (1 g) of soil sample was aseptically suspended into 10 mL of sterile distilled water in test tubes and vortexed properly to allow distribution of microorganism present in the sample. One Milliliter (mL) dilution 10^{-1} mL and 10^{-2} mL was aseptically taken from the suspension and transferred into sterile Petri plate, 0.1 mL of 100 % concentration of chloramphenicol was added into the Petri plate after which, the plates were swirled gently and incubated at 27 ± 2^{o}C for 72 hours (Adebola *et al*., 2014). The fungal isolates were identified using cultural, microscopic at X40 magnification and characterization as outlined by Cannon and Kirk (2008). Stock cultured were preserved in PDA and refrigerated at 4^{o}C.

3.5 Extraction and Identification of Nematodes from Soil

Pie-pan method was used in the extraction of nematode. The soil sample (25 g) was taken in a container (basin) and mixed thoroughly by hand. Hard particles and stones were removed by stirring the suspension and the soil was passed through a set of sieves of 20, 60 and 250 µm pore sizes. A filter paper was placed in a plastic sieve/basket which was placed on a plastic plate, and was ensured that the base of the sieve was fully covered by the filter paper. The soil sample (25 g) was placed on the filter paper in the sieve, water was added to the extraction plate (water will be poured gently into the plate between the edge of the sieve and the side of the plate and not directly on the soil) containing enough water. This assemble was kept for 48 hours or more if possible. After the extraction period, the sieve was removed and soil was disposed. The nematode suspension from the plate was poured into a labeled beaker and left to settle (Coyne *et al*., 2018). Different plant parasitic

nematodes present in the suspension were identified to the genus level under a light microscope at X40 magnifications according to their morphological characters (Yeates *et al*., 2011; Panesar and Marshall, 2012; Mai and Mullin, 2018).

3.6 *In vitro* Efficacy of Fungal Isolates against *Meloidogyne incognita* (Second Stage Juvenile J2s)

Agar discs from the 14 days old Potato Dextrose Agar cultures were inoculated into 300 mL flasks containing 200 mL Potato Dextrose Broth and shaken thoroughly which was maintained at 25^{o}C for 7 days. The culture filtrates was obtained by separating the liquid from the mycelia using a filter paper and microspore filter (Hassan *et al*., 2010). *In vitro* toxicity of the culture filtrate against *M. incognita* (J2s) was done by taken 5 mL culture filtrates of each isolate of fungi and 200/mL of nematode suspension, *M. incognita* (J2s) in a Petri dishes. The control was prepared by taken 5 mL distilled water and 200/mL nematode suspension without fungal culture filtrate. Three replicates of each were prepared. The percentage of immotile nematodes was viewed under light microscope at X40 magnification and calculated after 24 and 48 hours. Those that remained inactive were considered dead (Meyer *et al*., 2010). Mortality rate was calculated using Abbott's formula:

$$M = \frac{Mt - Mc}{100 - Mc} \times 100 \qquad 3.1$$

Where Mt means mortality percentage in treatment and Mc means mortality percentage in control.

3.7 Pathogenicity Test of *Meloidogyne incognita* on Tomato Plant

The method of Coyne *et al*. (2008) was adopted for the test. Fresh healthy tomato seeds were washed with sterile water for 5 minutes and dried on sterile plain sheet for 20 minutes. The seeds were sown in 7 Litre plastic buckets containing 5 kg of sterilized loamy soil. After 2 weeks of seed germination, plants in each bucket were thinned to one plant. The inoculum was applied in four holes about a meter distance round the stem of each plant with 1000 nematode suspension per 5 mL in each bucket and the control were inoculated with distilled water. The plant samples were observed for symptoms after 10 days of inoculation.

3.8 *In vivo* Evaluation of Isolated Fungi in the Control of *Meloidogyne incognita* on Tomato Plant

Healthy seeds of tomato were surfaced sterilized with 0.1 % sodium hypochlorite solution for two minutes followed by repeated washing in sterile distilled water. The seeds were sown in 7 Litre plastic buckets containing 5 kg of sterilized loamy soil, arranged in a completely randomized block design on a bench (Ogwulumba *et al*., 2010). After 2 weeks of seed germination, plants in each bucket were thinned to one plant. The inoculum was applied in four holes about a meter distance round the stem of each plant with 1000 nematode suspension per 5 mL in each bucket. A day after nematode inoculation, fungal culture extract was inoculated into the same holes while in the control treatments only distilled water was inoculated (Silva *et al*., 2017). Three replicate buckets were made per each treatment as well as the control. The plants were nourished regularly with sterile distilled water at 20 mL per bucket (Coyne *et al*., 2008).

3.9 Data Collection

Data were collected after two weeks of seed germination and at harvest after 13 weeks of maturity of the tomato plant. It was done by direct stand count and visual examination, growth and yield data included average plant height (tape), number of fruits/plant (by physical counting), number of galls/plant (by visual examination), number of leaves/plant (by physical counting) and the disease incidence (%) were taken.

$$\text{Percentage disease incidence} = \frac{\text{Number of infected leaves}}{Total\ number\ of\ leaves} \times 100 \qquad 3.2$$

3.10 Experimental Design

Buckets were laid in completely randomized block design (CRBD) on a bench in the screen house with four treatments and control (distilled water) with three replicates each.

3.11 Data Analysis

The data obtained from both the *in vitro* and in *in vivo* were subjected to Analysis of Variance (ANOVA) using Gen-Stat version 7.2 computer packages. Treatment means were separated using Least Significant Difference (LSD) test at 5 % level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Frequency occurrence of fungi isolated from soil sample

Four filamentous species of fungi (Plate IA & IB, Table 4.1 and Table 4.2), belonging to three different genera (*Aspergillus, Rhizopus* and *Penicillium*) were isolated from soil samples using serial dilution method. *Aspergillus niger* (47 %) being the most frequent and abundant species followed by *Aspergillus nidulance* (28 %), *Penicillium chrysogenum* (16 %) and the least was *Rhizopus stolonifer* with (9 %) occurrence.

Table 4.1: Percentage Occurrence of Fungi Isolated from Soil

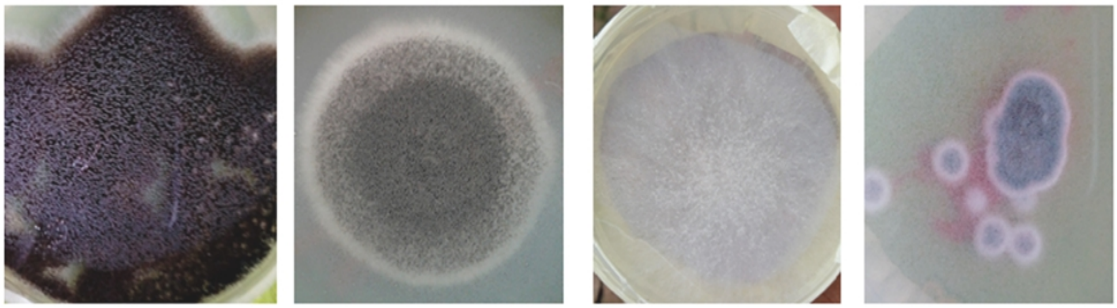
Fungi	Percentage Occurrence (%)
Aspergillus niger	47.00±0.67^{d*}
Aspergillus nidulans	28.00±0.17^{c}
Penicillium chrysogenum/notatum	16.00±0.67^{b}
Rhizopus stolonifer	9.00±0.18^{a}

Values are means of three replicates ± standard error. *Values followed by the same letters along the column are significantly different ($P < 0.05$).

Table 4.2: Characterization of Isolated Fungi

Fungi	Characteristics Features
Aspergillus niger	Rapidly growth colonies on potato dextrose agar (PDA) with abundant submerged mycelium, carbon black/deep brownish black conidial heads. Colourless to pale yellow conidial on reverse Petri dish with an initial globose and then radiate well defined columns (Plate IA) (Plate IB)
Aspergillus nidulans	Colonies fast growing reaching 6-7 cm diameter in 7 days, smooth walled conidiophores, small echinulate conidia. Quick quickly ripening ascospores, purple black in colour,with equatorial binding and large thick walled, globose bodies termed hulled cells forming an irregular layer aboutthe perithecia (Plate IA) (Plate IB)
Penicillium chrysogenum	Colonies are usually fast growing, green blue colour with white ring at the margine, sometimes white, mostly consisting of dense conidiophores. Conidiophores is hyaline, erect, branched and penicilately at the apexes with 2-3 metula, 3-4 verticilate phialides and catenulate conidia in each phialides, forming rather compact cylindrical (Plate IA) (Plate IB)
Rhizopus stolonifer	Colonies are very fast growing, 1-2 cm high, white at first, pale grey, dark brown grey, brownish black. Mycelium branched, non-septate, stolons and rhizoids present,sporangiophores arise from these points, sporangia globose, hemispherical, zygospores round or oval (Plate IA)(Plate IB)

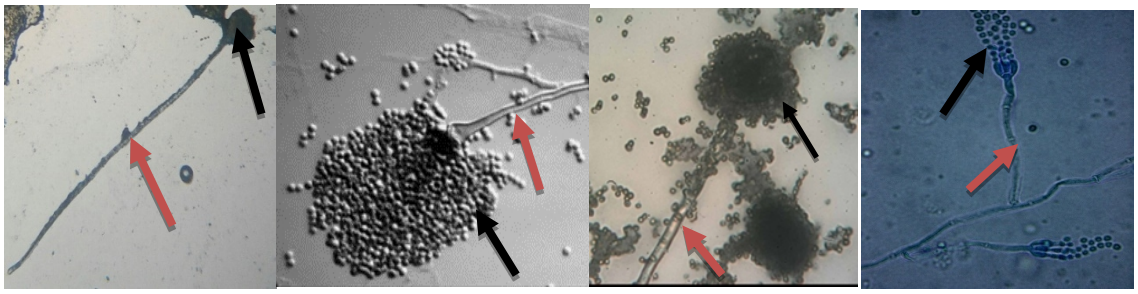
Source: **Altalhi (2019)**



Aspergillus niger *Aspergillus nidulans* *Rhizopus stolonifer* *Penicillium chrysogenum*

Plate IA: Culture Plates of Isolated Fungi

Source: Field Photograph



Aspergillus niger *Aspergillus nidulans* *Rhizopus stolonifer* *Penicillium chrysogenum*

Plate IB: Microphotographs of Isolated Fungi

The black arrows indicate the SPORES while the red arrows indicate the MYCELIA.

Source: Viewed under light microscope at X40 magnification

4.1.2 Characterization of isolated *Meloidogyne incognita*

The extracted *M. incognita* (J2s) varied in length 200 to 300 µm, labial region not offset, elevated labial disc and absent of lateral lips. Stylet ranged from 11 to 25 µm in length, basal knobs offset, rounded to transversely elongate. Hemizonid anterior or adjacent to excretory pore. Tail ranged 15 to 60 µm in length, tip rounded, anal body width 8 to 19 µm. lateral lines four with incisures, phasmids subterminal, dot-like near cloaca aperture (Plate II).

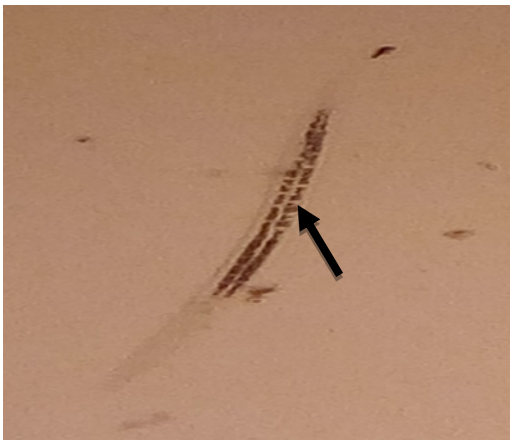


Plate II: ***Meloidogyne incognita*** **extracted from rhizospheric soil of infested tomato**

The arrow indicates the intestine.

Source: Viewed under light microscope at X40 magnification

4.1.3 *In vitro* efficacy of fungal isolates against second stage juvenile (J2s) of *Meloidogyne incognita*

The results of the *in vitro* efficacy of fungal isolates against *Meloidogyne incognita* in culture filtrate after 24 and 48 hours exposure period is presented in Table 4.2. From the observations it was clear that there was a significant difference ($P< 0.05$) in the mortality of *M. incognita* after 24 and 48 hours exposure to the fungal culture filtrates compared to control group. Mortality of *M. incognita* was significantly highest at $P< 0.05$ significant level in *Aspergillus niger* (39.28 %, 78.47 %) after 24 and 48 hours exposure period respectively, followed by *Penicillium chrysogenum* (18.59 %, 47.48 %) whereas, the minimum mortality was observed in *Rhizopus stolonifer* (8.06 %, 26.48 %) after 24 and 48 hours exposure period respectively, the recorded values of *P. chrysogenum* and *R. stolonifer* were significantly different ($P< 0.05$). On the other hand, no mortality was recorded in the control group after 24 hours exposure period however, 5.00 % mortality was recorded after 48 hours exposure period.

Table 4.3: *In vitro* Assessment of Nematicidal Activity in Culture Filtrate of Different Fungal Species against Second Stage Juvenile (J2s) of *Meloidogyne incognita*

Fungi	Mortality of *M. incognita* after	
	24 Hours	48 Hours
Aspergillus niger	39.28± 1.86^{e*}	78.47± 0.56^{e}
Aspergillus nidulans	29.12± 1.52^{d}	57.49±0.51^{d}
Penicillium chrysogenum	18.59± 1.53^{c}	47.48± 0.52^{c}
Rhizopus stolonifer	8.06± 1.52^{b}	26.48± 0.37^{b}
Control	0.00± 0.00^{a}	5.00± 0.00^{a}

Values are means of three replicates ± standard error. *Values in the same column followed by different letters are significantly different (P<0.05).

4.1.4 *In vivo* efficacy of fungal isolates against *Meloidogyne incognita* and growth parameters of tomato crop

The results of the *in vivo* efficacy of fungal isolates against *Meloidogyne incognita* is presented in Table 4.4 Plant height (cm) was highest in the group treated with *Aspergillus niger* (46.43 cm); which was not significantly different (P> 0.05) from the plant height recorded for the group treated with *Aspergillus nidulans* (42.23 cm). The study recorded the lowest plant height in the group treated with *Rhizopus stolonifer* (26.13 cm) and the control group.

Similarly, there was no significant different (P> 0.05) in the Number of fruits/plants in the group treated with *A. niger* (10.33) and *A. nidulans* (9.33). The Number of fruits/plants recorded for *P. chrysogenum* was significantly (P< 0.05) higher than that of control group, there was no significant different in the Number of fruits/plants recorded for the group treated with *R. stolonifer* (5.33) and the control group (3.67). On the other hand, the Number of galls/plants recorded for the control group (28.33) was significantly higher (P< 0.05) than the records of other treatment. Both *P. chrysogenum* (12.67) and *R. stolonifer* (16.67) recorded higher Number of galls than the two tested *Aspergillus* species.

The results of the Number of leaves/plants varies from 42.33 in the control group to 69.67 in the group expose to *A. niger* isolates. The study recorded no significant difference in the Number of leaves/plants for plant treated with *A. niger* (69.67) and *A. nidulans* (62.67). Similarly, the study recorded no significant difference in the Number of leaves/plants recorded for the control group (42.33) and *R. stolonifer* (46.33).

Table 4.4: Effects of Fungal Isolate against *Meloidogyne incognita* and Growth Parameters of Tomato Crop

Treatments	Plant height (cm)	Number of fruits Per plant	Number of galls per plant	Number of leaves per plant
A.niger	46.43 ± 0.92^{c*}	10.33 ± 0.88^{c}	3.67 ± 1.86^{a}	69.67 ± 1.20^{c}
A nidulans	42.23 ± 1.52^{c}	9.33 ± 0.88^{c}	5.33 ± 0.88^{a}	62.67 ± 1.20^{bc}
P chrysogenum	33.43 ± 3.44^{b}	6.67 ± 0.33^{b}	12.67 ± 1.45^{b}	59.00 ± 2.31^{b}
R. stolonifer	26.13 ± 1.39^{a}	5.33 ± 0.33^{ab}	16.67 ± 0.88^{b}	46.33 ± 3.53^{a}
Control	22.20 ± 1.08^{a}	3.67 ± 0.33^{a}	28.33 ± 1.76^{c}	42.33 ± 2.85^{a}

Values are means of three replicates ± standard error. *Values in the same column followed by different letters are significantly different (P< 0.05).

4.1.5 Pathogenicity test of *Meloidogyne incognita* on tomato plant

The result from pathogenicity test showed that the tomato plants leave became yellowish in colour and wilted, the roots were having enormous root galls and hairy roots after 10 days of inoculation. This confirms that *M. incognita* is the actual causative organism of the root galls of the tomato (Plate III).

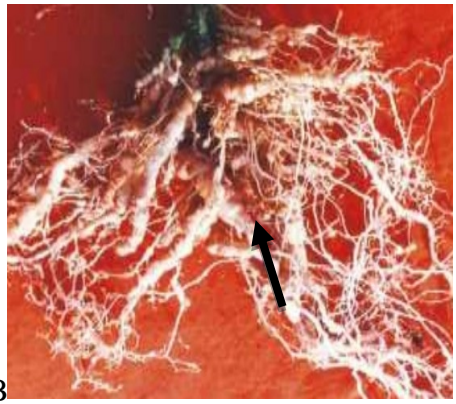
A  B 

Plate III: (A) Wilted tomato plant caused by *M. incognita*

(B) Enormous root galls caused by *M. incognita*

The arrows in A indicate the vine (short arrow) and the yellowish leave (long arrow)

The arrow in B indicates the galls.

Source: Field Photograph

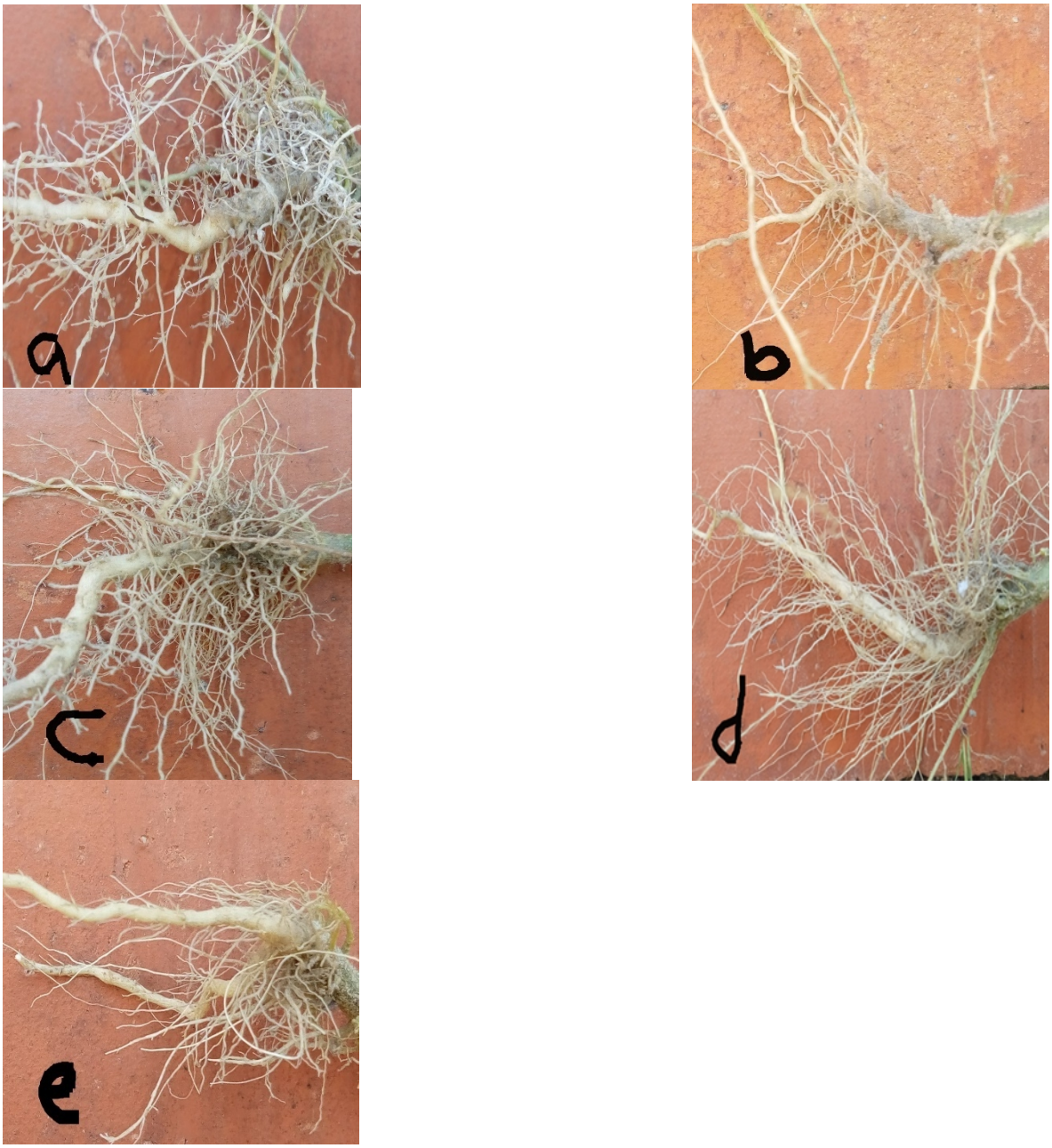
4.1.6 Disease incidence (%)

In table 4.5, the percentage disease incidence was 7.18, 9.55, 12.96, 20.31 and 39.46 % for *A. niger, A. nidulans, P. chrysogenum R. stolonifer* and Control (distilled water), respectively. The results recorded for Control (39.46 %) and *A. niger* (7.18 %) treatments are significantly different (P< 0.05).

Table 4.5: Disease Incidence Percentage of Tomato Plants After Harvest Inoculated with *Meloidogyne Incognita* Treated with Fungi Isolates in Screen house.

Treatments	Disease incidence (%)
A. niger	$7.18 \pm 0.88^{a*}$
A. nidulans	9.55 ± 0.79^{ab}
P. chrysogenum	12.96 ± 2.28^{b}
R. stolonifer	20.31 ± 2.19^{c}
Control	39.46 ± 1.53^{d}

Values are means of three replicates ± standard error. *Values in the same column followed by different letters are significantly different (P<0.05).



Plates IV: (a) Photographs of untreated tomato roots (control) with enormous root galls and hairy roots, (b) roots of tomato treated with *A. niger*, (c) roots of tomato treated with *R. stolonifer*, (d) roots of tomato treated with *P. chrysogenum* and (e) roots of tomato treated with *A. nidulans*.

Source: Field Photograph

4.2 Discussion

Based on the observable characteristics, four filamentous species of fungi, belonging to three different genera (*Aspergillus, Rhizopus* and *Penicillium*) were isolated from soil samples. *Aspergillus niger* had the highest percentage occurrence (47.00 %) followed by *Aspergillus nidulance* (28.00 %), *Penicilliun chrysogenum* (16.00 %) and the least was *Rhizopus stolonifer* with value of 9.00 %. The isolated and identified soil fungi were in conformity with the work of Abou-Zeid *et al*. (2011). Altalhi (2019) reported the most common genera of soil fungi are *Aspergillus, Penicillium* and *Rhizopus*. This finding is in conformity with the observation made by Frisvad *et al*. (2011) that *Aspergillus* spp contributed the broadest spectrum of species in the rhizosphere and *Aspergillus niger* the most frequent fungi. This result is in agreement with Liu *et al*. (2011) who isolated different fungal genera and species from the desert soils in Saudi Arabia. The present results also agreed with Abou-Zeid *et al*. (2011) who isolated 70 species and 31 genera of fungi from soil sample of Taif, Saudi Arabia and the most common genera were *Aspergillus, Fusarium, Penicillium* and *Rhizopus*. Xalxo *et al*. (2013) isolated twenty-seven (27) different fungal isolates belonging to various genera from different rhizosphere soil samples by using serial dilution method where the most frequent was *Aspergillus* accounting 44.4 % of the total isolates with *Aspergillus niger* being the frequent and abundant species. The second most common genera are *Trichoderma* followed by *Penicillium*, *Rhizopus* and *Fusarium*.

The effect of culture filtrates on *M. incognita* differed according to nematode and fungal biological groups as well as exposure time. In the present result, *A. niger* and *A. nidulans* has the highest rate of mortality of *M. incognita* after 24 and 48 hours (39.28, 29.12, 78.47

and 57.49 % respectively) followed by *P. chrysogenum* (18.59 and 47.48 %) and the least was *R. stolonifer* (8.06 and 26.48 %). The trend of mortality in the fungi filtrates observed in this study agreed with the report of Basyony (2018) who reported that filtrate of *Aspergillus niger* exhibited highest (100 %) mortality after 12 hours followed by *Aspergillus nidulans*. Similarly, Siddiqui and Shaukat (2014) recorded maximum mortality by filtrates of *Aspergillus nidulans* after 72 hrs. In concordance with Xalxo *et al*. (2013) who reported that the nematicidal efficacy of each fungal filtrate increased with duration of exposure. These results are in agreement with Marie *et al*. (2014) who reported that the *in vitro* experiment was conducted by using bio-agents like *Aspergillus niger, Paecilomyces lilacinus, Rhizoctonia solani* and *Trichoderma harzianum* of which *A. niger* was found toxic to eggs of *M. incognita*. Also in another case, Basyony (2018) reported that the culture filtrates of *Aspergillus niger, Pythium debarianum*, *Fusarium oxysporum, F. solani* and *F. moniliforme* were studied *in vitro* to see their influence on mortality of second stage juveniles (J2s) of the root-knot nematode, *M. incognita* and the filtrates of *A. niger* was most effective against second stage juveniles followed by *F. moniliforme*, *F. solani*, *F. oxysporum* and *P. debarianum*. However, this effect varied according to the type of growth media, filtrate dilutions and time of exposure. Nagwa *et al*. (2019) also reported the effect of six fungal filtrates, *Aspergillus niger, A. flavus, A. ochrachus, A. nidulans, Trichoderma harzianum* and *T. viride* were studied under laboratory conditions against *M. incognita* larvae. All these fungal filtrates significantly affected nematode mortality after 24hrs.

Satyandra and Nita (2010) who studied an *in vitro* experiment to determine the efficacy of indigenous fungi *Acremonium strictum, Aspergillus terreus, A. nidulans, A. niger, Chetomium aubense, Chladosporium oxysporum, Fusarium chlamydosporium, F. dimarum,*

F. oxysporum, F. solani, Paecilomyces lilacinus, Pochonia chlamydosporia, Trichoderma viride and *T. harzianum* on egg parasitism, egg hatching, mobility and mortality of root-knot nematode *M. incognita*, all tested fungi showed varied effects against the nematodes. In concordance with Ashoub *et al*. (2018) who conducted laboratory experiment to evaluate the ability of some fungi species. A proportional relation was found between mortality percentage and tested fungal culture filtrate concentrations at different exposure times. *Trichoderma viride* was the most effective species followed by *Aspergillus niger* and *Rhizoctonia* spp. in parasitizing nematode eggs and growth inhibition. The inhibitory activity of the *Aspergillus* genera could be attributed to their ability of producing toxins which could be harmful to the survival of the nematodes. In this study, increased in mortality rate was noticed as the duration time increases (Sankaranarayanan *et al*., 2016)

In the present study, the inoculation of *A. niger, A. nidulans, P. chrysogenum and R. stolonifer* enhanced plant growth parameters and reduced number of galls on tomato plant compared to the control in the screen house. The application of *A. niger* and *A. nidulans* filtrate significantly increased the average plant height (46.43 and 42.23 cm, respectively), number of fruits/plant (10.33 and 9.33, respectively) and number of leaves/plant (69.67 and 62.67, respectively) of the tomato plant more than the rest of the treatments. The increased plant parameters by the application of *Aspergillus* spp. could also be related to the decrease value for number of galls/root plant recorded (3.67 and 5.33, respectively). *P. chrysogenum* and *R. stolonifer* have a slight decrease for average plant height (33.43 and 26.13 cm, respectively), number of fruits/plant (6.67 and 5.33, respectively) number of leaves/plant (59.00 and 46.33, respectively) and an increase for number of galls/root plant. All the treatments used enhanced the growth parameters of the tomato plant and reduced gall

formation compared to the inoculated control. This result agrees with that of Jang *et al*. (2016) who reported that the culture filtrate of *A. niger* was highly active against *M. incognita* in inhibition of egg hatching and significant reduction in gall formation of tomato plant by 58.8 and 70.7 % which suggested that *A. niger* can be used as a microbial nematicide for the control of root knot nematode disease. In agreement with Sun *et al* (2011) who reported that incorporation of *A. niger* in seed coats suppressed gall formation and increased yield in tomato plant. Similar observation were also reported by several research workers in the past (Belay *et al*., 2016; Terra *et al*., 2018; Muhammad *et al*., 2019; Sikandar *et al*., 2019).

Based on the finding of the present study, indicated that *P. chrysogenum* significantly shows an increase in Plant height, Number of fruits and for Number of leaves in the tomato plant while a low value for Number of galls in the roots of the tomato plant was recorded. This signifies that *P. chrysogenum* showed plant growth promoting characters as well as have nematicidal activities with potential biocontrol agents against root knot nematode on tomato under greenhouse condition, in agreement with Sikandar *et al*. (2019) who stated that *P. chrysogenum* inhibited the development of nematodes at 60.30 %, reduce significant reproduction rate at 69.46 % of *M. incognita*, interfered in gall formation in root and enhanced plant growth parameters. Also, Siddiqui and Shaukat (2014) described that antagonistic fungi (*P. chrysogenum* and *A. niger)* efficiently enhanced the tomato growth and significantly reduced gall formation.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The findings of this research study conclude that there were differential occurrences of fungal species in soil samples.

It is also concluded from present research work that efficacy of the fungal culture filtrates increased with the increase in duration of exposure against second stage juvenile (J2s) *Meloidogyne incognita*.

Similarly, these fungal culture filtrates had differential effect on growth parameters of tomato crop, root-gall formation and disease incidence of *Meloidogyne incognita*.

5.2 Recommendations

From the findings of the present research work, recommendations are made as follows:

i. Some fungal species have potential to improve the plant growth parameters which could be used as bio-fertilizer.
ii. It is also recommended that some fungal culture filtrate have promising attributes to kill nematodes which could be used as bio-nematicides for eco-friendly management of plant parasitic nematodes especially root-knot nematodes.
iii. It is also recommended that there is need to carry out several research to find out active ingredient that promote plant growth and inhibit nematode population.

REFERENCES

Al- Fattah, A.A., Dababat, A.A. Richard, A., & Sikora, R.A. (2010). Use of *Trichoderma harzianum* and *Trichoderma viride* for the Biological Control of *Meloidogyne incognita* on Tomato. *Jordan Journal of Agricultural Science*, 3, 330-340.

Ashoub, A.H., Abd-Elgawad, A.A., Montasser, S. A., Mourad, M. H. & Gamal, M. (2018). Impact of some Fungi Species as Bio-control Agent against the Root-Knot Nematode, *Meloidogyne incognita*. *Australian Journal of Basic Applied Sciences*, 3(4), 3617-3624.

Abdissa, T., Amanti, C., Geremew, H. & Taha, M. (2010). Effect of Seedling Management on Yield and Quality of Tomato at Adami Tulu Jiddo Kombolcha District, Central Rift Valley of Ethiopia. *Africa Journal of Agriculture Resources*, 5, 3056-3059.

Abou- Zeid, A. M. & El-Fattah, R.I.A. (2011). Ecological Studies on the Rhizospheric Fungi of some Halophytic Plants in Taif Governorate, Saudi Arabia. *World Journal of Agricultural Science,* 3, 273-279.

Abubakar, U., Adamu, T. & Manga, S.B. (2016). Control of *Meloidogyne incognita* (Kofoid and White) Chitwood (root-knot nematode) of *Lycopersicon esculentum* (tomato) using cow dung and urine. Available online at http//:www. Cababstractsplus.org/abstract/Abstract.aspx?ACNO=20053156403

Acquaah, G. (2012). *Horticulture: Principles of plant genetics and breeding* (2nd Ed). Prentice Hall, New Jersey, Wiley Blackwell, 758

Adam, M., Goswami, J., Pandey, R.K., Tewari, J.P. & Goswami, B.K. (2014). Management of Root- knot Nematode on Tomato through Application of Fungal Antagonists, *Acremonium strictum* and *Trichoderma harzianum*. *Journal of Environmental Science and Health*, 43, 237–240.

Adam, M.A.M., Phillips, M.S. & Block, V.C. (2015). Molecular Diagnostic Key for Identification of Single Juveniles of Seven Common and Economically Important Species of Root-knot Nematode *(Meloidogyne* spp.). *Plant Pathology*, 56, 190-197.

Adebola, M.O., Dadi-Mahmud, N. J. & Yakima, M. K. (2014). Effect of Packaging Materials on Shelf-life Stability of Gari brought from Market in Lapai, Niger State, Nigeria. *Journal of Applied and Environmental Microbiology*, 2, 12-15.

Adebola, M.O., Mohammed, H.M. & Aliyu, D. (2014). Prevalence of Fungi in Central Market of Lapai, Niger State Nigeria. *International Journal of Applied Biological Research*, 6, 87-94.

Agrios, G.N. (2015). *Plant Pathology* (5th Ed). Burlington, MA, USA, Elsevier Academic Press, Amsterdam, 922.

Altalhi, A. D., (2019). Studies on Rhizospheric Bacteria and Fungi of some Plants in Taif area, Saudi Arabia. Egypt. *Journal of Applied Sciences*, 19, 164- 176.

American Phytopathological Society. (2012). Root-knot Nematode, http://www.apsnet.org/edcenter/intopp/lessons/Nematodes/Pages/RootknotNematode.aspx

Anonymous, 2012. *Documentation and Occurrence of Hot Spots for Economically Important Plant Parasitic Nematodes in the Maharashtra State*. Biennial Report (2009-2011) of All India Co-ordinated Research Project on Plant Parasitic Nematodes with Integrated Approach for Their Control, M.P.K.V, Rahuri presented in XVII Biennial Group Meeting held at Anand Agril, University, Anand (Gujrat), 7-26.

Anonymous, 2013. *Nematode biodiversity, identification of hot spot and pest free areas for economically important plant parasitic nematodes in the country*. Consolidated Biennial Report (2011-2012) of All India co-ordinated Research Project on Plant Parasitic Nematodes with Integrated Approach for their Control, IARI, New Delhi, presented in XI Annual Group Meeting held at M.P.K.V, Rahuri, (Maharashtra), 9-18.

Anwaar, S. A., & Mckenry, M. V. (2012). Incidence and Population Density of Plant Parasitic Nematodes Infecting Vegetable Crops and Associated Yield Losses. *Pakistan Journal of Zoology*, 44, 327–333.

Ardakani, A.S (2012). Toxicity of Silver, Titanium and Silicon Nanoparticles on the Root-knot Nematode, *Meloidogyne incognita*, and growth parameters of tomato. *Nematology*, 15, 671-677

Asaduzzaman, M., Alam, M. & Islam, M. (2010). Effect of *Trichoderma* on Seed Germination and Seedling Parameters of Chili. *Journal of Science Foundation*, 8, 141- 150.

Asare-Bediako, E., Showemimo, F.A., Buah, J.N. & Ushawu, Y. (2017). Tomato Production Constraint at Bolgatanga. *Journal of Applied Sciences,* 3, 459-461.

Ashraf, M. S. & Khan, T. A. (2017). Effect of Fungal Filtrates of some Opportunistic Fungi on Hatching and Mortality of Root-knot Nematode, *Meloidogyne incognita*. *Indian Journal of Nematology*, 35, 138-141.

Asif, M., Rehman, B., Parihar, K., Ganai, M.A. & Siddiqui, M.A. (2015). Effects of various physico-chemical factors on the incidence of root knot nematode *Meloidogyne* spp. infesting tomato in District Aligarh (Uttar Pradesh). *Indian Journal of Plant Sciences*, 10 (6), 234-243.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. & Vivanco, J.M. (2016). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, 57, 233-266.

Bakr, R.A., Mahdy, M.E. & Mousa, M.E. (2013). Efficacy of Soil Solarization and Post-Planting Mulch on Control of Root-Knot Nematodes. *Pakistan Journal of Nematology*, 31 (1), 71-76.

Basyony, A.G. (2018). Comparative Effects of Culture Filtrate of Soil- Borne Fungi on Mortality and Infectivity of Juveniles of *Meloidogyne incognita*. *Indian Journal of Nematology*, 37 (2), 109-114.

Bawa, J.A., Bashir, K.A. & Sani, Z.R. (2014). Pathogenicity Study of Southern Root Knot Nematodes (*Meloidogyne incognita* Chitwood) on Roma King Tomato Cultivar (CV). *International Research Journal of Biological Sciences*, 85, 2278-3202.

Belay, F., Alemu, L., Thangavel, S., & Gezehegne, G. (2016). Evaluation of some botanical and *Trichoderma harzianum* against root-knot nematode Mi (kofoid and white chitwood) in tomato. *Biology*, 4 (2), 75-83.

Benizri, E., Baudoin, E. & Guckert, A. (2011). Root colonization by inoculated plant growth rhizobacteria. *Biocontrol Science Technology*, 11(5), 557–574.

Bhattacharjee, R. & Dey, U. (2014). An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. *African Journal of Microbiology Research*, 8, 1749–1762.

Blacket, M.J. (2019). A History of the Introduction and Spread of Nematodes: In Mackenzie, D.R., Barfield, C.S., Kennedy, G.G., Berger, R.D. & Taranto, D.J. eds. The Movement and Dispersal of Agriculturally Important Biotic Agents, 15(4), 131-144.

Brussaard, L., Kuyper, T.W., & Goede, R.G.M. (2015). On the Relationships between Nematodes, Mycorrhizal Fungi and Plants: Functional Composition of Species and Plant Performance. *Plant and Soil*, 232, 155 - 165.

Burkett-Cadena, M., Kokalis-Burelle, N., Lawrence, K.S., Van Santen, E. & Kloepper, J.W. (2019). Suppressiveness of root-knot nematodes mediated by rhizobacteria. *Biological Control,* 47, 55-59.

Buskov, S., Serra, B., Rosa, E., Sorense, H. & Sorensen, J. C. (2019). Effects of Intact Glucosinolates and Products Produced from Glucosinolates in Myrosinase-Catalyzed Hydrolysis on the Potato Cyst Nematode (*Globoderaro stochiensis* cv. Woll). *Journal of Agricultural Food Chemistry*, 50, 690-695.

Cannon, P. F. & Kirk, P. M. (2008). *The fungi families of the world.* CABI Nosworthy Way, Wallingford Oxford Ire OX108DE U.K, 456.

Cao, Y., Zhang, Z.H., Ling, N., Yuan, Y.J., Zheng, X.Y., Shen, B.& Shen, Q.R. (2011). SQR 9 can control *Fusarium* wilt in cucumber by colonizing plant roots. *Biological Fertilizer Soils*, 47(5), 495-506.

Castillo, P. & Vovlas, N. (2009). *Pratylenchus (Nematoda Pratylenchidae) Diagnosis, Biology, Pathogenicity and Management. Nematology Monographs and Perspectives*. The Netherlands, Brill Academic Publishers, 529.

Castognone-Sereno, P. (2014). Genetic Variability and Adaptive Evolution in Parthenogenetic Root-knot Nematodes. *Heredity*, 96, 282-289.

Charchar, J.M. & Santo, G.S. (2019). Effect of soil temperature on the life cycle of *Meloidogyne chitwoodi* races 1 and 2 *M. hapla* on Russet Burbank potato. *Nematologia Brasileira,* 33, 154-161.

Charegani, H., Majzoob, S., Hamzehzarghani, H. & Karegar-Bide, A. (2012). Effect of Various Initial Population Densities of Two species of *Meloidogyne* on Growth of Tomato and Cucumber in Greenhouse. *Nematological Meditarrenian*, 40, 129-134.

Chitwood, D.J. (2016). Phytochemical Based Strategies for Nematode Control. *Annual Review Phytopathology,* 40, 221-249.

Compant, S., Clement, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology of Biochemistry*, 42(5), 669-678.

Coyne, D., Nichol, J. & Claudius-Cole, B. (2018). *Practical Plant Nematology: A Field and Laboratory Guide*, SP-IPM Secretariat. International Institute of Tropical Agriculture (IITA), Cotonou, Benin, 25-16.

Crawford, J.M., & Clardy, J. (2011). Bacterial Symbionts and Natural Products. *Chemical Communications*, 47, 7559-7566.

Crow, W.T. & Dunn, R.A. (2018). Introduction to Plant Nematology. Accessed on 14th March 2015 at entnemdept.ufl.edu/crow.htm

Dababat, A. A., Sikora, R. A. & Hauschild, R. (2011). Use of *Trichoderma harzianum* and *Trichoderma viride* for the Biological Control of *Meloidogyne incognita* on Tomato. *Soil Ecosystem Phytopathology and Nematology*, 71(3), 953-961.

Dababat, A.A. & Sikora, R.A. (2010). Use of *Trichoderma harzianum* and *Trichoderma viride* for the Biological Control of *Meloidogyne incognita* on Tomato. *Jordan Journal of Agricultural Sciences*, 3(3), 953-961.

Dallemole-Giaretta, R. (2014). Soil amendment with substrate containing mycelium and conidia of *Pochonia chlamydosporia* for the management of *Meloidogyne javanica*. *Ciência Rural*, Santa Maria, 44, 629-633.

De Waele, D. & Elsen, A. (2014). Challenges in Tropical Plant Nematology. *Annual Review of Phytopathology,* 45, 457-485.

Desaeger, J. R., Rao, M. R., & Bridge, J. (2019). *Nematodes and Other Soil-borne Pathogens in Agroforestry*. In: M. van Noordwijk, G. Cadisch & C.K. Ong, (eds.) Below-Ground Interactions in Tropical Agroecosystems, Concepts and Models with Multiple Plant Components, CABI, Wallingford, 263-283.

Devran, Z., Gotlieb, D., Oka, Y., Ben-Daniel, B. H. & Cohen, Y. (2011). Dry Mycelium of *Penicillium chrysogenum* Protects Cucumber and Tomato Plants against the Root-knot Nematode *Meloidogyne javanica*. *Phytoparasitica*, 31, 217-225.

Dias-Arieira, C.R., Marini, P.M., Fontana, L.F., Roldi, M. & Silva, T.R.B. (2012). Effects of *Azospirillum brasilense*, Stimulate and potassium phosphite to control *Pratylenchus brachyurus* in soybean and maize. *Nematropica,* 42, 170-175.

Dias-Arieira, C.R., Mattei, D., Puerari, H.H. & Ribeiro, R.C.F. (2015). Uses of organic amendments in the management of root-knot nematode in lettuce. *Horticultural Brasileira*, 13, 38-42.

Diedhiou, P.M., Hallmann, J., Oerke, E.C. & Dehne, H. W. (2018). Effects of arbuscular mycorrhizal fungi and a non-pathogenic *Fusarium oxysporum* on *Meloidogyne incognita* infestation of tomato. *Mycorrhiza*, 13, 199- 204.

Dinesh, K., Maheshwari, P., Trivedi, P.C. & Malhotra, A. (2013). Effects of *Pseodomonas aeruginosa* on the diversity of culturable micro-fungi and nematodes associated with tomato, Impact on root-knot disease and plant growth. *Soil Biology and Biochemistry*, 35, 1359- 1368.

Domsch, K. H., Gams, W., & Anderson, T. H. (2010). *Compendium of Soil Fungi.* Academic Press, London, United Kingdom.

Donkoh, S.A., Tachega, M. & Amowine, N. (2013). Estimating Technical Efficiency of Tomato Production in Northern Nigeria. *Journal of Experimental Agriculture,* 3, 56-75.

Escobar, C., Barcala, M., Cabrera, J. & Fenoll, C. (2015). Overview of Root-knot Nematodes and Giant Cells. *Advances in Botanical Research*, Elsevier. 73, 1-32.

Esheteu, B., Ferdu, A., & Tsedeke, A. (2014). Experience with Management of Major Plant Diseases in Ethiopia Proceedings on Facilitating the Implementation and Adoption of Integrated Pest Management (IPM) in Ethiopia. Melkassa Agricultural Research, October 13-15 2003, *Ethiopian Agricultural Research Organization*, 25-26.

Evans, A.A.F. & Perry, R.N. (2018). *Survival Mechanisms*. In Perry, R.N., Moens, M. & Starr, J.L. (Eds), Root-knot nematodes. Wallingford, UK, CAB International, 201-219.

FAO, (2010). Food and Agrriculture Organisation. (FOASTAT) Food and Agriculture Organization of Statistical Database. Accessed on 21th April 2015 at http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

FAO. (2012). Food and Agriculture Organization of the United Nations. Retrieved on 21 November, 2014 at http:// en.wikipedia.org/wiki/Tomato

Farahat, A.A., Al-Sayed, A.A. & Mahfoud, N.A. (2010). Compost and other organic and inorganic fertilizers in the scope of the root-knot nematode reproduction and control of *Meloidogyne incognita* infecting tomato. *Egyptian Journal of Agronematology*, 9, 18-29.

Ferris, H., Bongers, T., & De Goede, R.G.M. (2019). A Framework for Soil Food Web Diagnostics: Extension of the Nematode Faunal Analysis Concept. *Applied Soil Ecology*, 18, 13-29.

Frisvad J. C., Larsen, T. O., Thrane, U., Meijer, M., Varga, J., Samson, R. A, *et al*. (2011). Fumonisin and Ochratoxin Production in Industrial *Aspergillus niger* Strains. PLoS One. 6:e23496. doi: 10.1371/journal.pone.0023496 PMID: 21853139

Gams, W. & Bisset, J. (2017). *Morphology and Identification of Trichoderma*. In: *Trichoderma* and *Gliocladium*.GE. Harman and CP. Kubicek, eds, Taylor and Francis, London, 1, 3-34.

Ganaie, M.A. & Ahmadkhan, T. (2010). Biological potential of *Paecilomyces lilacinus* on pathogenesis of *Meloidogyne incognita* infecting tomato plant. *European Journal of Applied Science*, 2, 80-84.

Garcia, T. C., Liliane, E. V., Everaldo, A. L., Cladio, M. G. O. & Pedro, I.V.G.G. (2018). Diagnostic methods for identification of root-knot nematode species from Brazil. *Crop Protection*, 48(2), 2-11.

Janati, S., Geeta, S., Abdellah, H. & Mokrini, F. (2018). Occurrence of the root-knot nematode species in vegetable crops in Sonss region of Morocco. *Journal of Plant Pathology*, 4, 324-330.

Gitanjali, D. & Bora, L.C. (2018). Effects of some biocontrol agents against root-knot nematode (*Meloidogyne incognita*). *International Journal of Environment, Agriculture and Biotechnology*, 3(5), 1748-1755.

Greco, N. (2009). Alternatives to Methyl bromide to Control Plant Parasitic Nematodes in Greenhouses. Access on 20th August, 2015 at http://www.minagric.gr/greek/data/files2251/GRECO1.DOC

Hassan, M., Chindo, P. & Alegbejo, M. (2010). Management of Root Knot Nematodes (*Meloidogyne* spp.) on Tomato (*Lycopersicon lycopersicum*) using Organic Wastes in Zaria, Nigeria. *Plant Protection Science*, 46, 34-39.

Heve, W. K., Been, T. H., Schomaker, C. H. & Teklu, M. G. (2015). Damage thresholds and population dynamics of *Meloidogyne chitwoodi* on carrot (*Daucus carota*) at different seed density. *Nematology,* 17(5), 501-514.

Holterman, M., Karssen, G., Van den Elsen, S., Van Megen, H., Bakker, J. & Helder, J. (2009). Small Subunit rDNA-Based Phylogeny of the Tylenchida Sheds Light on Relationships among some High-Impact Plant-Parasitic Nematodes and the Evolution of Plant Feeding. *Phytopathology,* 99, 227-235.

Hortic, J. (2017). Management of Root Knot Nematode *Meloidogyne incognita* (Kofoid and white) Chitwood in Tomato *Lycopersicon esculentum* Mill through Poutry Manure and Repeseed Cake. *Journal of Horticulture and forestry,* 9, 59-65.

Huang, W. K. (2016). Testing various Biocontrol Agents against the Root-Knot Nematode (*Meloidogyne incognita*) in Cucumber Plants Identifies a Combination of *Syncephalastrum racemosum* and *Paecilomyces lilacinus* as being most effective. *Biological Control*, 92, 31-37.

Hunt, D.J. & Handoo Z.A. (2016). *Taxonomy, Identification and Principal Species*. In: Perry, R.N, Moens, M. & Starr, J.L (eds.), Root Knot Nematodes, CABI publishing Wallingford, UK, 55-97.

Hunt, D.J. & Handoo, Z.A. (2016). Root-knot nematode. Books google.com.

Hussain, M. & Zouhar, M. (2017). Effects of Nematophagous Fungi on Viability of Eggs and Juveniles of *Meloidogyne incognita*. *Pathology*, 15, 115-127.

Hussey, R.S. & Janssen G.J. W. (2016). Root-knot Nematodes, *Meloidogyne* species In: J.L., Starr, R., Cook, J. & Bridge (Eds.), Plant Resistance to Parasitic Nematodes, CABI, New York, USA, 43-70

Hwang, S.M., Park, S.M., Kim, J.C., Jang, K.S., Choi, Y.H. & Choi, G.J. (2014). Occurrence of *Meloidogyne incognita* Infecting Resistance Cultivars and Development of an efficient Screening method for resistant Tomato to the Mi-virulent Nematode. *Korean Journal of Horticultural Science Technology*, 32, 217-226.

Jada, M.Y. Gungula, D.T. & Jacob, I. (2010). Efficacy of Carbofuran in controlling Root-Knot Nematode (*Meloidogyne javanica*) on Cultivars of Bambara Groundnut (*Vigna subterranea* (L.) Verdc.) Yola, Nigeria. *International Journal of Agronomy,* 11, 5-10.

Jang, J. Y., Choi, Y. H., Shin, T. S., Kim, T. H., Shin, K.-S., Park, H. W., Kim, Y. H., Kim, H., Choi, G. J., Jang, K. S *et al*. (2016). Biological Control of *Meloidogyne incognita* by *Aspergillus niger* F22 Producing Oxalic Acid, PloS One 11: e0156230

Kalele, D. N., Affokpon, A. & Coosemans, J. (2010). Efficacy of *Paecilomyces lilacinus* Strain 251 against Root knot Nematodes in Tomato under Greenhouse Conditions. *Communications in Agricultural and Applied Biological Sciences*, 72, 209-213.

Karssen, G. &Moens, M. (2015). *Root-Knot Nematodes*. In: Perry, R.N. and Moens, M. (eds), *Plant Nematology*. CAB International, Wallingford, UK, 59-90.

Kennelly, M. (2014). Wilt, Nematode and Viral Diseases of Tomato. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. Access on 25th May, 2015 at www.oznet.ksu.edu

Keshari, A. K., & Gupta, R. (2015). Management of root-knot nematode, *Meloidogyne incognita* on tomato using extracts of indigenous plants of Nepal. *Annuals of Plant Protection Sciences*, 23, 131–134.

Khan, M.R. & Kounsar, K. (2018). Effect of Seed Treatment with certain Bacteria and Fungi on the Growth of Mungbean and Reproduction of *Meloidogyne incognita*. *Nematologia Mediterranea*, 28, 221-226.

Khan, Z., Kim, S.G., Jeon, Y.H., Kh, H.U., Son, S.H. & Kim, Y.H. (2019). A plant growth promoting rhizobacterium, Paenibacillus polymyxa strain GBR-1, suppresses root-knot nematode. *Bio-resources Technology*, 99(8), 3016–3023.

Kim, D. I., Park, J. D., Kim, S. G., Kuk, H., Jang, M. J., & Kim, S. S. (2019). Screening of some crude plant extracts for their acaricidal and insecticidal efficacies. *Journal of Asia-Pacific Entomology*, 8, 93–100.

Kokalis, B. N., David, M. B. & Erin, N. R. (2013). Evaluation of cover crops with potentials for use in Anaerobic soil disinfestation (ASD) for susceptibility to 3 species of *Meloidogyne*. *Biology*, 8, 32-48.

Kumar, V. & Jain, R. K. (2010). Management of root-knot nematode, *Meloidogyne incognita* by *Trichoderma viride*, *T. harzianum* and Bacterial Antagonist *Pseudomonas fluorescens* as Seed Treatment on Okra. *Indian Journal of Nematology*, 40, 226-228.

Kwara, B.K., Kwoseh, C.K. & Starr, J.L. (2014). Effectiveness of Root-Knot Nematode (*Meloidogyne* species) Resistant Tomato (*Solanum lycopersicum* L.) and Pepper (*Capsicum* species) Cultivars in Ghana. *Nematropica*, 44(2), 130-136.

Lee, E., Sargent, S.A. & Huber, D.J. (2019). Physiological changes in roma type tomato induced by mechanical stress at several ripeness stages. *Horticultural Sciences*, 42(5), 1237-1242.

Lee, J., Huh, N., Hong, J. H. & Kim, B.S. (2012). The antagonistic properties of *Trichoderma* spp. Inhabiting woods for potential biological control of wood-damaging fungi. *Korea University Liberary*, 66, 883-887.

Liu, R., Dai, M., Wu, X., Li, M. & Liu, X. (2011). Suppression of root-knot nematodes (Mi) [Kofoid& White chitwood] on tomato by dual inoculation with arbuscular mycorrhizal fungal and plant growth promoting rhizobacteria. *Biology*, 15(2), 78-82.

Mai, W.F. & Mullin, P.G. (2018). *Plant Parasitic Nematode*. A pictorial key to genera, 5thEd Ithaca, New York, Cornell University Press, 124.

Manzanilla-Lopez, R.H., Kenneth, E. & Bridge, J. (2013). *Plant Diseases Caused by Nematodes*. In: Chen, Z.X., Chen, S.Y., Dickson, D.W., editors. Nematology Advanced and perspectives. Nematode management and utilization. Wallingford, UK, CAB International, 2, 637–716.

Mao, L., Hajibabaei, M., Singer, G.A.C., Hebert, P.D.N. & Hickey, D.A. (2017). DNA Barcoding, How it Complements Taxonomy, Molecular, Phylogenetics and Population Genetics. *Trends in Genetics*, 23,167–172.

Marie, S. T., Johan, P. G., Reynaldo, C. Q., Cristobal, A.G. & Therry, M. (2014). Commercial Biological Control Agents Targeted against Plant Parasitic, Root-knot nematodes. *Agriculture, Agribusiness and Biotechnology*, 57(6), 167- 180.

Mascarin, G. M. & Junior, M. F. B. (2012). *Trichoderma harzianum* reduces Population of *Meloidogyne incognita* in Cucumber Plants under Greenhouse Conditions. *Journal of Entomology and Nematology*, 4, 54-57.

Meher, H.C., Gajbhiye V.T., Singh, G., Kamra, A. & Chawla, G. (2010). Persistence and Nematicidal Efficacy of Carbosulfan, Cadusafos, Phorate, and Triazophos in soil and uptake by Chickpea and Tomato Crops under tropical conditions. *Journal of Agricultural Food Chemistry*. 58(3), 15-22.

Mendes, R., Garbeva, P. & Raaijmakers, M.J. (2013). The rhizosphere microbiome, significance of plant beneficial, plant pathology and human pathogenic microorganism. *FEMS Microbiology Reviews,* 37(5), 634-663.

Meyer, S.L.F & Roberts, D.P. (2014). Combination of Biocontrol Agents for Management of Plant Parasitic Nematodes and Soil-borne Plant Pathogenic Fungi. *Journal of Nematology*, 34(1), 1-8.

Meyer, S.L.F., Huettel, R.N., Liu, X.Z., Humber, R.A., Juba, J. & Nitao, J.K. (2010). Activity of Fungal Culture Filtrates against Soybean Cyst Nematode and Root-Knot Nematode Egg Hatch and Juvenile Motility. *Nematology*, 6, 23-32.

Miyashita, N., Yabu, T., Kurihara, T. & Koga, H. (2014). The Feeding Behavior of Adult Root-knot Nematodes (*Meloidogyne incognita*) in Rose Balsam and Tomato. *Journal of Nematology*, 46, 296-301.

Moens, M., Perry, R. N. & Starr, J. L. (2015). *Meloidogyne* species. A Diverse Group of Novel and Important Plant Parasites in Root-knot nematodes Wallingford Oxford shire UK: CAB International.

Moosavi, M.R. & Zare, R. (2012). Fungi as Biological Control Agents of Plant Parasitic Nematodes. *Biological Control,* 12, 67-107.

Muhammad N. U., Saifullah M. A., Wajid K. & Bilal M. K. (2019). Evaluation of *Pochonia chlamydosporia* (Goddard) isolates for Suppression of *Meloidogyne incognita*, Root knot Nematode of Tomato. *Journal of Agricultural Science*, 11(5), 70-81.

Mukhtar, I., Hannan, A., Atiq, M. & Nawaz, A. (2012). Impact of *Trichoderma* species on seed Germination in Soybean. *Pakistan Journal of Phytopathology*, 24, 159-162.

Munos, S., Ranc, N., Botton, E., Berard, A., Rolland, S., Duffe, P., Carretero, Y., Le Paslier, M.C., Delalande, C., Bouzayen, M., Brunel, D. & Causee, M. (2011). Increase in tomato locule number is controlled by two single nucleotide polymorphisms located near Wuschel. *Plant Physiology*, 156(4), 2244-2254.

Nagwa, Abdl Bari, A., Aboul Eid, H. Z., Anter, E. A. & Noweer, E. A. (2019). Effects of Different Fungal Filtrates on *Meloidogyne incognita* Larvae in Laboratory Bioassay Tests. *Egyptian Journal of Agrometeorology*, 4(1&2), 46-69.

Nico, A. I., Jimenez-Diaz, R.M. & Castillo, P. (2010). Solarization of soil in piles for the control of *Meloidogyne incognita* in olive nurseries in southern Spain. *Plant Pathology,* 52(6), 770-778.

Noling, J.W. (2010). Nematode Management in Tomatoes, Peppers and Eggplant. U.S. Department of Agriculture, UF/IFAS Extension Service, University of Florida. Accessed on 13th April, 2015 at www.http://edis.ifas.ufl.edu/ng032

Noling, J.W. (2011). Movement and Toxicity of Nematicides in the Plant Root Zone. Accessed on 13th April, 2015 at www.http://edis.ifas.ufl.edu

Nurul, H., Muhammad, M.K. & Nashriyah, M. (2016). Occurrence and control of root-knot nematode in crops: A review. *Australian Journal of Crop Science*, 10(12), 1647-1654.

Nyczepir, A. P. & Thomas, S. H. (2016). Current and future management strategies in intensive crop production systems. In: Perry, R. N. Moens, M. Starr, J. L. (2009). Root-knot nematodes Wallingford Oxfordshire UK: CAB International.

Ogwulumba, S., Ugwuoke, K. & Ogbuji, R. (2010). Studies on *Meloidogyne javanica* infestation on roma tomato (*Lycoperiscon esculentum* Mill.) under different Soil Amendment. *Africa Journal of Biotechnology*, 9, 3280-3283.

Osei, K., Addico, R., Nafeo, A., Edu-Kwarteng, A., Agyemang, A., Danso, Y. & Sackey-Asante J. (2011a). Effect of some Organic Waste Extracts on Hatching of *Meloidogyne incognita* eggs. *African Journal of Agricultural Research*, 6(10), 2255-2259.

Panesar, T.S. & Marshall, V.G. (2012). *Monograph of soil nematodes from coastal Douglas*. Fire forests in British Columbia. Royal Roads University. Canada.

Peet, M. (2018). Crop profiles, Tomato. Archived from the original on 26 November. Retrieved 27 October 2008.

Perry R.N. & Moens M. (2013). *Plant nematology*: CAB International, ISBN – 10:0-85199-027-4. (accessed 2nd March 2015) Available at www. Cabi.org

Perry R.N., Moens M. & Starr J.L. (2019). Root Knot Nematodes. CAB International, ISBN 13:978 1 84593 492 7

Preedy, V.R & Waltson, P.R. (2019). *Tomatoes and tomato products: Nutritional, Medicinal and Therapeutic properties*. Enfield, NH, USA. Science publisher. ISBN 978-1-57808-534-7, 2-30.

Raveendra, H. R., Krishna Murthy, R. & Mahesh Kumar, R. (2011). Management of Root knot Nematode, *Meloidogyne incognita* by using Oil cake, Bio-agent, Trap Crop, Chemicals and their Combination. *International Journal of Science nature*, 2(3), 519 – 523.

Regina, M.D., Gomez, C., Fabia, S.O.L. & Valder, R.C. (2016). Methods and tools currently used for the identification of plant parasitic nematodes. *Nematology*, 3, 2174-2270.

Sankaranarayanan, C., Hussaini, S. S., Rangeshwaran, R. & Kumar, P.S. (2016). Efficacy of *Pseudomonas fluorescens* alone and in Combination with *Pasteuria penetrans* against Plant Parasitic Nematodes. *International Journal of Tropical Plant Diseases*, 19(2), 7-13.

Santhosh, J.E., Beena, B. & Ramana, K.V. (2016). Tropical soil microflora of spice-based cropping systems as potential antagonist of root-knot nematodes. Division of crop protection, *Indian Institute of Spice Research Calicut*, 10, 673-1210.

Sato, S., Tabata, S., Hirakawa, H., Asamizu, E., Shirasawa, K., Isobe, S., Kaneko, T., Nakamura, Y., Shibata, D., *et al*. (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, 485 (7400), 635-641.

Satyandra, S. & Nita, M. (2010). *In vitro* Studies of Antagonistic Fungi against the Root-knot Nematode, *Meloidogyne incognita*. *Biocontrol Science of Technology*, 20(3), 275-282.

Selim, M. E. M. (2010). Biological, Chemical & Molecular Studies on the Systemic Induced Resistance in Tomato against *Meloidogyne incognita* caused by the Endophytic *Fusarium oxysporum*, Fo162. Ph.D. University of Bonn.

Senthilkumar, P. & Ramakrishnan, S. (2011). Studies on the Compatibility of *Pseudomonas fluorescens, Trichoderma viride* and Carbofuran 3G, and their Influences on *Meloidogyne incognita* in okra. *Annuals of Plant Protection Sciences,* 12(1), 140-142.

Sharma, D. D., (2018), Effect of culture filtrate of bio control agents on larval mortality of fungi against the root-knot nematode, *Meloidogyne incognita*. *Biological control of Science Technololgy,* 20(3), 275-282.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A.,Kleifeld, O. & Spiegel, Y. (2011). Biological Control of the Root-knot Nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology*, 91, 687-693.

Siddiqui, I. A., & Shaukat, S.S. (2014). Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: Importance of bacterial secondary metabolite, 2,4-diacetylpholoroglucinol. *Soil Biology and Biochemistry*, 35, 1615–1623.

Sikandar, A.1., Zhang, M. Y.1., Zhu, X. F.1., Wang, Y. Y.2., Ahmed, M.3., Iqbal, M. F.2., Javeed, A.2., Xuan, Y. H.1., Fan, H. Y.1., Liu, X. Y.4., Chen, L. J.1. & Duan, Y. X.1. (2019). Efficacy of *Penicillium chrysogenum* Strain Snef1216 against Root-knot Nematodes in Cucumber under Greenhouse Conditions. *Applied Ecology and Environmental Research*, 17(5), 12451-12464.

Sikora, R. A., & Fernández, E. (2016). Nematode parasites of vegetables. In M. Luc, R. A. Sikora, & J. Bridge (Eds.), *Plant parasitic nematodes in subtropical and tropical Agriculture*, 319–392.

Silva, O. J., Marcus, V. S., Leonardo, L. F., Ferreira, S. B., & Rocha, M. R. (2017). Biological control agents in the management of *Meloidogyne incognita* in tomato. *Ciencia Rural*, 47, 10-23.

Singh, S., Rai, A. B., Singh, R., & Singh, A. K. (2011). Population Dynamics of Phytonematodes in Vegetable Crops. *Annuals of Plant Protection Sciences*, 19, 503–504.

Singh, T., & Patel, B. A. (2013). Management of Root-knot Nematode (*Meloidogyne incognita*) in Bottle Gourd using Different Botanicals in Pots. *Journal of Parasitic Diseases*, 17, 54-72.

Smant, G.,Helder, J. & Goverse, A. (2018). Parallel Adaptations and Common Host Cell Responses Enabling Feeding of Obligate and Facultative Plant Parasitic Nematodes. *Plant Journal*, 93, 686-702.

Sohlenius, B. (2011). *Soil Nematode Communities in Grasslands Effects of Plant Species*. Identity and Diversity. Ph.D. Thesis. Swedish University of Agricultural Sciences, Uppsala, 12.

Strajnar, P. & Sirca, S. (2011). The Effect of some Insecticides, Natural Compouds and Tomato cv. Venezia with Mi gene on the Nematode *Meloidgyne ethiopica* (Nematoda) reproduction. *Acta Agriculturaal Slovenica,* 95, 5-10.

Sun J. H.,Wang H. K., Lu F. P., Du L. X. & Wang, G.F. (2014). The Efficacy of Nematicidal strain *Syncephalastrum racemosum*. *Annual Microbiology*, 58, 369–373.

Sun, M.H., Gao, L., Shi, Y.X., Li, B.J. & Liu, X.Z. (2011). Fungi and Actinomycetes Associated with *Meloidogyne* spp. Eggs and Females in China and their Biocontrol Potential. *Journal of Invertebrate Pathology,* 93, 22-28.

Terra, W. C., Campos, V. P., Martins, S. J., Costa, L. S. A. S., da Silva, J. C. P., Barros, A. F., Lopez, L. E., Santos, T. C. N., Smant, G. & Oliveira, D. F. (2018). Volatile Organic Molecules from *Fusarium oxysporum* Strain 21 with Nematicidal activity against *Meloidogyne incognita*. *Crop Protection*, 106, 125-131.

Trudgill, D.L.& Blok, V.C. (2014). Apomictic, Polyphagous Root-Knot Nematodes: Exceptionally Successful and Damaging Biotrophic Root Pathogens. *Annual Revise Phyto-pathology,* 39, 53-77.

Villian, L., Hernández, A., Fargette, M. & Sarah, J.L. (2008). Characterization of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) from Coffee Plantations in Central America and Brazil. *Nematology,* 6(2), 193–204.

Von der Weid, I., Artursson, V., Seldin, L. & Jansson, J. K. (2017). Antifungal, root surface colonization properties of GFP tagged *Paenibacillus brasilensis* PB177. *World Journal Microbiology and Biotechnology*, 21(8), 1591–1597.

Wang, K.H., Hooks, C.R., & Ploeg, A. (2019). A Review: Protecting Crops from Nematode Pests: using Marigold as an Alternative to Chemical Nematicides. Cooperative Extension Service, College of Tropical Agriculture and Human Resources, University of Hawaii at Mänoa PD-35. (Accessed 22 August 2014) from http://www.ctahr.hawaii.edu/oc/freepubs/pdf/PD-35.pdf

Weimen, Y., Robert, T.R. & Kirkpatrick, T. (2019). Molecular characterization of root-knot nematodes (*Meloidogyne* spp) from Arkansas USA. *Scientific Reports*, 9, 156-180.

Wesemael, W.M.L., Viaene, N. & Moens, M. (2011). Root-Knot Nematodes (*Meloidogyne* spp.) in Europe. *Nematology,* 13, 3-16.

Westerdahl, W.M.L. & Becker, J.O. (2011). Intergrated Pest Management Guidelines: Cucurbits. (Accessed 22 August 2014) from http://www.ipm. ucdavis.edu/PMG/r116200111.html

Xalxo, P.C., Karkun, D. & Poddar, A. N. (2013). Rhizospheric Fungal Associations of Root Knot Nematode Infested Tomatos: In vitro Assessment of their Nematicidal Potential. *Research Journal of Microbiology*, 8, 81-91.

Xiang, N., Lawrence, K. S., Kloepper, J. W., Donald, P. A., McInroy, J. A. & Lawrence, G. W. (2017). Biological Control of *Meloidogyne incognita* by Spore-Forming Plant Growth-Promoting Rhizobacteria on Cotton. *Plant Disease,* 101, 774-784.

Yang, G., Zihou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang *et al*. (2016). Effects of tomato Root exudates on *Meloidogyne incognita*. PLosONE 11 (a): e0154675.doi: 10.1371/ journal pone. 0154675.

Yao, Q., Lu, X., Zhu, X., Wang, Y., Chen, L. & Duan, Y. (2014). Resistance against *Meloidogyne incognita* in Tomato Induced by Fermentation Liquid of *Penicillium chrysogenum* Strain 1216. *Acta Phytopathologica Sinica*, 44, 693-699.

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. & Georgieva, S.S. (2011). Feeding Habits in Soil Nematode Families and Genera: An outline for Soil Ecologists. *Journal of Nematology*, 25, 315-331.

Youdeowei, A. (2014). Integrated Pest Management Practices for the Production of Vegetables. GTZ. Integrated Pest Management Extension Guide 4. Published by The Ministry of Food and Agriculture (MOFA) Plant Protection and Regulatory Services Directorate (PPRSD), Ghana with the German Development Cooperation (GTZ).

APPENDICES

A. Anova for the Treatment

		Sum of Squares	Df	Mean Square	F	Sig.
PlantHeight	Between Groups	1271.351	4	317.838	29.303	.000
	Within Groups	108.467	10	10.847		
	Total	1379.817	14			
Numoffruits	Between Groups	91.600	4	22.900	20.206	.000
	Within Groups	11.333	10	1.133		
	Total	102.933	14			
Numofgalls	Between Groups	1182.000	4	295.500	48.179	.000
	Within Groups	61.333	10	6.133		
	Total	1243.333	14			
NumofLeaves	Between Groups	1561.333	4	390.333	22.606	.000
	Within Groups	172.667	10	17.267		
	Total	1734.000	14			

B. Anova of Disease incidence

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2042.296	4	510.574	61.938	.000
Within Groups	82.433	10	8.243		
Total	2124.729	14			