PATHOGENIC POTENTIAL OF ROOT- KNOT NEMATODE (*Meloidogyne arenaria*)TO COMMON ZINNIA (*Zinnia elegans*) AND ITS MANAGEMENT USING COFFEE HUSK AND BIOCHAR

M.Sc. Thesis

ZUBEYDA YASIN MOHAMMED

November, 2019

Jimma, Ethiopia

PATHOGENIC POTENTIAL OF ROOT- KNOT NEMATODE (*Meloidogyne arenaria*.) TO COMMON ZINNIA (*Zinnia elegans*) AND ITS MANAGEMENT USING COFFEE HUSK AND BIOCHAR

By

Zubeyda Yasin Mohammed

M.Sc. Thesis

Submitted to the School of Graduate Studies, Jimma University College of Agriculture and Veterinary Medicine, Department of Horticulture and Plant Sciences in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology

Major Advisor: Dr.Beira Hailu Meressa

Co-Advisor: Dr. Wakuma Bayissa Hundessa

Jimma, Ethiopia

February 2020

DEDICATION

I dedicated this thesis to my beloved father Mr. Yasin Mohammed.

STATEMENT OF AUTHOR

I, Zubeyda Yasin Mohammed, hereby declare that this thesis is my original work and all sources of materials used for writing this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. Degree at Jimma University College of Agriculture and Veterinary Medicine and is deposited in the University's Library to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Name: Zubeyda Yasin

Signature: _____

Place: Jimma University

Date of submission_____

BIOGRAPHIC SKETCH

The author Zubeyda Yasin was born from her Father Yasin Mohamed and her Mother Asha Kemal in Oromia region Wonji in 1996. She completed primary and secondary high school in Wonji Gefersa primary and secondary school. She completed her preparatory school at Wonji Gefersa preparatory school. Then in 2013 she joined Dilla University and obtained a BSc degree in Plant Science in July 2015. After her graduation, she was employed as a graduate assistant at Dilla University until she joined Jimma University College of Agriculture and Veterinary Medicine in September 2017 to peruse a master study in Plant Pathology.

ACKNOWLEDGMENT

First and foremost I would like to glorify and bring the greatest of all thanks to Allah for the strength he has given me to withstand all burdens and rigorous to complete this work.

I wish to express my most sincere appreciation and gratitude to my advisors Dr. Beira Hailu and Dr. Wakuma Bayissa without their valuable comments; unreserved guidance and professional expertise to completion of this work would not have been realized

I would like to thank specially my mother. The support of many good people, colleagues and friends who devoted their time and resources to assist me during my study period is greatly acknowledged**.** Without their kind help this study would have been hardly possible.

I sincerely also like to thank Dilla University for the sponsorship it provided to me to pursue my post-graduate studies and also thanks Jimma University for accepting me as M.Sc student and also for permission to use the laboratory facilities.

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CRD	Complete Randomized Design
EMN	Eggmass Number
FAO	Food and Agricultural Organization
FP	Final Population
J1	First stage Juvenile
J2	Second stage Juvenile
LN	Leaf Number
PAGE	Polyacrylamide Gel Electrophoresis
RFW	Root Fresh Weight
RGN	Root Gall Number
RKN	Root Knot Nematode
RF	Reproduction Factor
RL	Root Length
SAS	Statically Analysis System
SFW	Shoot Fresh Weight
SH	Shoot Height

TABLE OF CONTENTS

TABLE OF CONTENTS(C*ontinued*)

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF APPENDICES

Page

ABSTRACT

Root knot nematodes are capable of infecting over 3000 host plants including, ornamental plants. It is widely distributed and damaging Zinnia elegans flowers. Widespread dissemination and lack of information regarding this ornamental plant damage due to plant-parasitic nematodes is attributed to demand research for agronomic crops and an important avenue for distribution of other plant-pathogenic organisms. The aim of this study was to identify and evaluate the Pathogenecity of Meloidogyne arenaria on common zinnia, and to manage the nematode using coffee husk and biochar amendments. All experiments were conducted in Jimma University College of Agriculture and Veterinary Medicine (JUCAVM). The host status experiment was carried out on common zinnia plant on infected soil under greenhouse. Pathogenecity study and nematode pest management experiments per pot were laid out using completely randomized design (CRD) under greenhouse condition at JUCAVM. Seedlings with four true leaves were inoculated with J2 a week after transplanting. Data on number of eggmass and root gall, final nematode population, reproduction factor, plant height, root length, leaf number, fresh weight of shoots and roots were collected from each treatment in all experiment after two months of inoculation. The results of host status showed that the growth rate of plant (height, root length, leaf number, fresh weight of shoots and roots) were increased with time. Aboveground symptoms developed over time due to infection. The maximum nematode populations (26715), numbers of root gall (506) and eggmass (483) were noted 16th week after infection. The result of Pathogenecity study showed that the initial inoculum level increases the number of galls, eggmass and final nematode population. The highest number of galls122.0), eggmass (66.33) and final nematode population (25114.3) were noted on 16J2/g soil, but for nematode reproduction factor was achieved at inoculum level of 2 J2/g soil (6.45). Application of coffee husk and biochar amendment reduced the root galling and final populations of M. arenaria compared with control. The lowest nematode populations were recorded at 4:1 ratio from coffee husk and biochar (200,925) respectively while the highest was noted on non-amended plants (29695). Coffee husk was more effective than biochar in reducing population of nematodes. Amendment of soil with coffee husk and biochar may provide practical control of root knot nematodes as a part of integrated management system. However, further research is needed to evaluate their efficacy under field condition.

Key words: Eggmass, Final population, Reproduction factor, Meloidogyne spp, Root gall,

1. INTRODUCTION

Floriculture is a discipline of horticulture concerned with the cultivation of flowering and ornamental plants for gardens and for floristry, comprising the floral industry. It is concerned with commercial production, marketing, and sale of bedding plants, cut flowers, potted flowering plants, foliage plants, flower arrangements, and noncommercial home gardening (Mulugeta, 2009). Floriculture is one of the booming sectors in Ethiopia. The first private floriculture companies, Meskel flower and Ethio–flora, started activities around 1997 on a few hectares of land. Today, the Ethiopian InvestmentAgency has given permit to 251 investors in the floriculture sector (Negusse, 2006).

Common zinnia is is an annual flowering and native to North America (Mexico) but widely cultivated as ornamental plant for cut flowers and flowerbeds. It is grown in many places and naturalized in several places including scattered locations in South and Central America, the West Indies, the United States, Australia, and Italy (Kirkbride and Wiersma, 2007). It is grown because of its economic value, potential salt-tolerant cut flower crop to grow in greenhouse systems using recycled agricultural waste water and tolerance to temperature fluctuation (Carter *et al*., 2010). Z. elegans was introduced to Africa as an ornamental from North America. Among the 23 known Zinnia species in the genus Zinnia, only two species, *Z.elegans* and *Z. peru-viana* (L.) are known to occur in Ethiopia. Zinnia elegans is not known to be invasive, but have been recorded as an escape in the central parts of Ethiopia (Mesfin, 2004). *Z.elegans* are popular garden flowers and due to its attracted from seem interested by many gardeners. They are usually grow from seeds and preferable in fertile, humus rich and well drained soil in area in full sun (Salme *et al*, 2003).The Environmental condition of Ethiopia has conducive for production of many ornamental plant including common zinnia spp.

Over the past few years, the floriculture industry has become an important sector of Ethiopian agriculture, yet plant damage caused by plant-parasitic nematodes still receives little attention compared with other plant diseases. While fungal diseases and insects are already considered as a major pest problem on cut flowers in Ethiopia. Plant-parasitic nematodes have been ignored for a long time. Only recently, thirteen genera of plant-parasitic nematodes were reported to be associated with cut flowers produced in Ethiopia (Beira et al., 2014).

Diseases often reduce the quantity and quality of zinnia flowers, causing their production to be unprofitable. Fungal, bacterial, viral and nematodes are among the major pathogens that have economic impact on its production (Gombert*et al*., 2001). Plant parasitic nematodes are destructive soil pests that attack a wide range of plants that root-knot(*Meloidogyne incognita, M. arenaria,M. hapla*), lesion (*Pratylenchus vulnus*), ring (*Macroposthonia* and *Criconemoides* spp.), and stunt (*Tylenchorhynchus* spp.) nematodes cause considerable injury to annuals, perennials, herbs, and woody ornamentals including Zinnia elegans (Austin, 2005). The report showed that 11.1% of the ornamental plants are damaged by plant parasitic nematode in the world.

Two species of nematodes that infest *Z. elegans are: Meloidogyne incognita*, which causes root knot and *Aphelenchoides ritzemabosi*, which causes angular spots on the leaves. Root knot nematodes are capable of infecting of over more than 3000 plant species (Abad et al., 2003). Plants including all major field crops, vegetables, ornamental plants, fruit trees and some weed species with ability of compatible interaction of up to 8 weeks in the roots (Dubreuil, *et al*., 2011). The host range of root knot nematodes is so vast that it is difficult to find a common landscape and garden as non-hosts while it has been found that vegetables, bedding plants, shrubs as well as trees are all susceptible hosts (Olsen, 2000). *Meloidogyne javanica* and *Meloidogyne incognita* caused damage 9.17% and 18.7% (Costa *et al*,2001). Above ground symptoms of RKNs are similar to many other root diseases or environmental factors limiting water and nutrient uptake. These symptoms consist of wilting during periods of moisture stress, stunted plants, chlorotic or pale green leaves. Most characteristic symptoms; however, are those occurring on underground plant parts (McSorley and Frederick, 1994). Attacked roots swell at the point of infection and form knots or galls. Several infections may occur along the same area resulting in large fleshy galls. The appearance of galls will depend in part upon the host and the nematode species involved (Kiewnick, 2006). Generally, fast growing annuals will have a large flesh gall and woody perennials, small hard galls. Infected roots are retarded in growth and lack fine feeder roots. Rotting of roots may develop late in the season. When tubers, corms or other edible root portions are infected, small swellings or pimpling is evident on the surface (Kohl, 2011).

Although different species of root knot nematodes vary in their host-parasite relationships, all have basically the same life cycle. The infectious stage of *Meloidogyne* spp. is the second stage juvenile which occurs free in the soil. The second stage juvenile penetrates the plant at or near the root tip and become sedentary (Abad *et al*., 2003). An enzyme is released which causes the plant cells surrounding the head region to enlarge, forming giant cells which serve as a source of nourishment for the parasite. The female nematodes swell until they become pear-shaped or oval. During this time, the nematode undergoes two more molts. Females begin laying eggs around 20 days after penetration of the host. The average female will lay approximately 30 eggs a day for two weeks. The average life cycle is 25 days. Populations will build up rapidly when environmental conditions are favorable (Williams and Davis, 2001).

The management of root-knot nematode includes chemical, biological and cultural methods with different levels of successes. Although chemical nematicides are effective in nematode control, their high costs, non- availability at the time of need, the hazards they pose to human as well as on non-target organisms discourage users (Wachira *et al.,* 2009). Soil amendments with cruciferous residues and other organic manures are reported to be effective in reducing the population densities of many soil-borne plant pathogens (Hassan *et al*., 2010). The use of organic amendments in the form of manure, compost, stabilized biosolids and plant extracts is on the increase and has been attributed to their ability to decrease parasitic nematode populations and disease intensity on plants (Chen *et al.,* 2000). Application of soil organic amendments is not only beneficial to nematode management but also improves plant growth and productivity (Orisajo *et al.,* 2008). Effect of soil organic amendments on root-knot nematodes (*Meloidogyne* spp.) has not been adequately studied.

The lack of information regarding annual and perennial ornamental plants losses due to plant-parasitic nematodes is attributed to the demand for research to agronomic crops. Widespread dissemination of annual and perennial ornamentals presents an important avenue for distribution of *Meloidogyne* spp. and other plant-pathogenic organisms. Common zinnia is one of the ornamental plants used by ornamental plant production industries in Ethiopia. Root knot nematodes are among the major pathogens causing the stunted growth, loss of vigor, wilting, chlorosis and poor quality flower. However, no management system has been implemented as to control the pathogens. Chemical control using nematicides is slowly becoming extinct from the

market due to their toxicity and impacts on humans, livestock and environment, creating a need to invest in safer agricultural practices for management of root knot nematodes (Collange *et al*, 2011).

Integrated pest management option using naturally occurring bio-pesticides is eminent considering the effects of agrochemicals continue to inflict on the global environment and humanity (Oerke and Dehne, 2004). Cultural control is one of the broadest methods for management of RKNs and involves cropping systems, fallowing, solarization, and use of organic amendments, intercropping, age of transplantable nurseries, altering dates of planting, removal of infected plants and burning of crop residues (Khan *et al*., 2014). Among these alternative control strategies use of organic amendments in the control of root knot nematodes have been exploited and there is evidence of root knot nematode suppression through reduced multiplication and reduced diseases as evidenced by enhanced plant growth parameters (Parihar*et al*., 2012). Moreover, it can suppose the management of large amounts of wastes generated by urban settlements and agro-industrial processes, after their transformation by composting process, and in addition it can improve plant resistance and plant protection by stimulating root development by recycling plant nutritive elements (De Bertoldi, 2008).

Application of coffee waste in sandy soil increases the availability of phosphorus, retained soil water from 53 to 60%. It also promotes the retention of basic cations and immobilized manganese. Coffee waste also has the potential to be used as a liming material, NPK fertilizer and has the benefit of increasing water and nutrient retention (Kasongo *et al*., 2011). Biochar is a process where organic material is heated under low oxygen conditions it can add nutrients by itself or make them more available for plant uptake by enhance the decomposition of organic material and reduce decomposition rates of other organic material thereby increasing soil C concentration in the long run (Lehmann and Joseph, 2009). Whereas direct toxic effects of biochar exudates on nematode viability, infectivity or development(Huang *et al*., 2015).The use of such materials has merits over other methods due to their availability, low cost, pollution free and their capacity to improve soil fertility. Wondirad et al (2009) stated that most studies done in Ethiopia on root-knot nematode have yet focused mainly on generating baseline information rather than management. Therefore, this study was designed with the following objectives.

General objective

- To identify and evaluate the Pathogenecity of the root-knot nematode (*Meloidogyne spp*) on common zinnia (*Zinnia elegans*), and management of *Meloidogyne* spp using coffee husk and biochar amendments.

Specific objective

- To identify the *Meloidogyne spp* associated with common zinnia in JUCAVM
- To evaluate the *M. arenaria* associated with common zinnia
- To evaluate the impact of varying initial population densities on damage to common zinnia
- To evaluate the effectiveness of biochar and coffee husk amendments against root-knot nematodes

2. LITERATURE REVIEW

2.1. Origin and importance of common zinnia (*Zinnia elegans*)

Common zinnia *(Zinnia elegans* Jacq.), is an annual flowering plant and the best known of the genus Zinnia (Balick*et al*., 2000). It belongs to the family Compositae (Asteraceae). Zinnia has an erect stem with 10 to 100 cm in height and ovate leaves, flowers up to 10 cm in diameter across in solitary heads. The disk florets are yellow orange or purplish brown; the ray florets have every flower color, except blue and various shapes (Dar *et al*., 2002). Zinnia flowers exhibit uniform, bright colors, sturdy stems and have a long vase life with great commercial and aesthetic value (Dole, 1999).

Common zinnia is native to Mexico and Central America and now has world-wide importance as a garden plant and cut-flower (Kirkbride and Wiersma, 2007). Two species, *Z. elegans* and *Z. peru-viana* (L.) are known to occur in Ethiopia. *Z. elegans* is not known to be invasive, but has been recorded as an escape in the central parts of Ethiopia (Mesfin Tadesse, 2004). It is renowned for its extensive and striking color-range, long bloom-duration, lasting from late spring to early autumn, and tolerance of hot climates. Ideally, the full-range of bloom colors would be available for the cut-flower market without seasonal-dependency. Differences in plant stature are also important; with the shorter cultivars more appropriate for planting in annual beds, borders and containers (Ye *et al*., 2008).

Ornamental plants are grown for decorative purposes in gardens and landscape design projects, as house plants, for cut flowers and specimen display. Besides, ornamental plants play important role in human health and psychology. Human health depends on well-functioning ecosystems. We cannot live without the goods and services that nature provides to purify our air and water, maintain soil fertility, pollinate plants, break down waste, provide food and fuel and keep diseases in check. We live in a very artificial environment where we rely on mechanical systems. Alternatives to manufactured or engineered solutions improve occupant well-being, reduce operating costs and also improve environmental quality (Amingad and Lakshmipathy, 2014). Common zinnia grown because of its economic value, potential salt-tolerant cut flower crop to grow in greenhouse systems using recycled agricultural waste water and tolerance to temperature fluctuation (Carter *et al*., 2010). The Plants are known to have good medicinal properties and

used for various purposes. Roots of and whole plants of *Z.elegans* phytochemicals, antifungal and inhibited the growth of *Fusarium moniliforme* (Hafiza et al., 2002).

Figure 1. Common zinnia (*Z. elegans*): Photo taken from JUCAVM campus

2.2. Constraints of common zinnia production

The production of common zinnia is reduced due to fungal, bacterial, viral and nematodes are the prominent factors that affect its productivity (Gombert*et al*., 2001). Plant parasitic nematodes are destructive soil pests that attack a wide range of plants that root-knot (*M. incognita, M.arenaria. hapla*), lesion (*Pratylenchus vulnus*), ring (*Criconemoides* spp.), and stunt (*Tylenchorhynchus* spp.) nematodes cause considerable injury to annuals, perennials, herbs, and woody ornamentals including Zinnia elegans (Austin, 2005). Two species of nematodes that infest *Z. Elegans are: Meloidogyne incognita*, which causes root knot and *Aphelenchoides ritzemabosi*, which causes angular spots on the leaves (McSorley and Frederick, 1994).

2.3. Root-knot nematodes (*Meloidogyne* species)

Plant parasitic nematodes are microscopic organisms that cause significant damage to most plant species and are widely spread (AGRIOS, 2005). Plant parasitic nematodes are small in size (300

to 1000 micrometers) and are composed of several genera. Many genera described, ten have been found to be economic importance in Agriculture with root-knot nematodes (*Meloidogyne* spp.) on top of the list (Jones *et al*., 2013). Root-knot nematodes are microscopic worms that live in soil and feed on the roots of many common garden crops .The nematode gets its name because its feeding causes galls (swellings or "knots") to form on the roots of infected plants. Root-knot nematodes are scientifically classified in the genus *Meloidogyne* (Fox, 2001).

There are over 90 characterized species of economic importance in the *Meloidogyne* sp group (Lamovšek *et al.*, 2013). And these are obligate endoparasites that are capable of infecting nearly every species of higher plants in a wide range of geographical distribution (Adegbite 2011; Favery *et al*., 2016). The estimated global yield losses for arable crops associated with RKNs are estimated between 5-43% in the tropical and subtropical areas (Surendra *et al*., 2014). There are four major *Meloidogyne* spp that are common in the tropics *M. incognita, M. javanica, M. arenaria*, and *M. hapla* (occurring in the temperate regions) of which *M.incognita* is the most destructive among the species causing yield losses of up to 16% (Perry *et al*., 2009).

Root knot nematodes attack a wide range of plants, including many common vegetables, fruit trees, and ornamentals. They are extremely difficult to control and can be spread easily from site to site in soil and plant material. Infestations of these nematodes are fairly easy to recognize by digging up a few plants with symptoms, washing or gently tapping the soil from the roots, and examining the roots for galls (Jeff, 2015).

2.4. Distribution of Root-knot Nematode (*Meloidogyne* species)

Root knot nematodes are most abundant in the upper foot of soils until a few feet deep (Olsen, 2000). In cool climates where the temperature is around 0°C to 15°C or above, the most common *Meloidogyne* species is *M. hapla*. These species can be found in northern United States, northern Europe, northern Asia, Southern Canada in North America, and as well as in South America which is specific at latitude 40°S. While in Africa, it may be found at altitudes above 1500 meters. It is common in Victoria, Australia the southernmost state. For the tropic zone, the most common *Meloidogyne* species are *M. incognita* and *M. javanica* (Olsen, 2011). According to Truggill and Blok (2001) *M. incognita* is easily found in every temperate and tropical country, and it is possibly the single most damaging crop pathogen in the world. *M. javanica* is probably

the most common *Meloidogyne* species in many parts of tropical Africa, Australia and southern Asia. While *M. incognita* and *M. arenaria* are common and widespread in the same regions. Thus, between 35°S and 35°N latitudes of the world three main species of *Meloidogyne* were believed to continuous existence in warm countries, which are *M. javanica*, *M. incognita* and *M. arenaria* (Ralmi, 2016).

2.5. Damage Symptoms of Root-knot Nematode (*Meloidogyne* species)

Root-knot nematodes are usually first detected in localized areas within a field, greenhouse, high-tunnel, nursery, or home garden. Gradually, the area of infected plants expands in size and the entire planting can eventually be affected. Aboveground symptoms usually involve stunting, chlorosis and yellowing of leaves (nitrogen deficiency symptom and yield reductions that often worsen over time (Karssen, 2002).

Root-knot nematode symptoms induced expansion of root cells, swellings, or galls develop on the roots of infected plants. The galls vary in size from slight thickenings to lumps 5 to 10 cm across. Stems or leaves may be galled but this is rarely seen in plants growing outdoors. Galls caused by *Meloidogyne* hapla are much smaller than those caused by other species. All root knot galls damage the vascular tissues of roots and thus interfere with the normal movement of water and nutrients through the plant. They also increase the susceptibility of the root system to be invaded by disease-causing fungi and bacteria (Rahman, 2003). The degree of root galling generally depends on three factors: nematode population density, *Meloidogyne* species and "race," and host plant species and even cultivar. As the density of nematodes increases in a particular field, the number of galls per plant also will increase. Large numbers of nematodes penetrating roots in close proximity also will result in larger galls (Jepson, 1987). *Meloidogyne hapla* (the northern root-knot nematode) produces galls less than half the size of those produced by *M. incognita* (the southern root-knot nematode) on the same plant hosts. Finally, each crop responds differently to root-knot nematode infection (Perry and Starr, 2009).

Figure 2. Symptoms of *M. arenaria* on common zinnia. **(A)** Above ground symptoms and **(B)** root symptoms.

2.6. Life cycle of Root-knot nematodes (*Meloidogyne* species)

Root knot nematodes are obligate endoparasites that complete their life cycle inside the host plant feeding for survival and reproduction (Caillaud *et al*., 2008). The life cycle consists of egg, four juvenile stages and adult stage (Wesemael *et al*., 2014). The development of the first stage juvenile happens within the egg where first molting takes place and this is followed by a second juvenile stage (J2) that infect plant roots after seeking the host within the soil surrounding the root(Noling, 2009). The ability of *Meloidogyne* spp. to survive is enhanced by several physiological and biochemical adaptations, which includes delayed embryogenesis, quiescence and diapauses, and lipid reserves that prolong viability until the Juveniles is attained and invades a host (Perry *et al*., 2009).

The short lived males are vermiform and motile in nature and they move in the soil to copulate with females for reproduction. Males are not important as female root knot nematodes are capable of reproducing asexually. Adult female nematodes deposit eggs in a gelatinous protective matrix, closer to the outside of root surface. A single female nematode has capacity to lay over 500 to 1500 eggs during its life cycle which lasts for 3 months (Tiwari *et al*., 2009). Optimal conditions must prevail for egg laying and includes adequate moisture and warm temperatures. The duration of life cycle is from 17 to 57 days depending on the prevailing conditions of the host plant they infest and the environment. After the completion of

embryogenesis, the first juvenile stage remains inside the egg up to the time it molts into the second juvenile stage. The second stage of juveniles hatches freely in the soil in search of a suitable host plant where it will feed for survival. The second Juveniles are well equipped for parasitism of their host and will move to feed by means of a stylet, a retractable mouth part adapted for piercing and feeding (Tiwari *et al*., 2009). The second juvenile enters the root becomes sedentary and grows thick, feeds on the surrounding cells by inserting its stylet and saliva secretion. With this they are capable of manipulating key aspects of plant biology and are able to hijack host-cellular development to establish a feeding site (Curtis, 2007).

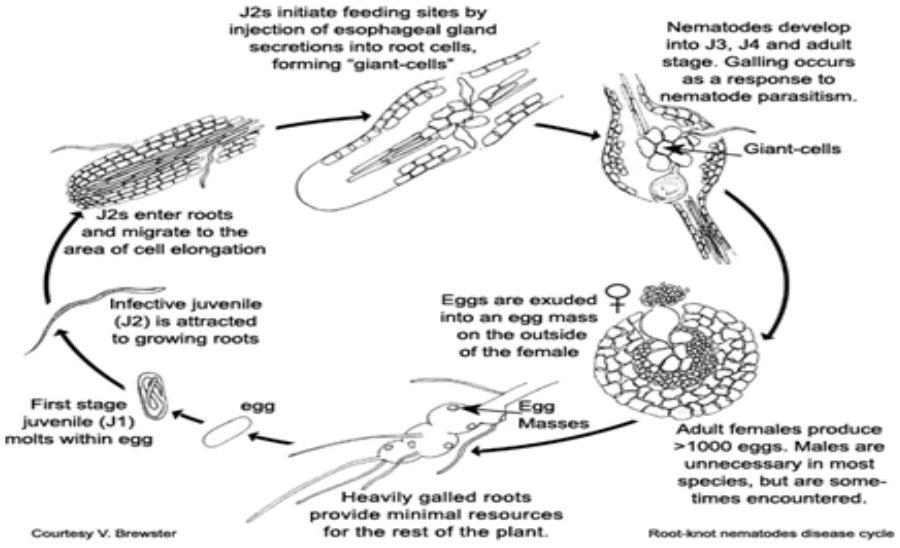


Figure 3. Complete life cycle of root knot nematode (Mitkowski and Abawi, 2003)

2.7. Identification of Root-knot nematodes (*Meloidogyne* species)

Accurate identification of root-knot nematodes is crucial for effective disease control and depends on rapid and accurate classification of the pathogens involved so that appropriate control measures may be taken. Morphological (perineal pattern of adult females; male, female and J2 labial region shape, and stylet morphology; length and shape of J2 tail and, in some cases, biochemical or molecular methodologies (Handoo and Subbotin, 2018). Detailed diagnostic characters differentiating *Meloidogyne* species was outlined by Eisenback (1985). For many years the form of the perineal pattern of the mature female and various morphometric and morphological features of the J2 were relied upon in species determination. To these were added features of the male, such as the form of the labial region, including the annulations, and form of

the stylet and basal knobs. Isozyme electrophoresis has discriminated a number of these otherwise cryptic species but it is PCR-based molecular methodologies that currently carry (Carneiro *et al*., 2004).

Isozyme and the genome of root-knot nematodes, mostly since the1970s, provided further evidence for the large diversity of species within the genus. Importantly, several new technologies have provided tools to assist in species identification. One of the most important has been the use of Isozyme phenotypes, using polyacrylamide gel electrophoresis (PAGE) of crude protein extracts and histochemicalstains for non-specific esterase, superoxidedismutase, malate dehydrogenase, and glutamateoxaloacetate transaminase (Esbenshade and Triantaphyllou, 1985).

2.8. Management of Root-knot nematode (*Meloidogyne* species)

2.8.1. Cultural control methods

Cultural control is one of the broadest methods for management of RKNs and involves cropping systems, fallowing, solarization, and use of organic amendments, intercropping, age of transplantable nurseries, altering dates of planting, removal of infected plants and burning of crop residues (Khan *et al*., 2014). Because nematicides are slowly being phased, alternative agronomic practices required to solve the nematode problems are encouraged as options for nematode control (Collange *et al*., 2011). Other cultural practices in the control of root knot nematodes include an incorporation of cover crops as fallows in rotations although their effects on nematodes is decimal and requires prolonged application (Baginsky *et al*., 2013).

2.8.1.1. Organic soil amendment

Organic amendments in the form of manure, compost, stabilized biosolids and plant extracts is on the increase and has been attributed to their ability to decrease parasitic nematode populations and disease intensity on plants (Ozores-Hampton *et al.*, 2012). It works by increasing the level of nutrient supply and improves the soil structure thereby increasing nitrogen availability and consequently improving plant health (Tabarant *et al*., 2011). Decomposing plant residues and other organic amendments release compounds or by products such as nitrogen and organic acids that may have nematicidal effects (Oka 2010; Thoden *et al.*, 2011).

Coffee husk is rich in organic matter (cellulose, hemicelluloses, pectin and lignin), and chemical nutrients such as nitrogen (N) and potassium (K) (Pandey *et al.,* 2000). Composting of coffee husk and other agricultural wastes reduce the consumption of natural resources and recycle nutrients, increase in soil organic matter and improve the physical, chemical and biological characteristics of soil (Westerman *et al*.,2005). Coffee husk contains some amount of caffeine and tannins, which can make it toxic and slow degradation in nature, resulting the disposal problem. However, coffee husk is rich in lignocelluloses materials, which makes it an ideal substrate for microbial processes and release toxic to plant parasitic nematode on survival, reproduction and direct paralyze by interring their body's (Neves *et al*., 2006).

Application of biochar to soil increases in cation exchange capacity and pH, improves the overall sorption capacity of soils and influence the toxicity, transport and fate of different heavy metals in the soil (Verheijen *et al*., 2009). Biochar increase the availability of major plant nutrients (Glaser *et al*., 2002). It is enhanced state of resistance and effective against a broad range of pathogens and parasites, including fungi, bacteria, viruses, and nematodes (Vallad *et al*., 2004).

Coffee husk and biochar have ability to reduce plant parasitic nematode due to the presence of tannins chemical compounds which release toxic to plant parasitic nematode on survival, reproduction and direct paralyze by interring their body's. Tannins have antihelmintic properties anti parasitic activity to root knot nematode (Hoste *et al*., 2006). Tannin greatly reduced nematode egg hatch percentage when increased concentrations. It is an attractant for the root-knot nematodes *M.arenaria* may serve to disorientate phytoparasitic nematodes causing them difficulties in locating the root systems and potentially reducing plant damage. Those import ants are in addition to improving the soil structure, and increasing the soil temperature (Maistrello *et al*., 2010).

2.8.2. Biological control methods

Biological control is application of microorganisms antagonistic to root-knot nematodes or compounds produced by these microbes could providean additional option for managing the damage caused by rootknotnematodes. Bioproducts contain a microorganism (bacterium,fungus, virus, protozoan or alga) as an active ingredient oftenreferred to microbial pesticides, they are host specific and theyare potential candidates with regard to integrated pest management(Arora

et al., 2000). Fungi and bacteria are among the mostdominant soil-borne groups in natural soil ecosystem and someof them have shown great potential as biological control agentsfor root-knot nematodes (Kerry, 2000). The free-living soil fungi Trichoderma spp. are potential nematode bio-control agents onmany food, vegetable and cash crops (Affokpon et al., 2011). Besides Trichoderma spp., Bacillus megateriumis common soil beneficial bio-fertilizer belonging to plant growthpromoting rhizobacteria have also been used for controlling root knot nematodes (Oliveira *et al*., 2009). The biological effects of the bio-active product of marine brownalgae of Ascophyllum nodosum have been proved economic andeco-friendly approach in reducing root-knot nematode infestation(Radwan, 2012).

2.8.3. Resistance breeding

Breeding for nematode resistance involves the same basic principles implored when breeding for resistances against plant pathogens of pyramiding resistance genes of interest so as to create new cultivar with wide and strong resistance to *Meloidogyne* species (Dong *et al*., 2008). Resistance breeding must be combined with desirable agronomic and horticultural traits of recommended cultivars. It is believed that two homozygous genes have been linked to condition nematode resistance in carrot and it poised that using this model of duplicate recessive epistasis may explain the reaction of resistant varieties and nematode in the derived crosses (Wang and Goldman, 1985). Breeding advancement has also revealed that several root knot nematode resistant genes have been discovered in different plants.

The candidate nematode resistant gene called Tfg-Mi was discovered and isolated from a resistant fenugreek line Giza 3 a root knot nematode gene from leguminosae by degenerate PCR amplification method and in combination with Race Amplification of cDNA ends (RACE) technique (Abbas *et al*., 2008). The developed lines by this technique have shown resistance to root knot nematodes *M. javanica* and *M. incognita*. Resistance (R) genes are assumed to detect Avr-Avirulent gene specific molecules for activation of defense against root knot nematodes in resistant plants (Hammond-Kosack and Jones, 1997). The recognition of nematodes, mediated directly or indirectly by plant R proteins through nematode secreted effectors evokes a resistance response, which is referred to as effector-triggered immunity (Hogenhout and Bos, 2011).

2.8.4. Chemical control methods

Chemical control is one of the oldest methods of controlling nematodes. Nematicides are chemicals used in the field of agriculture to mitigate the negative effects of plant parasitic nematodes on plant health and subsequently on crop productivity and/or quality (Becker, 2014). These chemicals are applied to the soil as fumigants when wet and is ploughed thoroughly very well for better incorporation (Araya and Mario, 2004). These chemicals have a correlated effect on soil type with regards to control as they are known to respond differently to soil type. These fumigants, besides controlling nematode, also help in controlling other soil-borne pathogens and weeds as they are most non-selective. Chemicals such as carbofuran, carbosulfan, and fenamiphos have been tried in controlling *M. incognita* and have shown good results in the treatment of seeds when dressed and soil fumigation (Khan, 2004).

In addition to broad-spectrum fumigants, nervous system toxins (including ox amyl and fenamiphos) have been shown to be extremely effective for controlling root-knot nematodes. Because both nematodes and humans have nervous systems, chemical treatments that target nematode nerves also are a potential danger to human nerves. These chemicals (carbamates and organophosphates) are extremely toxic to humans and other non-target organisms. Currently, these chemicals are the most economically feasible control method for root-knot nematodes. Because they are not toxic to plants, they are the only chemical options for established plants (Karssen, 2002).

2.8.5. Integrated Nematode Management

Root knot nematode is an aggressive and resilient pathogen so implementation of integrated management strategies is difficult. Nevertheless, a combination of management tactics, such as resistant cultivars, use of cultural practices and chemical soil treatments generally provide acceptable control of root knot nematodes. Root knot nematodes have been reported to decrease by soil organic amendments with oil cakes or by integrating oil cakes with other management techniques (Goswami et al 2006). An increase in nematicidal efficacy of microorganisms appears possible when such biocontrol agents integrate with either organic amendments or nematicides into an integrated control (Khan, 2010)

3. MATERIALS AND METHODS

3.1. Description of study site

This study was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) both under laboratory and greenhouse in 2018/19. Jimma is located in south western parts of Ethiopia which located at $7^0$33' latitude and 36^0 57' longitudes and at the altitude of 1710 m.a.s.l. The area receives an annual rain fall of 1500 mm.The maximum and minimum temperatures of the area are 26.89 c and 11.40 c respectively (FAO, 2018). Host status, Pathogenecity and management evaluation were conducted under greenhouse condition. Other activities including extraction, counting, processing to permanent mount and species identification was performed in Plant Disease Diagnosis Laboratory of JUCAVM.

3.2. *Meloidogyne* spp Isolation and purification

Root samples from infected common zinnia plants with root-knot nematode were collected from the JUCAVM. Ten single eggmass from the infected roots was picked up using forceps and needle and placed in to the Eppendorf tube having distilled water and kept on the laboratory benches at room temperature (25 ± 2 °C) and allowed to hatch for 3-6 days in 2018. Freshly hatched juveniles was prepared in suspension form and inoculated to tomato (variety Marglobe) seedling planted in 1000ml plastic pots filled with sterilized sandy and sub soil in the greenhouse of JUCAVM.

Two months after inoculation, when egg masses are formed in the tomato roots, the inoculated plants were uprooted and their roots gently washed free of soil. Again the eggmass from single plant was collected from pure culture of single eggmass established under greenhouse and placed to the Eppendorf tube having distilled water and kept on the laboratory benches at room temperature and allowed to hatch for 3-6 days. The pure culture was used for morphological characterization *Meloidogyne* species.

3.3. Morphological identification of *Meloidogyne* spp

The species of root-knot nematode was identified on the basis of perineal pattern of adult females (Eisenback, 1985). Perineal patterns of female *Meloidogyne* spp was prepared from fresh root materials that was derived from single eggmass culture following the procedure adopted

from Taylor and Netscher (1974). Root galls with mature females were selected and root tissue was teased apart with forceps to separate out the adult females. The adult females were collected in lactic acid in a Petri dish with needle. The body wall of female near the neck was ruptured and the body tissue was gently pushed out. The female was placed in a drop of lactic acid in Petri dish. The lactic acid facilitates the removal of body tissues that adhere to the body wall. The body was cut in half (equatorially) and the part with perineal pattern was removed from the drop of lactic acid.

The perineal pattern was trimmed to square and placed back to a drop of lactic acid. The perineal patterns was transferred to a fresh drop of glycerin on a clean glass microscopic slide with the interior surface of the cuticle was placed against the glass. The cover slip was gently placed on the glycerin drop and sealed with nail polish and the slide was labeled and ready for microscopic examination. Examination of perineal patterns for species identification was performed with compound microscope by using identification keys available in laboratory.

4. 2. *Meloidogyne arenaria* associated with common zinnia (*Z.elegans)*

Seeds were collected from common zinnia in JUCAVM campus. The seeds were planted in 3cm x 5-cm plastic trays in a mix of sterilized sand and field soil. Soil sample was collected from infected soil from the Rhizosphere where Common zinnia has showed damage symptom at a depth of 15–20 cm in Jucavm. The collected soil was cheeked for the nematode density before filling the pots. There were 43 J2/g of soil. 36 pots were filled with the soil in to which two weeks old common zinnia seedling was transplanted. The host-status of root knot nematode on common zinnia was monitored every week for 1-18 weeks.

3.4.1. Staining of infected root tissue

The every week infected root was carefully washed with water and cut in small then socked in bleach (5 % NaOCl) for 4 min to soften the tissue then rinsed with tap water. The root was put in a beaker filled with acid fuchsin solution (875 ml of lactic acid, 63 ml of glycerol, 62 ml of water, 0.1g of acid fuchsin) and boiled for about 30s then cooled. Stain tissue was then washed with tap water.

3.4.2. Data Collection

Data was collected every week from two plants of common zinnia (*Zinnia elegans*). Plant height (the plant height was measured by using ruler from above the ground part of the root till to tip of shoot), root length (the root length was measured by using ruler below the ground parts of the main root), leaf number (by counted all the leaf found on the shoot part), fresh weight of shoots (shoot weighted above ground part of the plant by using synthetic balance) and fresh weight roots (root weighted by below ground part of the plant using synthetic balance)were recorded before staining. Number of root gall, eggmass per root system, egg per eggmass were counted and final nematode population (Pf = total number of root knot nematode extracted from the soil and root) also recorded after staining. The growth rate calculated W_n-W_{n-1}/time were w = week.

3.5. Damage Potential of *Meloidogyne arenaria* to common zinnia

3.5.1. Preparation of *Meloidogyne arenaria* for inoculum

For inoculation, pure culture of eggs and second stage juvenile from heavily infected roots of tomato were extracted. The roots were rinsed gently with tap water to remove adhering soil material and chopped to small pieces placed on plastic tube. 5% sodium hypochlorite (NaOCl) solution was added to the bottle up to covering of the root tissue and constitute shacked gently for 4min (Hussey and Barker, 1973) to dislodge eggs from egg mass. Eggs were collected on 250μm sieves over 38μm sieve and rinsed with tap water. Every 24hrs, added fresh water on hatched out juveniles 3-6 days at room temperature and stored in refrigerator at 5°C until used for inoculation. For enumeration, the root knot nematode suspension was thoroughly air bellowed to make homogenous distribution of nematode before taking 1ml of suspension immediately in to counting dish. An average of three counts was taken to determine the density of nematodes in the suspension under compound microscope at 40X. The suspension was concentrated to the required number nematode inoculation.

3.5.2. Planting material and growing condition

Seeds of common zinnia were collected from JUCAVM campus. The seeds were planted in 3cm x 5-cm plastic trays in a mix of sterilized sand and soil. The growth media was (sand and field soil) was dry sterilized in oven at 111℃ for 30 minutes. Sterilized soil mix was used to fill 18 pots. The seedling were allowed to grown until the development of three to four leaves and then

one seedling per pot was transplanted and regularly watered following the demand up to inoculation.

3.5.3 Greenhouse experiment and experimental design

The Pathogenecity *M.arenaria* was carried out under greenhouse condition on CRD design with three replication different density (0, 1, 2, 4, 8 and 16 J2 per gram of soil) of *M.arenaria* suspension react on common zinnia elegans.

3.5.4 *Meloidogyne arenaria* inoculation

Common zinnia seedlings with four true leaves were inoculated with infective second stage juvenile of *M. arenaria* a week after transplanting. Four holes around the stem of the plant were made into six densities (0, 1, 2, 4, 8 and 16 J2 per gram of soil) were inoculated by using pipette then the holes were covered with soil properly by hand.

3.6. Management of *Meloidogyne arenaria* on common zinnia

3.6.1. Preparation of *Meloidogyne arenaria* for inoculum

For inoculation, pure culture of eggs and second stage juvenile from heavily infected roots of tomato were extracted based on procedure mentioned in (3.5.1).

3.6.2. Organic matter preparation

Two organic amendments were prepared for management of root knot nematode; biochar and coffee husk. Coffee husk was obtained from the coffee processing station near to JUCAVM campus.

Banana leaves for biochar production was collected from Jimma University College of Agriculture and Veterinary Medicine then drayed. The biochar was produced at 500°C pyrolysis temperature. After the pyrolysis process, the biochar was grounded to small granules and pass through 2mm sieve in order to have the same particle size as that of the soil.

Each coffee husk and biochar amendments were mixed with sterilized field soil with the following ratio (0:1, 1:1, 2:1, 3:1and 4:1).

3.6. 3.Planting material and growing condition

Seeds of common zinnia were collected from JUCAVM campus. The seeds were planted in 3cm x 5-cm plastic trays in a mix of sterilized sand and soil. The growth media was (sand and field soil) was dry sterilized in oven at 111℃ for 30 minutes. Sterilized soil mix with each coffee husk and biochar amendments with the following ratio (0:1, 1:1, 2:1, 3:1and 4:1) was used to fill each pot. The seedling were allowed to grown until the development of three to four leaves and then one seedling per pot was transplanted and regularly watered following the demand up to inoculation of nematodes.

3.6.4. Greenhouse experiments and experimental design

The management of *M.arenaria* using different ratio of (0:1, 1:1, 2:1, 3:1and 4:1) for each coffee husk and biochar amendment experiment was carried out under greenhouse condition on CRD design with four replications.

3.6.5. Inoculation of *Meloidogyne arenaria*

Management of *M. arenaria* by using for each coffee husk and biochar amendment except control (none amended) treatments of common zinnia seedlings with four true leaves were inoculated J2 week after transplanting. Four holes around the stem of the plant were made then inoculated in 1lit pot with 2 J2 per gram of soil by using pipette. Finally the holes were covered with soil properly by hand.

3.7. Nematode extraction from potting soil

Eight weeks after inoculation, the greenhouse experiments were terminated and nematodes were extracted from 100ml of soil per pot for all experiments. Soil was placed over single layer of tissue paper on plastic sieve of 250μm on Oostenbrink dish. The setup was kept for 48h at room temperature without disturbance in modified Baermann tray method (Hooper, 1990). Nematodes were collected from each dish using 38μm aperture stainless steel sieve in to beaker. Suspension from each dishes were collected and allowed to settle in a beaker at room temperature. The volume of each suspension was standardized to 10ml. Each suspension was homogenized by blowing air with a pipette after which1ml of suspension was taken in to a counting slide and

counting was done under 40X magnification compound microscope. The density of nematode was expressed as the number of nematode per volume of soil per pot.

3.8. Nematode extraction from root

Nematodes were extracted from common zinnia root as follows. Roots were washed gently free of adhering soil and chopped in to small pieces and placed on plastic bottle and 5% sodium hypochlorite(NaOCl) solution was added to the bottle up to covering of root tissue and the constitute were shacked gently for 4 min (Hussey and Barker, 1973) to dislodge egg from eggmass. Eggs were collected on 38μmsieve over 250μm–pore sieves and rinsed with tap water. Suspension was allowed to settle and stored at room temperature for three days for hatching. Suspension containing both eggs and J2 were counted using counting slide with the aid of compound microscope.

3.9. Data Collected

Data was collected for Pathogenecity and management of *M. arenaria* experiments two months after seedling inoculation of common zinnia. Plant height (the plant height was measured by using ruler from above the ground part of the root till to tip of shoot), root length (the root length was measured by using ruler below the ground parts of the main root), leaf number (by counted all the leaf found on the shoot part), fresh weight of shoots (shoot weighted above ground part of the plant by using synthetic balance) and fresh weight roots (root weighted by below ground part of the plant using synthetic balance). Final nematode population, number of root gall, number eggmass per root system, number of egg per eggmass and reproduction factor from each treatment observations in experiment. Root gall index will be rated on scale of 0 to 5, are 0 = no galls, 1 = l to 2, 2 = 3 to10, 3 = 11 to 30, 4 = 31 to 100, and 5 = >100 galls per root system (Taylor and Sasser, 1978). The control plants was generally not galled and was used to compare root morphology with inoculated plants.

3. 9. Data analysis

Common zinnia growth parameter and nematode reproduction parameter data were subjected to ANOVA and means were separated using least significance difference tests at the $P < 0.05$ level to test significance level. All statistical analyses were performed using SAS 9.3 version (SAS, 2013). For each treatment, the reproduction factor was calculated with the following formula.

RF=$\frac{Pf}{Pi}$, where Pf = total number of root knot nematode extracted from the soil and root and Pi =initial population of root knot nematodes inoculated per pot were calculated (Ferris and Noling, 1987). The number of nematode per pot and per root system and RF values was used for statistical analysis. Before statistical analysis, data was checked for homogeneity of variance and the data were normal distributed.

4. RESULTS AND DISCUSSION

4.1. Identification *Meloidogyne* spp

From perineal pattern morphology confirmed that the species identified was *Meloidogyne arenaria* (Fig 4). The perineal pattern of this spp had low dorsal arch slightly intended near lateral fields to form rounded shoulder. Distinct later slightly wavy and bend towards the vulva. The perineal pattern of the *M.arenaria* found in this study completely similar to the pervious result obtained by (Eisenback 1985 and Garcia *et al*., 2012).

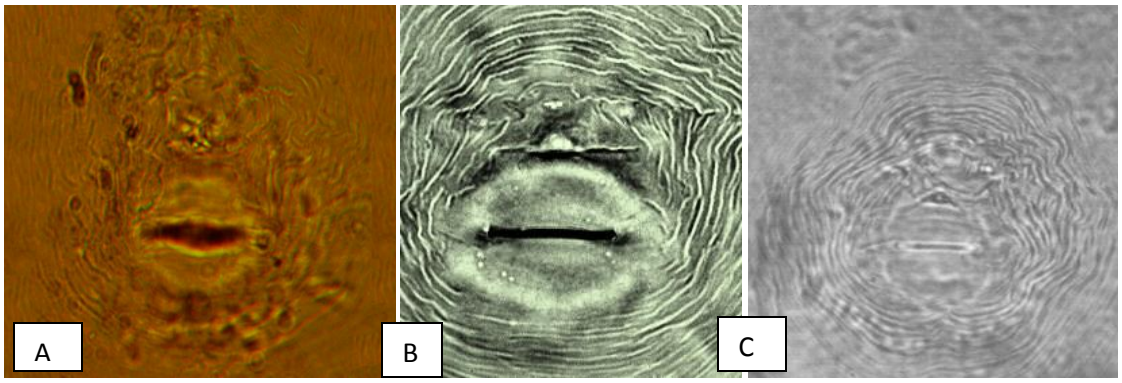


Figure 4. Perineal pattern of female *Meloidogyne arenaria*. (A) Perineal pattern under this study (B) Eisenback 1985) and (C) (Garcia *et al*., 2012.

4. 2. *Meloidogyne arenaria* associated with common zinnia

The result showed that growth parameter including plant height, leaf number, shoot fresh weight and root fresh weight from first to last week increase. The growth rate between each week also showed zigzag line but the values vary (Fig. 5,6 and7). Aboveground symptoms developed slowly over time. Symptoms consist of stunting, leaf yellowing and wilting (appendix 8). Rahman (2003) described that root knot nematode infected plants show symptom of nutrient deficiency such as slow or stunted growth, yellowing of the leaves, wilting of the plant and the worse situation the plant dies. These galls interfere with the roots ability to absorb water and nutrients, and provide locations for other disease-producing organisms, such as fungi or bacteria, to enter the plant. Results from changes in the root tissue as root-knot nematodes feed. Giant cells formed during the feeding process disrupt the development of the root's vascular system and impair the root's ability to remove water and nutrients from the soil efficiently; sugars and

carbohydrates produced in the leaves are diverted to the nematodes' feeding sites rather than to the developing (Anwar *et al*., 2007). Symptoms of damage induced by root-knot nematode (*Meloidogyne* spp.) include development of chlorosis on leaves, stunted growth, and root galling (Adegbite, 2011).

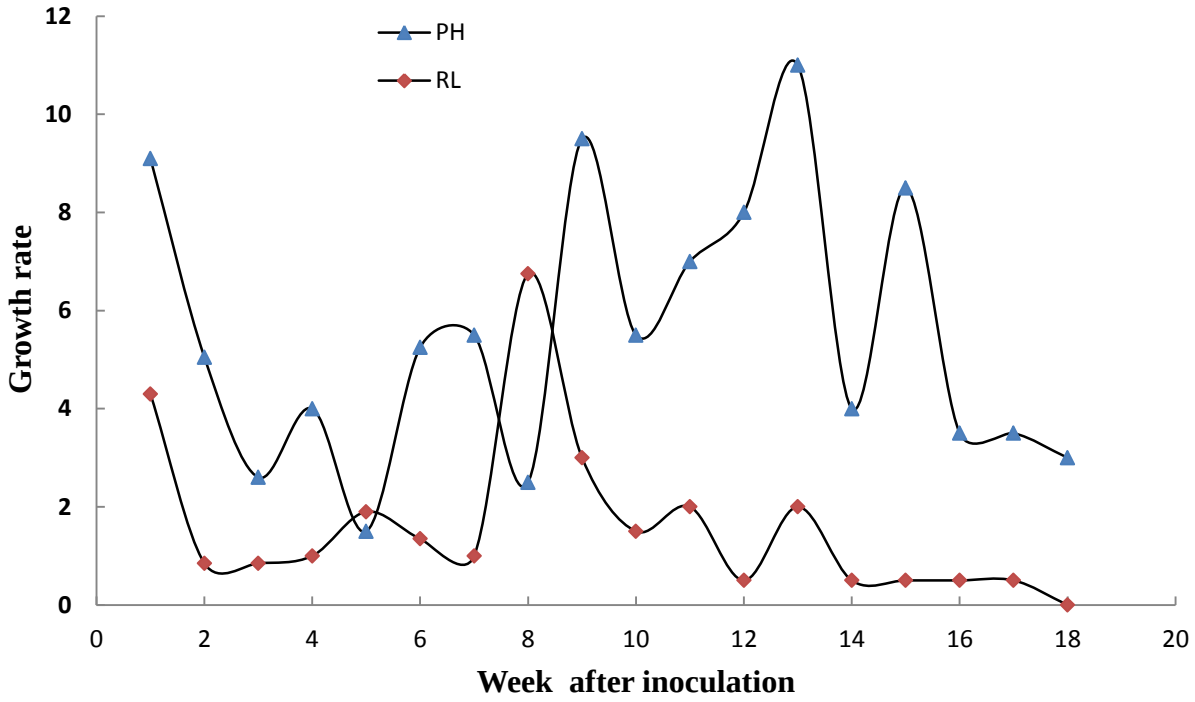


Figure 5.The effect of *M. arenaria* on growth rate of common zinnia week after inoculation on **PH**= plant height and RL= root length

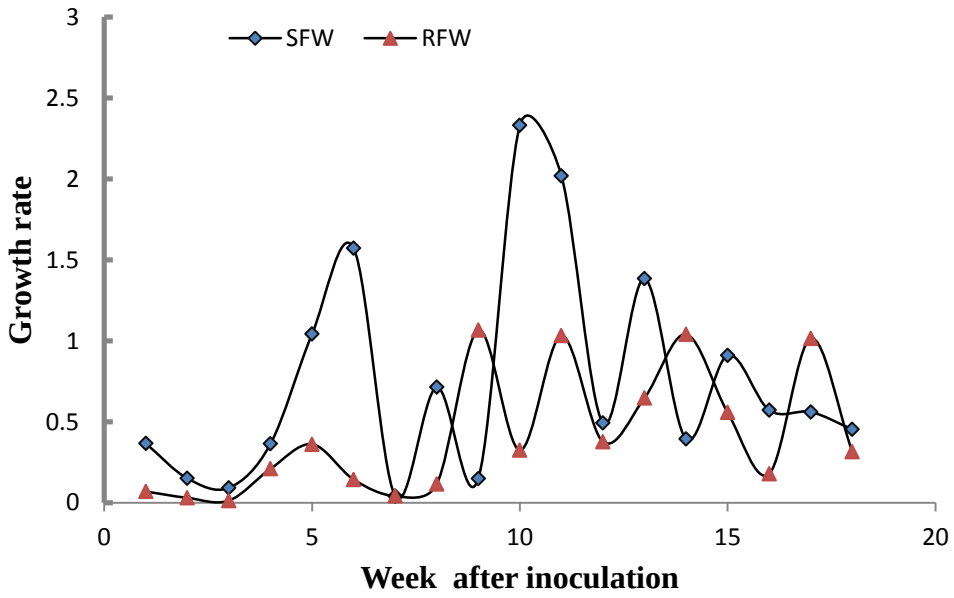


Figure 6. The effect of *M. arenaria* on growth rate of common zinnia week after inoculation on plant. SFW = shoot fresh weight and RFW = root fresh weight

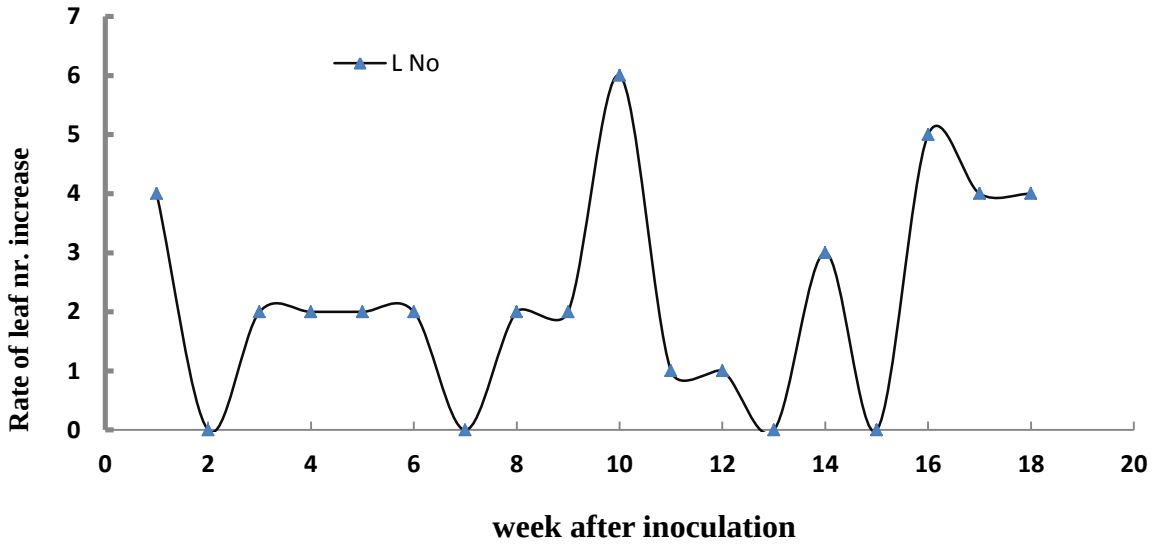


Figure 7. The effect of *M. arenaria* on growth rate of leaf number on common zinnia week after inoculation on. **LN** = leaf number

4.2.2. Reproductive potential of *Meloidogyne arenaria* on common zinnia

The result indicated that the nematode populations were increase when the week increases. The maximum nematode populations, numbers of root gall and eggmass formation (26715, 506 and 483) respectively were noted in16th week after transplanted. The gall formation was started from the second week (18), this might be nematodes induced expansion of root cells, swellings, or galls developed on the roots of infected plants. The galls were varying in size from slight thickenings to lumps when the number of week increases. The galls vary in size from pin-head to large size due to different levels root knot infestations on host plant and at times they may coalesce to form large secondary galls (Mohiddin & Khan, 2014). Number of root gall, egg mass and final population highly increase when the week increase but after 17 weeks (418, 375 and18231) the nematode reproductive parameters were declined to some extent (Fig.9 and 10). On the other hand, a reduction in reproductive value at the highest initial population could be linked to the destruction of root system by the parasitism of root knot nematode which led to competition for food and nutrition among the developing nematodes within the root Rhizosphere (Kumar and Haseeb, 2006) and also due to in ability of juveniles to find out new infection sites for subsequent generations (Hussain *et al*., 2011).

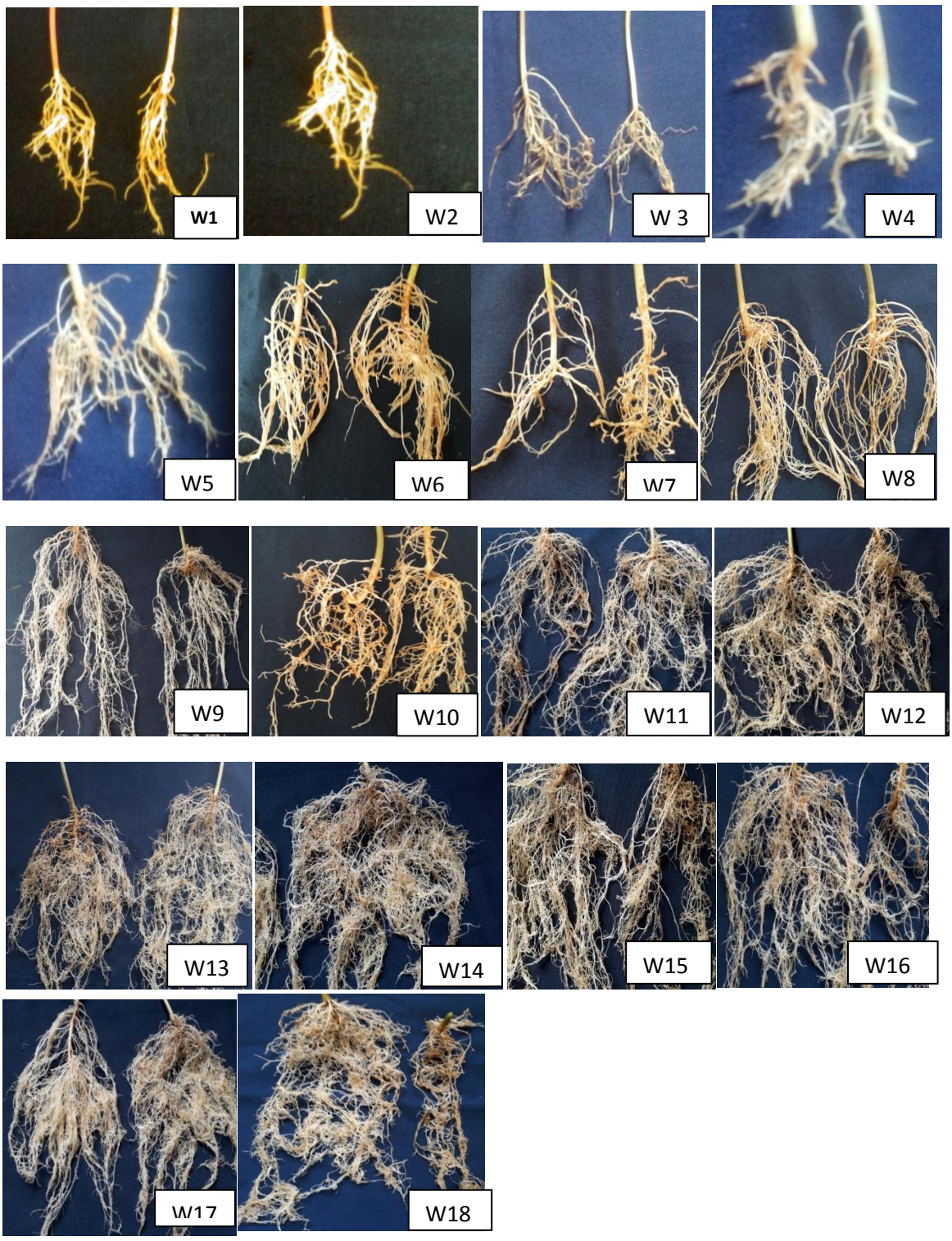


Figure 8. Root galls varying in number and size from slight thickenings to lumps when the number of week increases. **W** = week

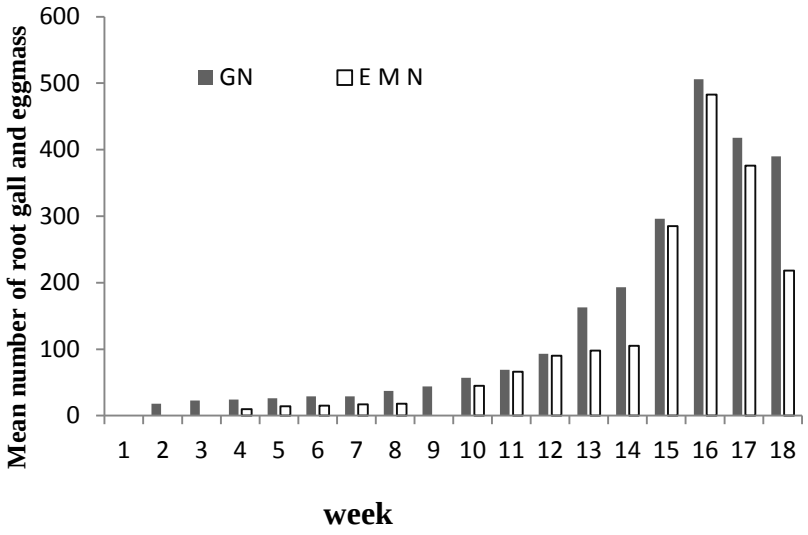


Figure 9.Effect of *Meloidogyne arenaria* on root gall and eggmass number on common zinnia per week.**GN**= gall number and **EMN** = egg mass number

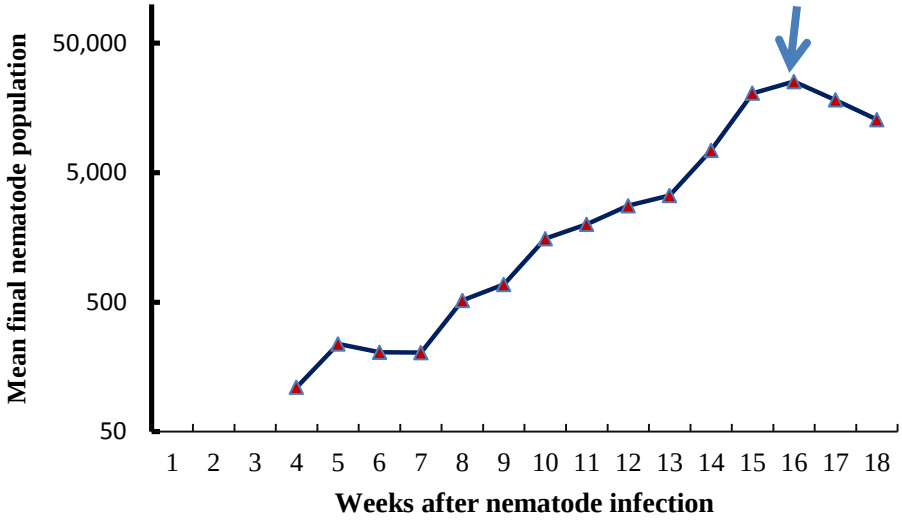


Figure 10. Mean number of final population density of *Meloidogyne arenaria* /pot on the Common Zinnia per week

4.3. Pathogenecity potential of *Meloidogyne arenaria* on common zinnia

The effect of a varying nematode population was apparently shown on all plant growth parameters of the test plant (Table 1).

There was significant variation (p= 0.0001) in mean plant height among treatments on common zinnia. The maximum plant height was recorded from un-inoculated control (0 J2/g soil) 73.33cm followed 1 J2/g soil. The minimum plant height was recorded from 16 J2/g soil (36.60cm) which was no significantly different from 8 J2/g soil inoculated treatment.

Leaf number also resulted in a significantly different (p=0.0001) among treatment. The maximum mean leaf number was from the un-inoculated control (0 J2/g soil) 28.66 followed by 1 J2/g soil (27 leaves). The minimum mean leaf number was recorded from16 J2/g soil (12.66 leaves) inoculated plants. Moreover, a significant difference (p=.0001) on mean fresh shoot weight of common zinnia among treatments was observed. All inoculated plants resulted in a significantly reduced fresh shoot weight compared to the control plants.The maximum mean fresh shoot weight was recorded from control plants(16.66g), while the minimum (5.29g) was from plants inoculated with the highest initial population(16 J2/g soil)which was not significantly different from plants inoculated with2, 4, and 8 J2/g soil.

Mean fresh root weight of common zinnia was significantly (p=0.0001) affected by difference in varying initial nematode population. The highest mean fresh root weight was recorded from the control plants similar to the aforementioned growth parameters (14.03g), followed 11.72from plants inoculated with 1 J2/g soil. The lowest mean fresh root weight was recorded on those plants that were inoculated with the highest initial density (16 J2/g soil) i.e.4.57gbutwas not significantly different from plants inoculated with 4 and 8 J2/g soil which resulted in 5.77gand 4.25g, respectively.

Mean root length of common zinnia also showed significance difference when inoculated with varying initial nematode densities. The maximum mean root length was recorded from 0J2/g soil (31.66cm).The minimum mean root length was recorded on plant inoculated with 16 J2/g soil(20.33cm) which was not significantly different from those inoculated with8 J2/g soil (20.66cm).

The result indicated that there was a corresponding decrease in plant height, root-shoot fresh weight of plants and number of leaves with increasing inoculum densities. Joymati (2009) revealed that an increase in nematode inoculum was associated with progressive reduction in various plant growth parameters of *Allium porrum* and *Centella asiatica*. The growth parameter like shoot and root dry weight, number of leaves showed variation at different inoculum levels. A considerable increased reduction in the shoot- root length, shoot- root dry weight and shoot-root fresh weight of *Menthaarvensis* cv. Joymati was previously reported with an increase in initial population densities of *M. incognita*/pot Perveen *et al.* (2001). Total sugar, phenol, chlorophyll a, b and total chlorophyll content in leaves were also indicated to decrease with increase in initial inoculum level of *M. incognita*. The work of Noling (2009) revealed that under heavy nematode infestation, crop transplants may fail to develop, maintaining a stunted condition that could result in a poor stand development.

Johnson *et al.* (2003) observed a significant reduction in growth parameters (shoot and root length, shoot and root weight and number of leaves) of *Gladiolus* and *Carnation* when inoculated with different inoculum levels. In the present study, inoculum densities of *Meloidogyne* arenaria greatly affected root length of common zinnia. Reduction in root length was due to extensive damage to the root system as a result of nematode feeding on giant cells, causing root cession of growth. The root galling together with and reduced root length should have affected the overall growth and development of the plant by limiting their ability to absorb water and nutrients from the soil, which may eventually result in stunting and reduction in fruit yield (Hussain *et al*., 2011). Increases in nematode populations and subsequent corresponding reductions in growth have been reported by (Vovla *et al*., 2008).

Table.1.Effects of initial inoculum density of *M.arenaria* on growth parameter of common zinnia

Pi (J2/g soil)*	Shoot height (cm)	Leaf count	Fresh shoot weight (g)	Fresh root weight (g)	Root length (cm)
0	73.33±1.53[a]	28.66±1.15[a]	16.66±0.58[a]	14.03±1.13[a]	31.66±1.15[a]
1	60.16±1.04[b]	27.00±1.00[b]	11.56±0.97[b]	11.72±0.97[b]	29.00±1.00[b]
2	44.66±0.76[c]	21.66±0.58[c]	6.49±1.25[c]	7.91±0.68[c]	24.33±1.15[c]
4	39.00±1.00[d]	19.00±1.00[d]	6.19±0.63[c]	5.77±0.83[d]	23.66±0.58[c]
8	37.16±0.76[e]	15.33±0.58[e]	5.33±0.39[c]	4.65±0.73[d]	20.66±1.53[d]
16	36.60±0.53[e]	12.66±0.58[f]	5.29±0.86[c]	4.57±0.93[d]	20.33±0.58[d]
CV %	1.78	4.10	10.52	12.00	4.05
LSD	1.57	1.54	1.64	1.75	1.83

Means within a column followed by the same letter are not significantly different at $p < 0.05$Lsd test.*Pi= Initial nematode population

4.3.1. Population dynamics of *Meloidogyne arenaria* inoculated to common zinnia

There was significance difference (p=.0001) in root gall number among initial population densities on common zinnia. The root gall(Y) increase with initial population density of nematode (X) times 24.55. Root gall number increasing by the slop of y=24.55x -31.26). The highest mean root gall number was recorded on plants inoculated with the highest initial population density (16 J2/g soil) that resulted in 122 root galls followed by plants inoculated with 8 J2/g soil. Control plants (0 J2/g soil) showed no root galls (Fig 11).

Eggmass number exhibited a significant variation (p=.0001) among plants inoculated with different population levels.The eggmass (Y) increase with initial population density of nematode (X) times 12.70. Eggmass number increasing by the slop of y=12.70x- 15.24). The highest mean eggmass number was recorded from the test plants inoculated with the highest initial population (16 J2/g soil) i.e. 66.33.The minimum mean of eggmass number was observed from un-inoculated treatment (0 J2/g soil) 0.00(Fig 11).

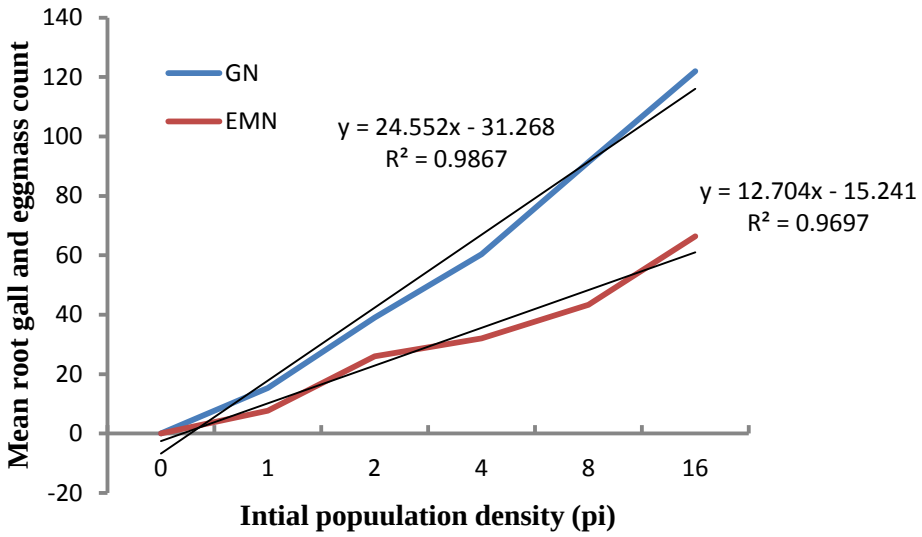


Figure 11. Effect of initial population density (pi) of M. *arenaria* on root gall and eggmass number in common zinnia .**GN** =gall number and **EMN** = eggmass number

The root gall and eggmass index on common zinnia significantly varied among plants depending on the initial population density inoculated. Both root gall and eggmass index increased with increase in initial inoculum density. The maximum mean root gall and eggmass index were observed from the treatment inoculated 16J2/g soil (5.00) and (4.00), respectively. None of these parameters were recorded from the control un-inoculated plants (Fig 12).

Khanna and Chandel (1997) have previously reported the effect of initial nematode population density on in *Gladiolus* cv. Sylviain that, reproduction factor decreases with increase in inoculum level. In contrast, root-gall index increased with a corresponding increase in nematode inoculum levels which might be associated with increase in less reproductive females.

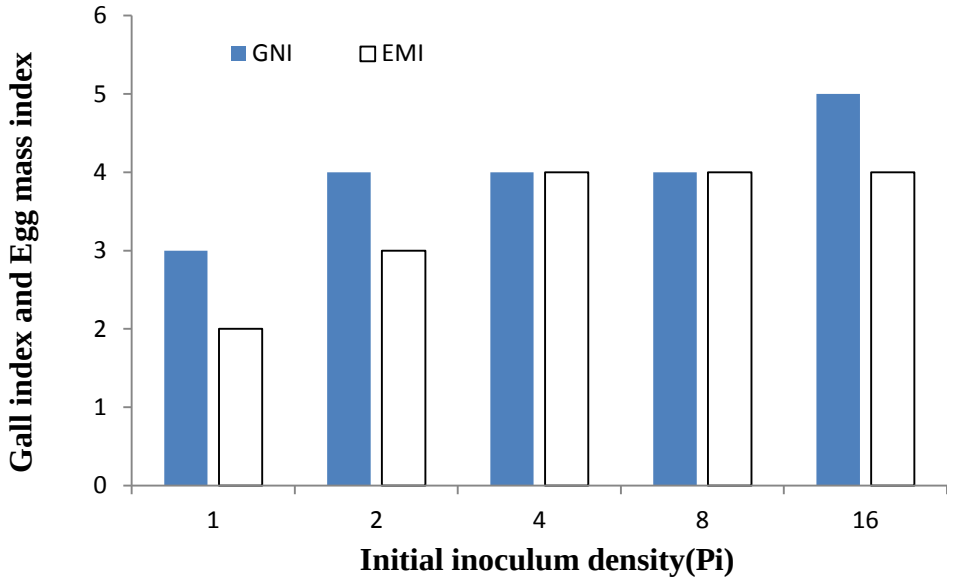


Figure 12. Effect of initial inoculum density on gall and eggmass index on common zinnia.***RGI**= gall index and **EMI**= eggmass index. 0 = no galls; 1 = 1 to 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls; 5 = more than 100 galls per root system (Taylor and Sasser, 1978).

Final nematode population was dependent on the initial population density (p=.0001, R^2= 0.92). The final population density of the nematode(Y) increase with initial population density of nematode (X) times 4814.The final population of the nematode growth rate increasing by the slop of y=4814x -5392). The maximum mean final population (25114.3 J2 and eggs) was recorded from plants inoculated with the highest Pi (16 J2/g soil),followed by treatments inoculated with 8 J2/g soil, 4 J2/g soil, 2 J2/g soil and lower mean being from those inoculated with 1 J2/g soil. No nematode was retrieved form un-inoculated test plants (Fig 13).

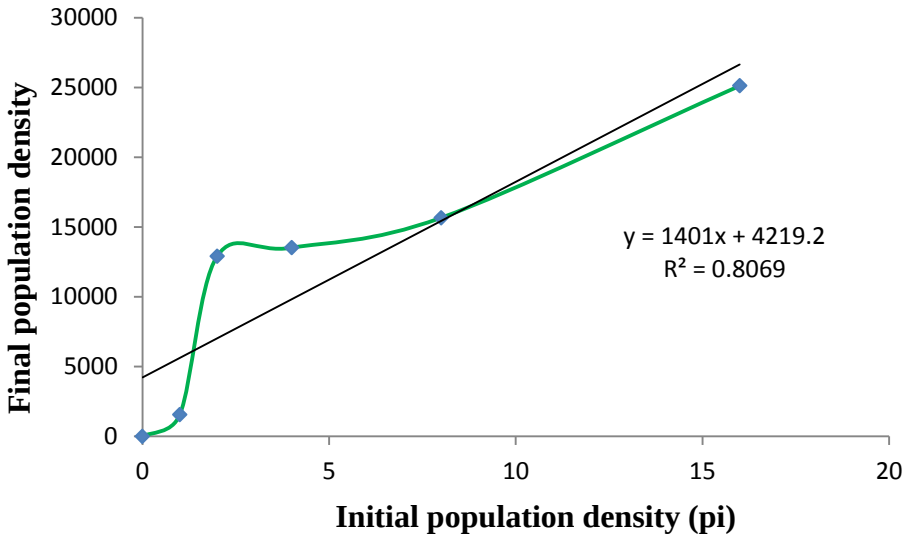


Figure 13. Relationships between initial population densities (Pi) and final population densities (Pf) of M. *arenaria* nematode in common zinnia

Significant difference (p=0.0001, R^2 = 0.015) was observed on reproduction factor of nematode among treatments. The highest mean reproduction factor was recorded from the treatment inoculated with 2 J2/g soils (6.45) and the lowest mean reproduction factor being recorded on plants inoculated with 1 or 16 J2/g soil. Generally, a reduction in rate of nematode multiplication was observed as the inoculum levels have increased from 4-16 J2/g soil (Fig 14).The reproduction of root nematodes are affected by different things including, soil type, amount of inoculum, the host resistance, soil temperature, cropping pattern ,type management to control the nematode, the alkalinity and acid of soils. The amount of inoculation is not always directly proportional with the reproduction of the nematode. Sometimes the amount of initial inoculum increase the reproduction of the nematode will be decrease due to intra specific computations. Destruction of root system by plant parasitic nematodes led to competition for nutrition and food among emerging nematodes within root system. High rate of multiplication of nematodes with low level of inoculum might be due to encouraging factors like plenty of food, reduced competition level and the ability of hosts to support these populations (Mohammed *et al*., 2015)

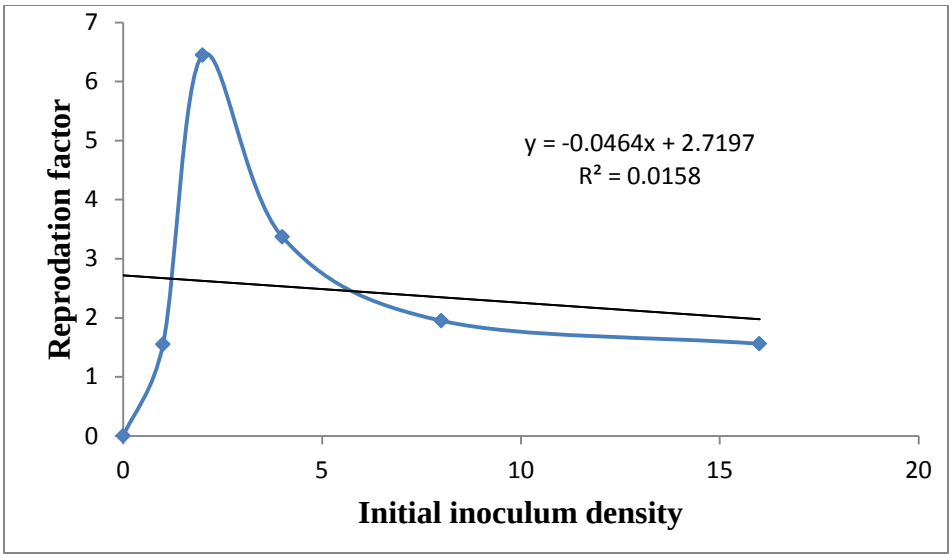


Figure14. Effect of initial population densities (pi) of M. *arenaria* on reproduction factor in common zinnia

Over all, maximum root gall, number of eggmass and final nematode population were recorded from the highest inoculum density. Singh and Hassan (2002) observed that the development of root galls, egg masses and nematode population increased with the increase in initial inoculum level of *Meloidogyne incognita* on bottle gourd. The root-gall index also increased with corresponding increase in inoculum level, while, the reproduction factor was decreased, which might be attributed to reduced egg production of adult females which have already created root galls. Higher rate of multiplication at low levels of inoculum could possibly be due to positive factors like abundance of food, low competition and the ability of host to support these levels of population (Das, 2013). The fact that lower reproduction value was recorded from plants inoculated with 1 J2/g soil might have come about from low infection rate of the few initial population. Variation on number of root galls at different initial inoculum levels of *M. incognit*a on *Allium porrum* and *Centella asiatica* was reported by Joymati (2009). On the other hand, a reduction in reproductive value at the highest initial population could be linked to the destruction of root system by the parasitism of root knot nematode which led to competition for food and nutrition among the developing nematodes within the root Rhizosphere (Kumar and Haseeb, 2006).

4.4. Management of *Meloidogyne arenaria* using decomposed coffee husk and biochar amendment on common zinnia

4.4.1. Effect of different coffee husk and biochar ratio amendment on growth parameter of common zinnia.

The plant height was significantly influenced by coffee husk ratio. There was difference at p=0.0001 among treatments. The maximum mean plant height was observed from 4:1 coffee husk ratio (79.25 cm). While the minimum was noted from 2:1coffee husk ratio (62.50cm) (Table 2).

Leaf number of common zinnia was significantly influenced by coffee husk ratio at (p=.0001) among each treatments. The highest mean Leaf number was observed from 1:1 coffee husk ratio (38.75). The lowest mean leaf number was observed from non amended coffee husk ratio (1:0) 22.00 (Table 2). Similar, shoot fresh weight was significantly influenced by coffee husk ratio at (p=.0001) among each treatments. The maximum mean shoot fresh weight was observed from 1:1 coffee husk ratio (33.64 g) followed by the treatment amended coffee husk ratio 4:1. The minimum shoot fresh weight in fact was noted from non amended coffee husk ratio (1:0)15.30g (Table 2).

There was significant variation at (p=.0001) of coffee husk ratio among each treatments on root fresh weight of common zinnia. The highest mean root fresh weight was noted from the pot amended coffee husk ratio 4:1(20.73 g). While the lowest mean root fresh weight was observed from non amended coffee husk ratio (1:0) 13.20g (Table 2).

Different treatments resulted in a significant (p=.0001) difference in root length of common zinnia. The highest mean root length was observed from the treatments amended coffee husk ratio with 2:1(32.50 cm). The lowest mean root length was noted from non amended coffee husk ratio (1:0) 20.25 cm (Table 2).

Amending coffee husk increases the growth parameter of common zinnia compared to none amended. Coffee husk is organic matter which is highly biodegradable. Therefore, after it breaks down it avails all the nutrients for plant growth (Caron et al. 2015). Coffee husks when added into the soil also improve soil chemical properties (Fairhurst 2012). These chemical properties include water holding capacity, aeration and cation exchange capacity. Kasongo et al (2011) also

reported that addition of coffee husks in soil promote the retention of basic cations and immobilized manganese as improving the pH of soil due its liming properties. Benefits of addition organic amendment, such as improved soil fertility, increased soil organic matter, and improved water-holding capacity, are apparently quite important in improving plant tolerance to nematode damage and infection (Renco et al., 2000).

Plant height, leaf number and shoot fresh weight were influenced by different ratio of biochar amendment. The greatest mean plant height was from biochar amended with ratio 3:1(72.00 cm), leaf number 4:1(26.75) and shoot fresh weight 1:1(25.74g).While the least were recorded from treatments 2:1(55.75cm), 3:1(19.50) and 0:1(15.30g) (Table 2).

Level of biochar significantly influenced root fresh weight of common zinnia. The maximum mean fresh weight recorded from biochar applied with the ratio of 1:1(20.40g). The minimum mean was also observed from non amended biochar 0:1 (13.20g) (Table 2).

The result indicates biochar amendment increased growth parameter of common zinnia. Graber *et al* (2010) reported as biochar can improve plant productivity directly as a result of its nutrient content and release characteristics, as well as indirectly, via: improved retention of nutrients (ii) improvements in soil pH, increased soil cation exchange capacity, improved soil physical properties, including an increase in soil water retention and alteration of soil microbial populations and functions. (Glaser et al. 2002) also reported biochar has significantly improved soil tilth, nutrient availability to plants, and plant productivity. Vaccari *et al*. (2015) found that the use of biochar on tomatoes increased plant growth compared to non-amended control.

Table 2. Effect of different ratio of coffee husk and biochar amendment on growth parameter of common zinnia.

Organic matter	Shoot height (cm)		Leaf count		Shoot fresh weight (g)		Root fresh weight(cm)		Root length(cm)	
	Coffee Husk	Biochar	Coffee Husk	Biochar	Coffee Husk	Biochar	Coffee Husk	Biochar	Coffee Husk	Biochar
0:1	64.00±0.82^{c}	64.00±0.82^{c}	22.00±0.82^{e}	22.00±0.82^{b}	15.30±0.51^{d}	15.30±0.51^{d}	13.20±0.66^{d}	13.20±0.66^{c}	20.25±0.96^{e}	20.25±0.96^{c}
1:1	67.50±1.29^{b}	61.50±0.58^{d}	38.75±0.96^{a}	22.25±1.26^{b}	33.64±0.95^{a}	25.74±0.54^{a}	17.35±0.94^{b}	20.45±0.97^{a}	28.25±0.96^{b}	25.75±0.96^{a}
2:1	62.50±0.58^{d}	55.75±0.65^{e}	31.25±0.96^{c}	20.00±0.82^{c}	24.28±1.20^{c}	15.41±0.89^{dc}	16.24±0.16^{c}	7.41±0.80^{d}	32.50±0.58^{a}	22.25±1.89^{b}
3:1	65.25±0.96^{c}	72.00±11.15^{a}	27.0±0.82^{d}	19.50±0.58^{c}	24.0±0.85^{c}	23.94±0.46^{b}	15.21±0.35^{c}	15.65±0.84^{b}	22.25±0.96^{d}	23.25±0.96^{b}
4:1	79.25±0.96^{a}	65.50±0.91^{b}	35.0±0.82^{b}	26.75±0.96^{a}	26.23±0.65^{b}	16.45±0.71^{c}	20.73±0.75^{a}	14.51±0.52^{b}	26.50±0.58^{c}	23.75±0.5^{b}
CV%	1.35	1.33	3.12	4.42	3.45	3.56	4.30	5.70	3.08	4.94
LSD	1.41	1.31	1.48	1.50	1.31	1.06	1.09	1.25	1.23	1.75

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$Lsd test.
(0:1, 1:1, 2:1, 3:1and 4:1) composition of each coffee husk and biochar ratio with soil.

4.4.2. Effect of different coffee husk and biochar ratio amendment on *Meloidogyne arenaria* reproductive parameter on common zinnia.

Final nematode population on common zinnia significantly (p=.0001) varied among treatments depending on different coffee husk and biochar ratio amendments. The highest final nematode population was recorded from non amended coffee husk and biochar amendments 0:1(29695.00) and the lowest final nematode population being recorded from coffee husk and biochar amended with the ratio of 4:1 (200.00) and (925.00) respectively (Fig 15).

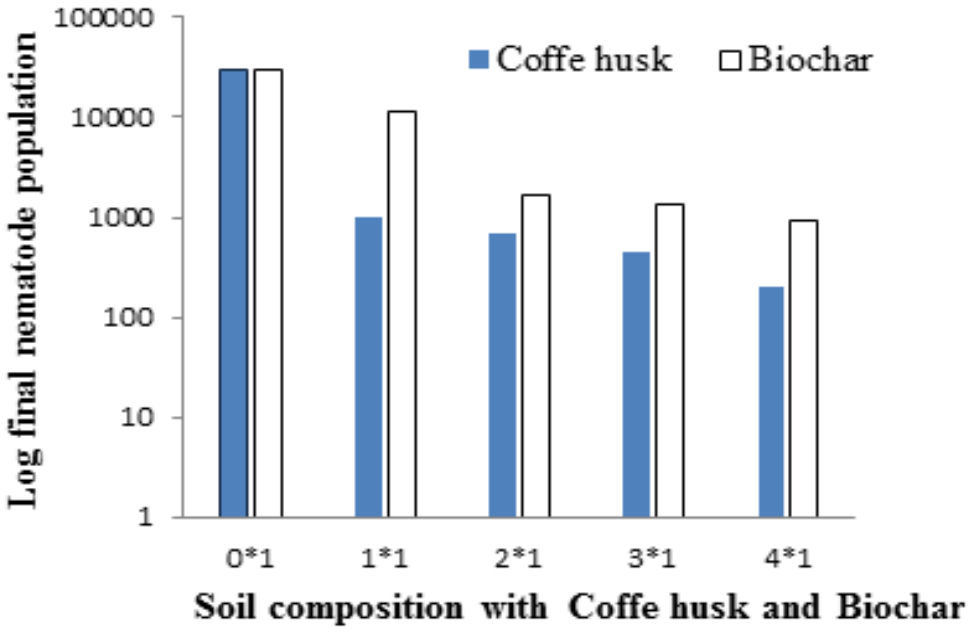


Figure 15. Effect of different coffee husk and biochar ratio amendment on *M.arenaria* final population on common zinnia. (0*1, 1*1, 2*1, 3*1and 4*1) composition of each coffee husk and biochar ratio with soil and *= ratio

The result showed that amending coffee husk and biochar ratio influenced reproduction factor on common zinnia (p=.0001). There was significantly difference among each treatment. Maximum mean reproduction factor for both amendments was recorded from non amended coffee husk and biochar treatments (14.84) and minimum reproduction factor was noted from amended coffee husk and biochar ratio with 4:1(0.10) and (0.46) (Fig 16).

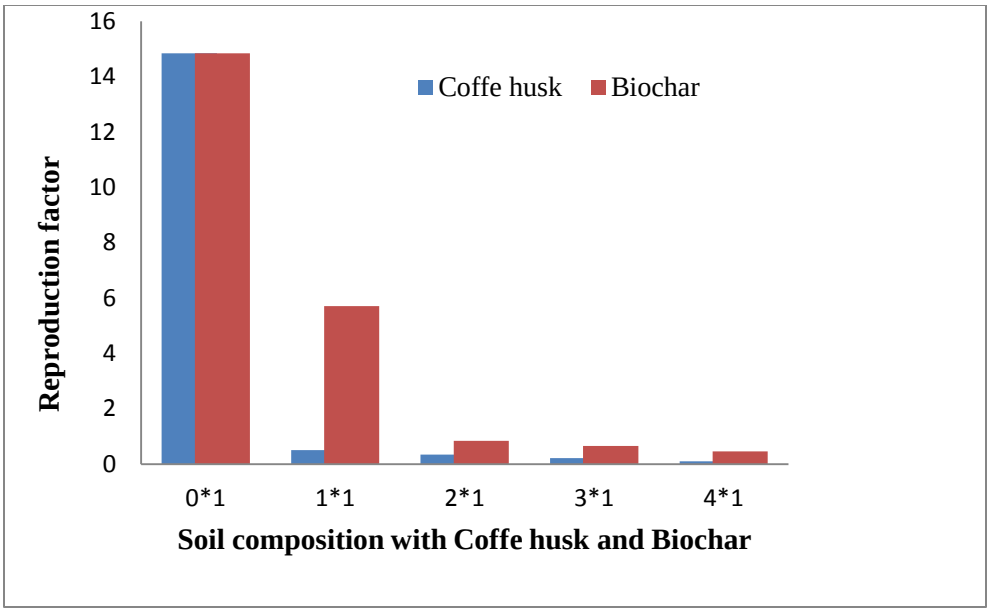


Figure 16. Effect of different coffee husk and biochar ratio amendment on *M.arenaria* reproduction factor on common zinnia. (0*1, 1*1, 2*1, 3*1and 4*1) composition of each coffee husk and biochar ratio with soil and *= ratio

The study showed that amending the soil with coffee husk materials increasing the rate of organic amendments enhanced the reduction of nematode populations. Increasing dosages of husk ratio organic amendment typically increased its efficiency in nematode control. According to Berkerlaar (2001) nematode problems are worse in soils with low amounts of organic matter content than in soils with high amounts. The study showed that amending the soil with Coffee husk suppressed the populations of *M.*arenaria both in the soil and on the roots of common zinnia. This is in agreement with previous findings Nico *et al*. (2004), who used poultry manure and decomposed agro-industrial waste products, namely composed dry cork, dry grape marc, dry olive marc and rice husk, as soil amendments for the management of *Meloidogyne* spp. Nico *et al*. reported that composted agro-industrial waste reduced the populations of *Meloidogyne* spp. Soil amendments with different types of organic manures are effective in reducing the population densities of many soil-borne plant pathogens including root-knot nematode (Hassan et al., 2010).

In the present research, mixing biochar in the soil and potting medium resulted in reduction of damage caused by root knot nematode on common zinnia. When the ratio of biochar increased the effectiveness of the control. Elad Y *et al.* (2011) reported that the suppression of soil pathogens by biochar may stem from several mechanisms, including improved nutrient solubilization and uptake, which helps enhance plant growth and resistance to the stresses of

pathogens; microbe stimulation, which promotes direct competition or parasitism against pathogens; or induced plant defense mechanisms. Biochar was found to have a high sorption capacity for dichloropropene, a strong anti-nematode fumigant. As a result, biochar-amendment to the soil can increase the required dose of dichloropropene to efficiently control nematodes (Graber *et al*., 2011). Biochar added to the potting medium the most effective at reducing nematode development, whereas direct toxic effects of biochar exudates on nematode viability, infectivity or development (Huang *et al*., 2015).

Coffee husk and biochar have ability to reduce plant parasitic nematode due to the presence of some chemical compounds. Among the chemical compound both have tannins which release toxic to plant parasitic nematode on survival, reproduction and direct paralyze by interring their body's. Tannins have antihelmintic properties especially for gastrointestinal nematodes in ruminants and anti parasitic activity (Hoste *et al*., 2006). Tannin greatly reduced nematode egg hatch percentage was gradually reduced as the applied concentrations increased. Tannic is an attractant for the root-knot nematodes *M.arenaria* may serve to disorientate phytoparasitic nematodes causing them difficulties in locating the root systems and potentially reducing plant damage. Those import ants are in addition to improving the soil structure, and increasing the soil temperature (Maistrello *et al*., 2010).

5. SUMMARY AND CONCLUSION

Common zinnia *(Z.elegans)* is widely cultivated ornamentals produced for cut flowers and flowerbeds. Diseases often reduce the quantity and quality of zinnia flowers, causing their production to be unprofitable particularly observed at JUCAVM. Root knot nematodes (*Meloidogyne* spp) are important economic pests of many cultivated crops and currently chemical nematicides used for control are being phased out due to their high toxicity. Findings of the study indicated that the root knot nematode that is causing damage to the ornamental crop, common zinnia (*Zinnia elegans*) is the *Meloidogyne arenaria*.

M.arenaria associated with common zinnia indicated that the growth and development of the plant (plant height, leaf number, shoot fresh weight and root fresh weight) is hampered due to infection over time. Aboveground symptoms have developed slowly over time, while root damage symptom, the gall formation, started from the second week of infection. The root galls were varying in size from slight thickenings to lumps when the number of week increases. The maximum nematode populations, numbers of root gall and eggmass (26715, 506 and 483) respectively were noted in16th week after infection but after 17 weeks the nematode reproductive parameters declined to some extent.

The relationship between initial *M. arenaria* population density and damage to the plant was revealed. Generally, all the inoculum levels reduced the plant height, number of leaves, shoot and root fresh weights and root length compared to the non inoculated plants. The highest growth parameters: plant height, number of leaves, root length and fresh weight of shoots and roots (73.33 cm, 28.66, 31.66 cm, 16.66 g and 14.03 g) respectively were recorded on non-inoculated plant while the lowest plant growth was in fact achieved with a nematode density of 16 J2/g soil. The highest number of galls, eggmass and final nematode population (122.0, 66.33 and 25114.3) respectively were noted on 16 J2/g soil but for nematode reproduction factor was achieved at inoculum level of 2 J2/g soil (6.45). Increasing the nematode inoculum level resulted in corresponding increase in number of galls, eggmass and final nematode population but nematode reproduction decreased with an increase the nematode density. The reductions in growth parameters and nematode infestations were found to be proportional to the inoculum level.

Therefore *Meloidogyne arenaria* is pathogenic to common zinnia *at* all inoculum levels and the damage is most severe at16 J2/g soil.

The management of *M. arenaria* using organic amendments showed that coffee husk and biochar amendments potting medium in common zinnia, could potentially reduce the damage caused by *M. arenaria* thereby protects the plants from the nematode infection. Therefore, management of coffee husk is more effective than biochar to increase plant growth and reduce population of nematodes. The highest was noted on non amended plant (29695) while the lowest nematode populations were recorded at 4:1 ratio (200,925) respectively. With increase in ratio of coffee husk and biochar amendment increase in the effectiveness root knot nematode control.

In conclusion, organic amendments may have nematode suppressive effects, depending on many interactions, including the type of compounds released, the dosages, the soil characteristics, and the level of nematode population. Moreover, nematode control requires a large amount of organic amendment. Therefore, coffee husk and biochar amendments could assume economic relevance, as alternative products to be used in sustainable strategies for nematode management. The results from this study demonstrate that coffee husk and biochar amendment could either kill root-knot nematodes or interfere with hatching and affect their capacity to invade and develop within their roots and effect to decrease populations of root-knot nematodes. The use of organic amendment as a tool for control of root knot nematodes and other soil pathogens is beneficial because it is a natural material, low cost from which nutrients and nematicidal substances are released gradually.

Recommendation and future line work

- Lack of information on plant-parasitic nematodes associated with annual and perennial ornamental plant including common zinnia attributed to the demand for research pertaining to agronomic and an avenue for distribution of plant pathogenic pathogen therefore should be considered.
- Amendment of soil with coffee husk and biochar provide practical control of root knot nematodes as a part of integrated management system.
- For biochar products, including as a disease control agent can help promote the adoption of biomass pyrolysis as an important tool in both mitigation and adaptation to climate change. The majority of available studies were done in laboratory or greenhouse conditions, with few cases addressing the potential application at field scale in realistic agricultural systems.

6. REFERENCES

Abbas, A., Taha, H. S., &Handa, A. K., 2008. Root-knot nematode resistance gene from fenugreek (Trigonell afoenum-graecum) confers nematode resistance in tomato. **11(2)**:139–158.

Abad, P., Favery, B., Rosso, M.N. and Castagnone-Sereno, P, 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Mol. Plant Path.***4**: 217–224.

Affokpon, A., Coyne, D.L., Htay, C.C., Agbèdè, R.D., Lawouin, L., Coosemans, J., 2011. Biocontrol potential of native Trichoderma isolates against root-knot nematodesin West African vegetable production systems. *Soil Biol*. **43**:600–608.

Amingad, V. and Lakshmipathy, M., 2014. Agricultural & Horticultural Sciences.

Anwar, S.A., Javed, N., Zia, A., Kamran, M., Hussain, M. and Javed, M., 2007. Root knot nematode reproduction and galling severity on thirteen vegetable crops.

Araya. Mario, L. A., 2004. Response to consecutive Nematicide Applicaltions Using the Same Product in Musa AAAcv. Grande Naine Originated from in vitro Propagation Material and Cultivated in Virgin soil.

Arora, R., Battu, G.S., Ramakrishnan, N., 2000. Microbial pesticides: current status and future outlook. In: Dhaliwal, G.S., Singh, B. (Eds.), Pesticides and Environment. Commonwealth Publishers. 344–395p.

Austin Hagan, 2005. By the Alabama Cooperative Extension System Department of Plant Pathology and Microbiology, Texas A and M University.

Baginsky, C., Contreras, A., Covarrubias, J. I., Seguel, O., &Aballay, E., 2013. Control of plant-parasitic nematodes using cover crops in table grape cultivation in Chile. *Cien cinvestigagrar*. **40(3)**: 567–577

Balick, M. J., M. H. Nee and D.E. Atha, 2000.Checklist of the vascular plants of Belize. Memoirs of the New York Botanical Garden. **85**: 1–246.

Becker, J. O., 2014. Encyclopedia of Agriculture and Food Systems. Encyclopedia of Agriculture and Food Systems.

Beira H., Dehne, H.W. and Hallmann, J., 2014. Plant-parasitic nematodes of commercial cut-flowers in Ethiopia. Int. J. Nematol. **24(1):**1-10.

Caillaud, M.-C., Dubreuil, G., Quentin, M., Perfus-Barbeoch, L., Lecomte, P., de Almeida Engler, J., Favery, B., 2008. Root-knot nematodes manipulate plant cell functions during a compatible interaction. *J. Plant Physiol*. **165(1)**: 104–13.

Carneiro, R.M.D.G., Tigano, M.S., Randig, O., Almeida, M.R.A. and Sarah, J.L, 2004. Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) on coffee from Brazil, Central America and Hawaii. *Nematol.* **6**:**287**–298.

Caron J, Price JS, Rochefort JC, 2015. Physical properties of organic soil: adapting mineral soil concepts to horticultural growing media and histosol characterization. Vadose Zone Journal. **14** :6.

Chen, J., Abawi, G.S. and Zuckerman, B.M., 2000. Efficacy of *Bacillus thuringiensis*, *Paecilomyces marquandii* and *Streptomyces constaricanus* with and without organic amendment against *Meloidogyne hapla* infecting lettuce. *J. Nematol.* **32**:70-77.

Collange, B., Navarrete, M., Peyre, G., Mateille, T., &Tchamitchian, M., 2011. Root-knot nematode (Meloidogyne) management in vegetable crop production: The challenge of an agronomic system analysis. *J. Crop Prot*. **30 (10)**:1251–1262.

Costa, M. J. N., Oliveira, S., Coelho, S. J. and Campos, V. P. 2001.Nematodes in ornamental plant roots. Cienc. agrotee, **25(5)**:1127-1132.

Curtis, R. H. C., 2007. Plant parasitic nematode proteins and the host-parasite interaction. *Brief Funct Genomic Proteomic*. **6(1)**:50–58.

Dar, Q.A.H., T.K. Chattopadhyay, R.L. Misra and M. Sanyat, 2002.Studies on growth characters of Zinnia. *Indian Soc. Orn. Hort.* 203–5p.

Das, P., 2013**.** Pathogenecity and management of root knot nematode (*Meloidogyne incognita*) infecting chilli, M.Sc. (Ag.)Thesis, Orissa University of Agriculture and Technology, Bhubaneswar.

Debertoldi, M., 2008. Production and utilization of suppressive composts: environmental, food and health benefit. In: Insam H., Franke-Whittle &Goberna M. (Eds).Microbes at Work from Wastes to Resource.153 –170p.

DLV-plant. Handbook for greenhouse rose production in Ethiopia. CBI, Wageningen, The Netherlands.2011.

Dong, W. B., Holbrook, C. C., Timper, P., Brenneman, T. B., Chu, Y., &Ozias-Akins, P., 2008.Resistance in Peanut Cultivars and Breeding Lines to Three Root-Knot Nematode Species. *Plant Dis.* **92**:631–638.

Dubreuil, G., Deleury, E., Magliano, M., Jaouannet, M., Abad, P., &Rosso, M.-N., 2011.Peroxiredoxins from the plant parasitic root-knot nematode, Meloidogyne incognita, are required for successful development within the host. *Int J Parasitol Parasites*. **41(4)**:385–96.

Duponnois, R., Chotte, J. L., Sall, S., & Cadet, P., 2001. The effects of organic amendments on the interactions between a nematophagous fungus Arthrobotrys oligospora and the root-knot nematode Meloidogyne mayaguensis parasitizing tomato plants. *Biol Fertil Soil.* **34(1)**: 1–6.

Eisenback, J. D. and Triantaphyllou, H. H, 1991. Root-knot Nematodes: *Meloidogyne* species and races. In: Manual of Agricultural Nematology, W. R. Nickle. (Ed). Marcel Dekker, New York. 281 – 286p.

Eisenback, J.D, 1985. Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). In: Sasser, J.N. and Carter, C.C. (Eds) *An Advanced Treatise on* Meloidogyne. *Biology and Control*. A cooperative publication of the Department of Plant Pathology and the United States Agency for International Development, North Carolina State University Graphics, Raleigh, North Carolina.**1**: 95–112.

Elad Y, Cytryn E, MellerHarel Y, LEW B, 2011. Graber ER. The biochar effect: plant resistance to biotic stresses. Phytopathol Mediterr. **50**:335–49.

Esbenshade, P.R. and Triantaphyllou, A.C, 1985. Identification of major *Meloidogyne* species employing enzyme phenotypes as differentiating characters. In: Sasser, J.N. and Carter, C.C. *An Advanced Treatise on* Meloidogyne .*Biology and Control*. North Carolina State University Graphics, Raleigh, North Carolina. **1** :135–140.

Fairhurst T, 2012. Handbook for Integrated Soil Fertility Management. Africa Soil Health Consortium, Nairobi.

Food and Agriculture Organization Corporate Statistical Database, 2018.

Fox, Joseph A, 2001. Nematode Control in the Home Garden. Mississippi State University Extension Service Publication. 483p.

Garcia, L.E. and Sanchez-Puerta, M.V., 2012.Characterization of a root-knot nematode population of Meloidogyne arenaria from Tupungato (Mendoza, Argentina). *J. Nematol*. **44(3)**:291.

Gombert L, Windham M, Hamilton S, 2001.Evaluation of disease resistance among 57 cultivars of zinnia. HortTechnology**. 11(1)**:71–74.

Glaser B, Lehmann J, Zech W,2002.Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal review. *Biol Fert Soils*. **35:** 219–230.

Goswami B K, Pandey R K, Rathour K S, Bhattacharya C and Singh L, 2006. Integrated application of some compatible biocontrol agents along with mustard oil seed cake and furadan on Meloidogyne incognita infecting tomato plants. J Zhejiang Univ Sci, **7**: 873-75.

Graber E.R., Harel Y.M., Kolton M., Cytryn E., Silber A., DavidD.R., Tsechansky L., Borenshtein M., Elad Y., 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Biology and Control*, **337:481**-496.

Graber ER, Tsechansky L, Khanukov J. Sorption, 2010.Volatilization and efficacy of the fumigant 1, 3-dichloropropene in a biochar-amended soil.SoilChemistry.**75**:1365–73.

Hafiza MA, Parveen B, Ahmad R, Hameid K, 2002. Phytochemicals and antifungal screening of Medicago sativa and Zinnia elegans v. J. Biological Sci. **2(2)**: 130-132.

Hammond-Kosack, K. E., & Jones, J. D. G., 1997.Plant Disease Resistance Genes. *ANNU REV PLANT BIOL*, **48(1)**:575–607.

Handoo, Z.A. and Subbotin, S.A., 2018. Taxonomy, identification and principal species. Cyst nematodes. *Wallingford, UK, CAB International*, 365-397.

Harnett, W., & Kennedy, M. W., 2001.Parasitic nematodes molecular. *Current Sociology*. 4p.

Hassan M, P Chindo, M Alegbejo, 2010. Management of root knot nematodes (Meloidogyne spp.) on tomato (Lycopersicon Lycopersicon) using organic wastes in Zaria, Nigeria. *Plant Prot. Sci*. **46:34**–39.

Hogenhout, S. A., and Bos, J. I. B., 2011.Effector proteins that modulate plant-insect interactions. *Curr Opin Plant Biol***. 14(4)**: 422–428.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M. and Hoskin, S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol*. **22(6)**: 253-261.

Hooper, D.J, 1990. Extraction and processing of plant and soil nematodes. In: *Plant Parasitic*, 45-68.

Huang, D., Liu, L., Zeng, G., Xu, P., Huang, C., Deng, L., Wang, R. and Wan, J., 2017. The effects of rice straw biochar on indigenous microbial community and enzymes activity in heavy metal-contaminated sediment. *Chemosphere*. **174**: 545-553.

Hussain, M.A., Mukhtar, T. and Kayani, M.Z., 2011.Assessment of the damage caused by *Meloidogyne incognita* on okra (*Abelmoschus esculentus*).*J.Anim. Pl. Sci*. **21**: 857-861.

Hussain, M.A., Fatima, I., Mukhtar, T., Aslam, M.N. and Kayani, M.Z., 2015. Effect of inoculum density of root-knot nematode Meloidogyne incognita on damage potential in eggplant. *Mycopath*. **13**:33-36.

Jepson, S.B, 1987. Identification of Root-Knot Nematodes (*Meloidogyne* Species). CAB International, Wallingford, UK.

Johnson, S.B.N., Cannayane, I. and Rajendran, G, 2003. Studies on pathogenic level of *Meloidogyne incognita* on *Gladiolus* and *Carnation*. *C.Nematol.* **14 (1/2)**: 75-78

Joymati, L. 2009. Pathogenecity of root knot nematode *Meloidogyne incognita* on two different indigenous crops of Manipur. *Indian J. Nematol*, **39 (2)**: 122-125.

Karssen, 2002. The Plant-Parasitic Nematode Genus *Meloidogyne* Goeldi, 1892. (Tylenchida) in Europe. Brill Academic Publishers, Boston, MA.

Kasongo RK, Verdoodt A, Kanyankagote P, Baert G, Ranst EV ,2011.Coffee waste as an alternative fertilizer with soil improving properties for sandy soils in humid tropical environments. Soil Use and Management. **27**:94-102.

Kerry, B.R., 2000. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. Annual Review of Phytopathology. **38**: 423–441.

Khan, M. R., 2004.Chemical approach for managing root-knot nematode, Meloidogyne incognita race 2, infecting jute. 195–199p.

Khanna, A.S. and Chandel, S.S, 1997. Damage potential of *Meloidogyne incognita* in *Gladiolus* cv. Sylvia. *Indian J. Nematol.* **27:253**-254.

Kiewnick S, Sikora RA, 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinu*sstra in 251. *Biol Control*. **38(2)**:179–187.

Kirkbride, J. H. and J. H. Wiersma, 2007.Proposal to conserve the name *Zinnia elegans* against *Z. violacea*. *Taxon.* **56(3)**: 958-959.

Kohl LM, 2011. Astronauts of the nematode world: an aerial view of foliar nematode biology, epidemiology, and host range.

Kumar, V. and Haseeb, A, 2006.Pathogenic potential of root-knot nematode, *Meloidogyne incognita* on chilli cv. Jwala. Proc. "8th Indian Agri. Scient. And Farmers Cong."BHU, 38.

Lamberti, F. and C.E. Taylor, 1979. Root-Knot Nematodes (*Meloidogyne* Species).Academic Press, New York.

Luc, M., Sikora, R.A. and Bridge, J. Orion, D. and Kritzman, G., 1991.Antimicrobial activity of *Meloidogyne javanica* gelatinous matrix. *Nematologica.* **14**: 481–483.

Madulu, J. &Trudgill, D, 1994. Influence of temperature on *Meloidogyne javanica*. *Nematologica.* **40**: 230 - 243.

Maistrello, L., Vaccari, G. and Sasanelli, N., 2010. Effect of chestnut tannins on the root-knot nematode Meloidogyne javanica. *Helminthol.* **47(1)**:48-57.

McSorley, R. and Frederick, J, 1994. Response of Some Common Annual Bedding Plants to Three Species of Meloidogyne. *J. Nematol*, **26 (4):**773-777.

Mesfin Tadesse, 2004.Asteraceae (Compositae). *Flora of Ethiopia and Eritrea*. The National Herbarium, Addis Ababa University, Addis Ababa and Uppsala. Nematodes *in Subtropical and Tropical Agriculture*. **4(2)***:* 1–408.

Mitkowski NA, Abawi GS, 2003. .Root-knot nematodes. The Plant Health Instructor. DOI: 10.1094/PHI-I-2003-0917-01 Revised2011.

Mohiddin, F. A., & Khan, M. R., 2014. Root-knot nematode: Ignored soil borne plant pathogen causing root diseases of chickpea. **2(1)**: 4–10.

Mulugeta G., 2009. Ethiopian floriculture and its impact on the environment. *Mizan law review*. **3(2)**: 240-270.

Neves, L., Oliveira, R., Alves, M.M., 2006.Anaerobic co-digestion of coffee waste and sewage sludge, Waste Management. **26**: 176-181.

Negusse K., 2006. Floriculture industry in Ethiopia: Trends, prospects and challenges. *Jimma University college of agriculture and veterinary medicine*.

Nico A.I., Jimenez-Diaz R.M., Castilla P., 2004.Control of root knot nematodes by composed agro industrial wastes in potting mixtures. Crop Protection. **23**:581–587.

Noling, J. W., 2009. Nematode Management in Tomatoes, Peppers and Eggplant. *University of Florida, IFAS Extension*. 1–15p.

Oerke, E. C., &Dehne, H. W., 2004. Safeguarding production - Losses in major crops and the role of crop protection. *J. Crop Prot*. **23(4)**:275–285.

Oliveira, D.F., Carvalho, H.W.P., Nunes, A.S., Campos, V.P., Silva, G.H., Campos, V.A.C., 2009. Active substances against Meloidogyne exigua produced in a liquid mediumby Bacillus megaterium. *Nematol Bras*. **33**: 271–277.

Olsen MW, 2000.Root-knot Nematode. University of Arizona, Arizona Cooperative Extension, AZ1187, 1–3.

Olsen, M. W, 2011. Root-knot Nematode. *Agriculture*, 1–3.

Oka, Y., Tkachi, N., Shuker, S., and Yermiyahu, U., 2007. Enhanced nematicidal activity of organic and inorganic ammonia-releasing amendments by Azadirachta indica extracts. *J. Nematol.* **39**:-9–16.

Orisajo S.B, Afolami S.O, Fademi O, Atungwu J.J, 2008. Effects of poultry litter and carbofuran soil amendments on Meloidogyne incognita attacks on cacao. ***J****. Appl. Biosci.* **7**: 214 – 221.

Ozores-Hampton, M., McSorley, R., and Stansly, P.A, 2012. Effects of long-term organic amendments and soil sanitation on weed and nematode populations in pepper and watermelon crops in Florida. *J. Crop Prot*. **41**:106–112.

Parihar, K., Rehman, B., &Siddiqui, M. A., 2012. Impact of Organic Additives for Sustainable Management of Root-Knot Nematode in Bottle Gourd. **1(4)**: 102–105.

Pandey, A; Soccol, C.R., Nigam, P., Brand, D., Mohan, R., Roussos, S., 2000, Biotechnological potential of coffee pulp and coffee husk for bioprocesses. *Biochem. Eng.* **6 (2)**: 153-162.

Perry, R.N., M. Moens and F.J. Starr, Eds., 2009.Root-Knot Nematodes. CAB International, Wallingford, UK.

Perveen, K., Haseeb, A. and Shukla, P.K, 2001. Influence of different initial inoculum densities of *Meloidogyne incognita* on the growth and oil yield of *Menthaarvensis*. Gomti. National Congress on Centenary of Nematology in India. Appraisal and Future Plans. 80p.

Radwan, M.A., Farrag, S.A.A., Abu-Elamayem, M.M. and Ahmed, N.S., 2012. Biological control of the root-knot nematode, *Meloidogyne incognita* on tomato using bioproducts of microbial origin. *APPL SOIL ECOL*. **56**:58-62.

Rahman L., 2003. Root knot diseases and its control. Agfact, 1–10.

Ralmi, N.H.A.A., Khandaker, M.M. and Mat, N., 2016. Occurrence and control of root knot nematode in crops: a review. *Aust. J. Crop Sci*. **11 (12)**: 1649.

Renco, M.; Sassanelli, N.; and Salamum, P., 2000.The effect of two compost soil amendments based on municipal green and penicillin production wastes, on plant parasitic nematodes. Helminthological. **46 (3)**:190-197.

Sharon, E., and Spiegel, Y. 1993. Glycoprotein characterization of the gelatinous matrix in the root-knot nematode *Meloidogyne javanica. J. Nematol*. **25**:585–589.

Singh, P.K. and Hassan, 2002.Pathogenecity of root-knot nematode *Meloidogyne incognita* on bottle gourd *Lagenariasciceraria*.*J. Mycol. Pl. Pathol*. **32:153**.

Starr J.L, J. Bridge and R. Cook, 2002.Plant Resistance to Parasitic Nematodes. CABI Publishing, Cambridge MA.

Tabarant, P., Villenave, C., Risède, J. M., Roger-Estrade, J., & Dorel, M., 2011. Effects of organic amendments on plant-parasitic nematode populations, root damage, and banana plant growth. *Biol Fertil Soils*. **47(3)**: 341–347.

Taylor, A.L. and J.N. Sasser., 1978.Biology, identification and control of root-knot nematodes (*Meloidogyne* species).North Carolina State Univ. Graphics, Raleigh.

Thoden, T. C., Korthals, G. W., and Termorshuizen, A. J., 2011.Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management. *J. Nematol.* **13**:133–153.

Tiwari, S., Tech, V., Eisenback, J. D., Youngman, R. R., & Entomologist, E., 2009.Root-knot Nematode in Field Corn. *Management*.

Trudgill DL, Blok VC, 2001. Apomictic, polyphagous root knot nematode: exceptionally successful and damaging biotrophic root pathogens. *Annu Rev Phytopathol*. **39**:53-77.

Trudgill, D. L., 1995. An assessment of the relevance of thermal time relationships to nematology. Fundamental and Applied Nematology.**18**:407 - 417.

Vallad, G. E., and Goodman, R. M. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Sci*. **44**:1920-1934.

Verheijen, F.G.A., S. Jeffery, A.C. Bastos, M. Van DerVelde and I. Diafas, 2009.Biochar Application to Soils - A Critical Scientific Review of Effects on Soil Properties, Processes and Functions, 149.

Viaene, N.M., 1998. Management of *Meloidogyne hapla* on lettuce in organic soil with sudangrass as a cover crop. *Pl. Dis*. **82**:945-952.

Vovlas, N., Troccoli, A., Minuto, A., Bruzzone, C., Sasanelli N. and Castillo, P., 2008. Pathogenecity and host-parasite relationships of *Meloidogyne arenaria* in sweet basil. *Pl. Dis*. **92**: 1329-1335.

Wachira PM, JW Kimenju, Okoth SA, RK Mibey, 2009. Stimulation of nematode destroying fungi by organic amendments applied in management of plant parasitic nematode. *Asian J. Plant Sci*. **8**:153-159.

Wesemael, W. M. L., Taning, L. M., Khan, A., Viaene, N., and Moens, M., 2014. Life cycle of the root-knot nematodes *Meloidogyne* chitwoodi, *M. fallax* and *M. minor*on potato and consequences for damage development.

Westerman, P.W., Bicudo, J.R., 2005. Management considerations for organic wastes use in agriculture, Bioresource Technology. **96**: 215-221.

Will`iams-Woodward, J. L. and R. F. Davis, 2001. *Meloidogyne incognita* and *M. arenaria* reproduction on dwarf hollies and lantana. *J. Nematol*. **33(4)**:332-337.

Wondirad M, Tesfamariyam M, 2002. Root-knot nematode on vegetable crops in central and western Ethiopia. Pest Manage. *J.ia Ethiop*. **4:19**-28.

Ye, Y.M., Zhang, J.W., Ning, G.G. and Bao, M.Z., 2008.A comparative analysis of the genetic diversity between inbred lines of Zinnia elegans using morphological traits and RAPD and ISSR markers. *Sci. Hortic.* **118(1)**:1-7

7. APPENDICES

Appendix 1. ANOVA skeleton for the effect initial inoculum density of *Meloidogyne arenaria* on growth parameter of common zinnia level of 0.05

		Growth parameters									
		PH*		LN*		SFW*		RFW*		RL*	
Source	**DF**	Mean square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F
Trt	5	676.68	<.0001**	120.18	<.0001**	63.79	<.0001**	48.57	<.0001**	61.92	<.0001**
Error	10	0.74		0.72		0.81		0.93		1.02	
Total	17										
Cv		1.78		4.10		10.52		12.00		4.05	
Lsd		1.57		1.54		1.64		1.75		1.83	

* =SH=plant height, LN = leaf number SFW=shoot fresh weight, RFW=root fresh weight, RL= root length **=highly significant

Appendix 2. ANOVA skeleton for the effect of initial t inoculum density Meloidogyne *arenaria* reproductive of common zinnia level of 0.05

		Meloidogyne *spp* reproduction							
		GN*		EMN*		FP*		RF*	
Source	**DF**	Mean square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F
Trt	5	6414.80	<.0001**	1747.82	<.0001**	263916400	<.0001**	14.81	<.0001**
Error	10	9.16		1.05		1357009		0.02	
Total	17								
Cv		5.53		3.51		10.16		5.90	
Lsd		5.50		1.86		2119.3		0.26	

*=GN = gall number, EMN= egg mass number, FN = final population and RF= reproduction factor **= highly significant

Appendix 3. ANOVAskeleton for the effect of coffee husk amendment on Meloidogyne arenaria growth parameter of common zinnia levelof0.05

		Growth parameters									
		PH*		**LN***		**FSW***		**FRW***		**RL***	
Source	**DF**	Mean square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F
Trt	4	180.17	<.0001**	172.92	<.0001**	171.14	<.0001**	31.19	<.0001**	94.67	<.0001**
Error	12	0.84		0.92		0.72		0.50		0.64	
Total	19										
Cv		1.35		3.12		3.45		4.30		3.08	
Lsd		1.41		1.48		1.31		1.09		1.23	

* =SH=plant height, LN = leaf number SFW=shoot fresh weight, RFW=root fresh weight, RL= root length **=highly significant

Appendix 4. ANOVA skeleton for the effect of coffee husk amendment on *Meloidogyne arenaria* nematode reproduction of common zinnia level of 0.05

		M.*arenaria* reproduction							
		GN*		**EMN***		**FP***		**RF***	
Source	**DF**	Mean square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F
Trt	4	583.20	<.0001**	369.80	<.0001**	677879170	<.0001**	169.46	<.0001**
Error	12	0.13		0.33		10357		0.002	
Total	19								
Cv		6.76		13.42		1.58		1.58	
Lsd		0.56		0.88		156.79		0.07	

*=GN = gall number, EMN= egg mass number, FN = final population and RF= reproduction factor **= highly significant

Appendix 5. ANOVA skeleton for the effect of biochar amendment on *Meloidogyne arenaria* growth parameter of common zinnia level of 0.05

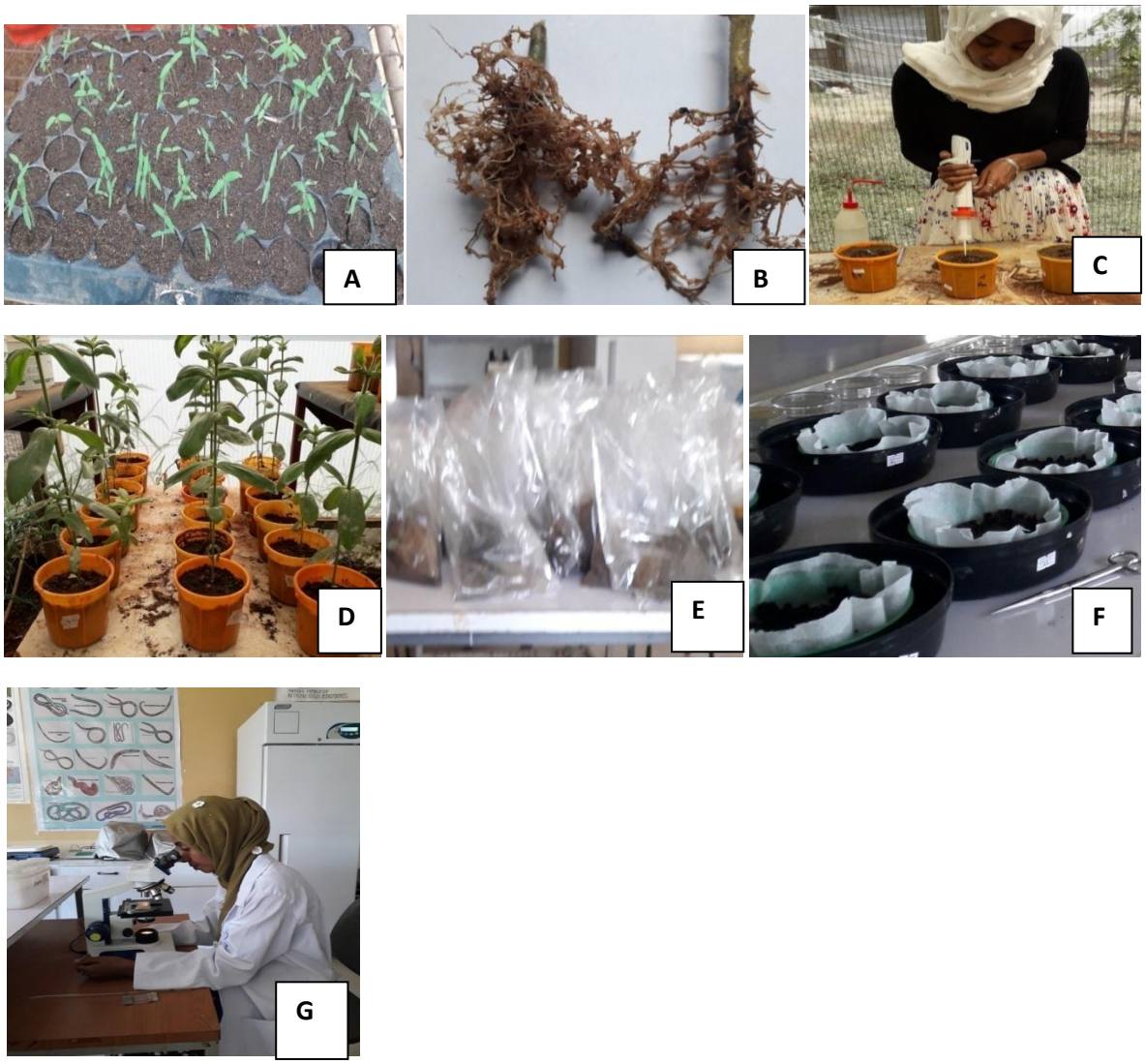
		growth parameters									
		PH*		**LN***		**FSW***		**FRW***		**RL***	
Source	**DF**	Mean square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F
Trt	4	140.25	<.0001**	32.82	<.0001**	102.28	<.0001**	87.66	<.0001**	16.30	0.0003**
Error	12	0.72		0.95		0.47		0.66		1.30	
Total	19										
Cv		1.33		4.42		3.56		5.70		4.94	
Lsd		1.31		1.50		1.06		1.25		1.75	

* =SH=plant height, LN = leaf number SFW=shoot fresh weight, RFW=root fresh weight, RL= root length **=highly significant

Appendix 6. ANOVA skeleton for the effect of biochar amendment on *Meloidogyne arenaria* reproduction of common zinnia level of 0.05

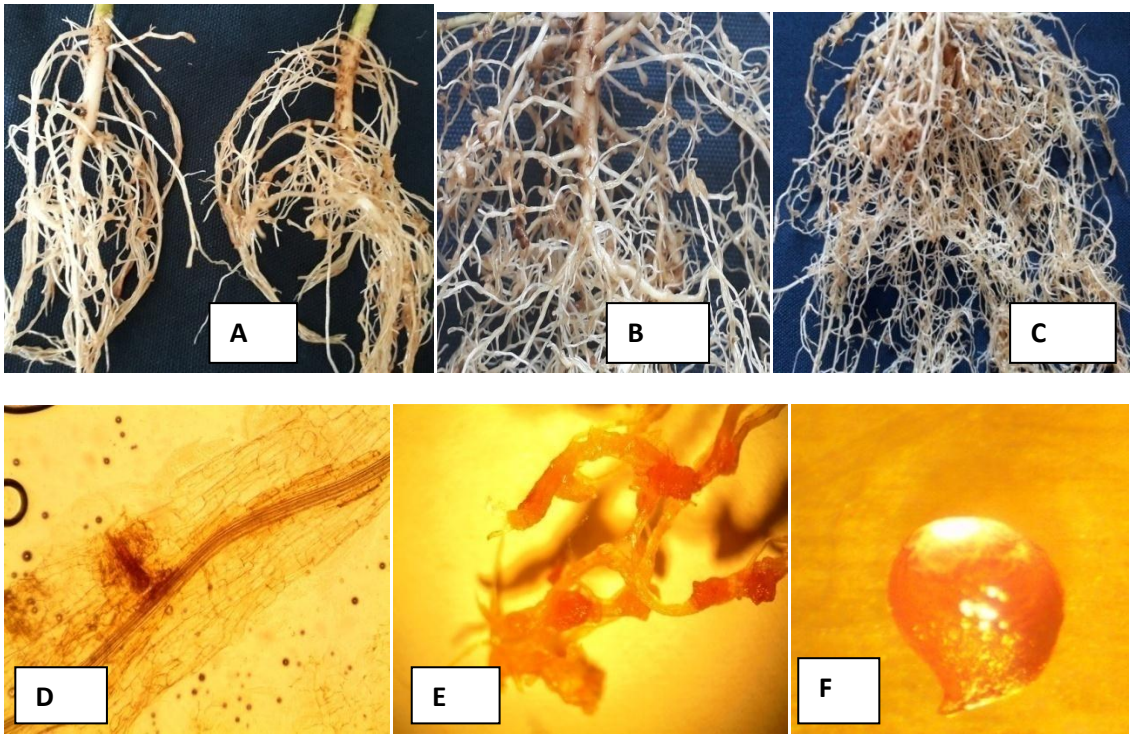
		M.*arenaria* reproduction							
		GN*		**EMN***		**FP***		**RF***	
Source	**DF**	Mean square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F	Mean Square	Pr>F
Trt	4	541.92	<.0001**	355.80	<.0001**	611443870	<.0001**	143.62	<.0001**
Error	12	0.19		0.43		8690		0.002	
Total	19								
Cv		6.08		10.88		1.03		1.00	
Lsd		0.67		1.01		139.71		0.07	

*=GN = gall number, EMN= egg mass number, FN = final population and RF= reproduction factor **= highly significant

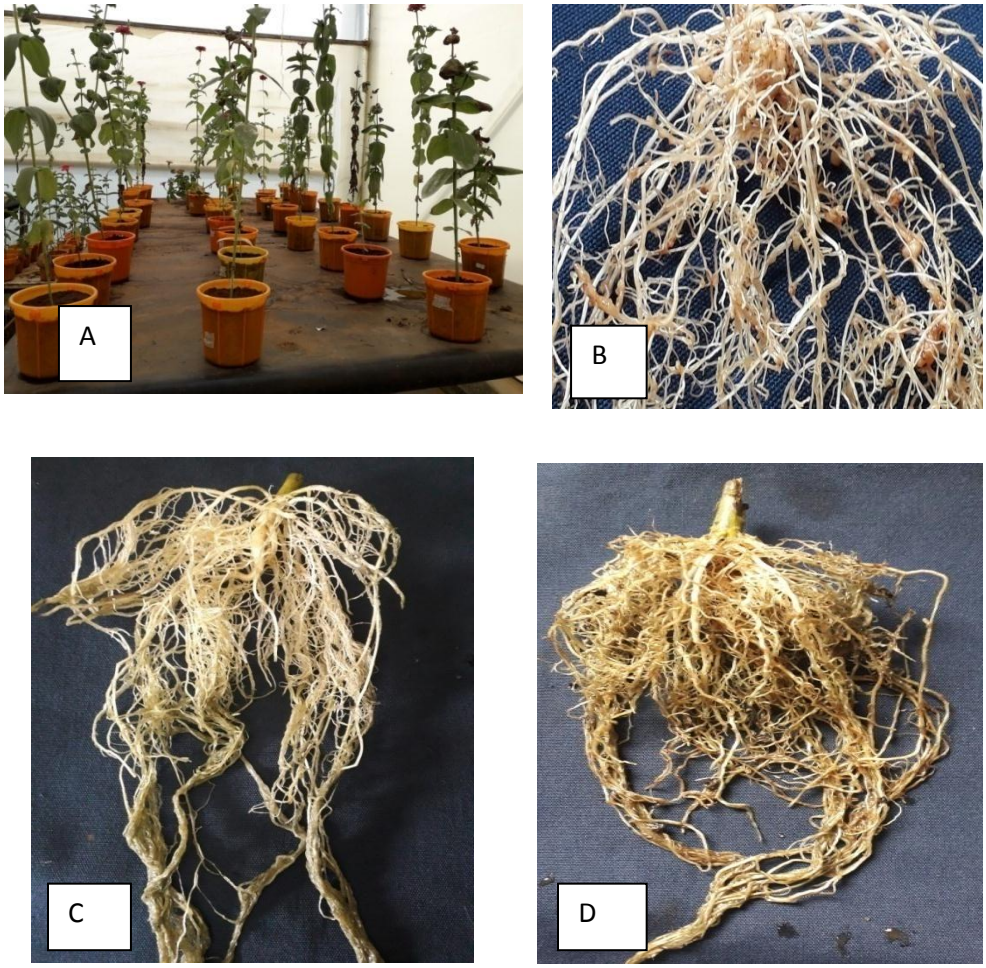


Appendix 7.Photographs taken when conducting experiments under greenhouse and laboratory conditions. (**A)**Seeds planted plastic trays,**(B)** pure culture root knot nematode from tomato root,(**C)** inoculation of J2 root knot nematode on common zinnia,**(D)** inoculated plant after some week,**(E)** sample from root and soil **F)** extracting samples on modified Baermann tray method and **(G)** counting root knot nematodes.

Appendix8. Effect of *Meloidogyne* spp on growth of common zinnia per week .The symptom showed stunting, yellowing the leaves and wilting.



Appendix 9.Reproductive potential of *M.* arenaria on common zinnia per week.(A**, B**and **C)** root galls varying in size from slight thickenings to lumps when the number of week increases,(**D**) infected root tissue (**E)** stained egg mass and (**F)** female *M.arenaria*



Appendix 10.Management of *M.*arenaria using decomposed coffee husk and biochar amendment on common zinnia.(**A**) Experiment in greenhouse,(**B)** non amended plant showed root gall and (**C** and **D)** biochar and coffee husk amended plants exhibits health root