PHYTONEMATODES ASSOCIATED WITH TOMATO (*Solanum lycopersicum* L.) AND SCREENING OF TOMATO VARIETIES AND USE OF COFFEE HUSK FOR MANAGEMENT OF *Meloidogyne* a*renaria*

MSc. THESIS

TAREKEGN MENGISTU MEKURIA

NOVEMBER, 2019

JIMMA, ETHIOPIA

Phytonematodes Associated With Tomato (*Solanum lycopersicum* L.) and Screening of Tomato varieties and use of Coffee Husk for Management of *Meloidogyne* a*renaria*

By

Tarekegn Mengistu

MSc. Thesis

Submitted to the Department of Horticulture and Plant Sciences, College of Agriculture and Veterinary Medicine, Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science (MSc) in Plant Pathology

Major Advisor: Dr. Beira H. Meressa

Co- Advisor: Dr. Wakuma B. Hundessa

November, 2019

Jimma, Ethiopia

APPROVAL SHEET

Jimma University College of Agriculture and Veterinary Medicine

Thesis Submission for External Defense Request Form (F-07)

Name of student: Tarekegn Mengistu **ID No**: RM 1213/10

Program of the study: Plant Pathology (MSc)

Title: **Phytonematodes associated with tomato (*Solanum lycopersicum* L.) and screening of tomato varieties and use of coffee husk for management of *Meloidogyne* a*renaria***

I have incorporated the suggestion and modification given during internal defense and got the approval of my advisors. Hence, I hereby kindly request the department to allow me to submit my thesis for external defense.

Name _____ Signature _____ Date _____

We, the thesis advisors, have verified that the student has incorporated the suggestions and modifications given during the internal thesis defense and the thesis is ready to be submitted. Hence, we recommend the thesis to be submitted for external defense.

Major Advisors' Name: Dr. Beira H. Meressa Signature _____Date _____

Co- Advisors' Name: Dr. Wakuma B. Hundessa Signature _____ Date _____

Decision/suggestion of department graduate council (DGC)

_____

Chairperson of DGC _____Signature _____Date_____

Chairperson of CGC _____Signature _____Date_____

DEDICATION

I dedicate this thesis to my mother and father: Amarech Gudeta and Mengistu Mekuria

STATEMENTS OF THE AUTHOR

I declare and affirm that this thesis work is my own original work and it has not been presented and will not be presented to any other University for a similar or any other degree award. Brief quotations from this thesis are allowable without special permission provided that accurate citation of source is made.

Name: Tarekegn Mengistu Mekuria

Signature: _____

Date of Submission: _____

BIOGRAPHICAL SKETCH

The author, Tarekegn Mengistu was born on 25th July, 1995 in SNNPR, Gamo Gofa zone, Chencha woreda. He attended his primary school in Dorze primary Schools from 2001 to 2009 and secondary and preparatory school at Chencha Secondary and preparatory High school from 2010–2013. He joined Dilla University in 2014 and graduated with Bachelor of Science Degree in Plant Science in 2016. After he graduated, he joined Dilla University as graduate assistance. In October 2017, he got an award by the Ethiopian Ministry of Education to pursue a master in Plant Pathology at Jimma University, College of Agriculture and Veterinary Medicine.

ACKNOWLEDGEMENT

First of all, I would like to thank almighty God who protected me in peace and healthy to come up with this work and I would like to express a particular thankfulness to my advisors, Dr. Beria H. Meressa and Dr. Wakuma B. Hundessa who have afforded me constant guidance and encouragement throughout my work. Under their guidance, I successfully overcame many difficulties and learned a lot about how to work with the small worms. They were always helpful, kind and optimistic and they have played an enormous role in my academic achievement. Moreover, they were always open for discussion and gave me plenty of freedom to develop all my interests in different aspects of academic issues which I sincerely appreciate.

Special thanks go to Dilla University college of Agriculture and Natural resource for giving me the opportunity to pursue the Master's program. I appreciate the support accorded to me by my colleagues and friends (Teshome Molla, Zubeyda Yasin and Enish Belete) especially at PDDL of JUCAVM. My appreciation also goes to colleagues and friends (Selamawit Araya and Ayele Gucho) for their technical support. I would like to thank Horticulture and Plant science department of JUCAVM for offering me access to use laboratory and greenhouse facilities.

And last but not least heartfelt thanks go to my beloved parents for their encouragement to accomplish my study. You have been so supreme; you have nurtured my studies and always supported my dreams. You made me into who I am. Thank you!

LIST OF ABBREVIATION AND ACRONYMS

CH	Coffee husk
CSA	Central Statistical Agency
EHDA	Ethiopian Horticulture Development Agency
FP	Final population
J2	Second Juvenile
NEM	Number of eggmass
NRG	Number of root gall
PDDL	Plant Disease Diagnostic Lab
RF	Reproduction factor
RFW	Root fresh weight
RKN	Root knot nematodes
SAS	Statistical Analysis Systems
SDW	Shoot dry weight
SFW	Shoot fresh Weight
SH	Shoot height
SM	Soil to sand mix

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF APPENDIXES

Page

ABSTRACT

Nematodes are a costly burden in agricultural crop production. The root knot nematode is one of the most economically important plant parasitic nematode groups and is widely distributed and damaging tomato. The occurrence, biodiversity and distribution of nematodes in major tomato growing areas around Jimma, in particular to Dedo and Karsa districts were not conducted. Moreover, information on nematode management using host resistance and coffee husk amendments is unknown. The aim of this study was to assess the diversity of Phytonematodes associated with tomato production system in two districts and evaluate tomato varieties for their reaction to Meloidogyne arenaria and test coffee husk as an option towards management of M. arenaria on tomato. The survey was conducted at Dedo and Karsa district of Jimma zone. A total of forty composite soil samples and plant roots were collected from farmer's field from eight kebeles. Fourteen tomato varieties (Moneymaker, Roma VF, Fetan, Melkasalsa, Metadel, Cochoro, Bishola, Gelila, Gelilema, APR d2 tomato, Chali, Margilobe, Melkashola and Miya) were tested for the reaction to M. arenaria and the effect of coffee husk application on M. arenaria was evaluated on three tomato varieties. The experiments were set using RCBD under greenhouse condition. Seedlings with four true leaves were inoculated with infective second stage juveniles a week after transplanting. Data on number of eggmass and root gall, gall index, final nematode population, reproduction factor, shoot height, fresh and dry weight, and root length and weight were collected after ten week of inoculation. ANOVA was done using SAS 9.3 version and means were separated using Lsd at p= 0.05. A total of nine nematode genera associated with tomato was recorded and identified viz. Meloidogyne, Pratylenchus, Helicotylenchus, Scutellonema, Rotylenchulus, Aphelenchus, Criconema, Cyst nematode (Globodera spp) and Paratylenchus. Meloidogyne, Pratylenchus, Helicotylenchus, and Scutellonema were the most frequently encountered genera (100%). Cyst nematode (Globodera spp) and Aphelenchus spp were detected and reported here for the first time from Ethiopia. The highest mean disease incidence was found from Babo kebele. Among tested tomato varieties, except Melkashola, all were found to be susceptible and have high numbers of eggmasses, root galls, final nematode population and reproduction factor. Variety Melkashola was identified as resistant host for M. arenaria with reproduction factor value of 0.14 *at p<0.05. Application of Coffee husk reduced the reproduction rate of M. arenaria and enhanced the growth of tomato plant as compared to non-amended treatment. An increase in coffee husk proportion in treatment of combinations resulted in reduction of number of eggmass and root gall, final population and reproduction factor. The present study revealed that tomato was infested with several PPNs and both Melkashola variety and coffee husk were used as an alternative option to manage M. arenaria. However, the severe infections on tomato plants and growth impairment observed in the farmers' field, calls for an immediate attention and implementation of feasible management strategies. Further studies are necessary to test this variety for more seasons to determine the durability of resistance and more attempts in coffee husk amendments are needed to confirm actual rates and timing of amendments and repeating the experiments under field condition to help us draw promising conclusion.*

Key words: Eggmass, root gall, reproduction factor, plant parasitic nematode, variety.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family which is dicotylenous perennial and annual plant (Van Eck, 2018) and is a diploid plant with 2n=2x=24 chromosomes (Fentik, 2017). It is indigenous to the Peru and Ecuador region in South America (Saavedra *et al*., 2017).

In 2017, the worldwide production of tomatoes reached 170.8 million tons. China, India and the United States are the leading producer of tomato in the world (FAO, 2017). Tomato has enormous economic value reaching billions of dollars (Van Eck, 2018). It is a source of nutrients and secondary metabolites which contains minerals, vitamins C and E, β-carotene, lycopene, flavonoids, organic acids, phenolic and chlorophyll and it has role in human healthy (Flores *et al*., 2017).

In Ethiopia, tomato is one of the most important and widely grown vegetable crops, both during the rainy and dry seasons for its fruit and the production is mainly concentrated in northern and central rift valley areas by smallholder farmers, commercial state and private farms (Emana *et al*., 2014). It is largely grown in the eastern and central parts of the mid to lowland areas of the country. Large scale production of tomato appears to be in the upper Awash valley, under irrigated and rain-fed conditions whereas small scale production for fresh market is a common practice around Koka, Ziway, Wondo-Genet, Guder, Bako, Jimma, Wellega and many other areas (Gemechis *et al*., 2012). It was recognized as an important product for both local and export markets and providing a cash crop for small scale producers in developing countries including Ethiopia (Tewodros and Asfaw, 2013). In the year of 2017/18 the total production of tomato in Ethiopia was about 27,774.54 ton harvested from 5,235.19 ha of land; with the productivity of about 5.31 t ha^{-1} (CSA, 2018) (Fig. 2). About 881.37 t/ha of tomato was harvested from 130.56 ha of land with productivity of 6.74 t/ha in Jimma Zone (CSA, 2017). However, several abiotic and biotic factors were identified as production constraints responsible for the low level of productivity in Ethiopia (Lemma, 2002; Ambecha *et al*., 2007) as compared to world average 36 M T/ha (FOASTAT, 2014).

Plant parasitic nematodes (here after, nematodes) are one of the biotic factors remain a major challenge to crop production in the world (Talwana *et al*., 2008) causing impact on the delivery of global food security (Jones *et al*., 2013). On a global scale, annual economic losses based on 37 life sustaining crops, is US$ 358.24 billion (Abd-Elgawad, 2014). Amongst the many genera of nematodes causing an economic impact, *Meloidogyne* spp. are responsible for a large part of the annual losses of $157 billion globally (Abad *et al*., 2008). *Meloidogyne* spp cause more than 50 % losses to tomatoes in USA (Natarajan *et al*., 2006). It is one of the major pathogens of tomatoes and limits fruit production (Sikora and Fernandez, 2005; Nicol *et al*, 2011). Hence, tomato is plagued by a wide range of PNN. In Ethiopia, it is heavily attacked by root-knot nematode (RKN), and the species *Meloidogyne incognita* is the dominant in Rift Valley (Lemma, 2002), central and eastern Ethiopia (Wondirad and Mekete, 2002; Seid *et al*., 2017). Economic losses due to *Meloidogyne* spp. are not only confined to yield reductions but also to an increase on production costs for farmers. Apart from the direct losses resulting from root deformation, nematode infections also predispose to hosts to break resistance to other pathogens and reduce yield quality and quantity (Hunt and Handoo, 2009).

Different management options were examined to keep the RKN population below damaging level (Barker and Koenning, 1998; Coyne *et al*., 2009). For instance, Natarajan *et al*. (2006) used cold aqueous extract of African marigold, *Tagetes erecta* for control of *M. incognita.* Aqueous suspension of rapeseed cake and BioNem WP were also evaluated by Belay *et al*. (2013) against RKN in the laboratory, greenhouse and field conditions. Sunil *et al*. (2007) evaluated six varieties of tomato for the reaction to *M. incognita* in which all varieties were found to be susceptible to varying degree. Seid *et al*. (2017) evaluated the reaction of 23 tomato cultivars and 10 breeding lines against to *M. incognita* and *M. javanica,* in which none of these materials were immune to both nematode species. The PPNs were also inhibited by application of organic matter like poultry manure (Ogwulumba *et al*., 2009), rice husk, saw dust and refuse dump (Hassan *et al*., 2010) and combined application of poultry liter and manure with rapeseed cake (Shiferaw *et al*., 2014; Shiferaw *et al*., 2017). Sohrabi *et al*. (2018) evaluated the effect of four plant growth-promoting Rhizobacteria. *Pseudomonas fluorescens, P. striata, Bacillus subtilis*, and *Paenibacillus polymyxa* strains significantly reduced the reproductive factor of *M. javanica.*

Very few nematological researches have been done in Ethiopia so far when compared to other plant pathogens. Since few years some progresses have been shown particularly on vegetable and ornamental crops. Attempts have been made to study the distribution of the RKN genus (Mandefro and Mekete 2002). However, no exhaustive work representing all production areas and available varieties of various crops is available. For instance, the occurrence, biodiversity and. distribution of nematodes in major tomato growing areas around Jimma, in particular to Dedo and Kersa districts were not conducted. Moreover, information on nematode management using host resistance and coffee husk amendments is unknown.

Since few years, chemical treatments have been restricted which led to reduced management options or application of more expensive control measures (Wesemael *et al*., 2011). Therefore, host plant resistance and coffee husk applications are important management options of nematodes in the light of increased awareness of environmental and human health hazards and economically feasible ways of controlling RKNs. Among these management options, the use of coffee husk applications are getting attention for generally positive agronomical effects and encourage soil biological activity (Nagaraju *et al*., 2010), they are also cheaper than synthetic nematicides and their application can be compatible with existing practices of resource poor farmers and can be easily adopted by most farmers in a safe manner. Beside this decomposed coffee husk released chlorogenic acid, tannin and phenolic compounds during decomposition process and these compounds are toxic to nematodes (Cruz, 2014; Bondesson, 2015). Therefore, this study was designed with the following objectives.

General objective

- To assess the diversity of PPNs associated with tomato production system in two districts and evaluate tomato varieties for their reaction to *M. arenaria* and test coffee husk application as an option towards management of *M. arenaria* on tomato.

Specific objectives

- To determine the distribution and prevalence of PPNs in tomato field in the study area.
- To identify and characterize the *Meloidogyne* species and other nematode genera.
- To evaluate the reaction of selected tomato varieties to *M. arenaria.*
- To evaluate the effect of coffee husk application on *M. arenaria* population.

2. LITERATURE REVIEW

2.1. Importance of tomato in Ethiopia

Tomato is one of the most popular and widely grown vegetables around the world. It is cultivated in tropical, sub-tropical and temperate climates (Perry *et al.*, 2009). It ranks 1st with respect to world vegetable production and accounts for 14 % (over 100 Mt year-1) $1.6 billion market (Bauchet and Causse, 2010). Tomato has enormous economic value. It has a part in purification of blood and curing of digestive ailments (Kaushik *et al*., 2011) and consuming of tomatoes reduce the risk of some conditions such as cancer, osteoporosis, neurodegenerative diseases and cardiovascular problem (Bhowmik *et al*., 2012). It has detoxification effect in the body due to the presence of sulfur and chlorine (Capel *et al*., 2017).

Ethiopia's wide range of agro-climatic conditions and soil types makes it suitable for the production of both warm and cool season vegetables (EHDA, 2012). Vegetable crops are suitable for production under intensive systems, where some farmers produce two to three times within a calendar year in Ethiopia (Emana and Gebremedhin, 2007). Tomato is a widely grown vegetable crop in Ethiopia. It is consumed in every household in different modes, mainly in Walo, Hararge, Shawa, Jimma and Wellega is an important co-staple food (Gemechis *et al*., 2012). In Ethiopia, the vegetable subsector has a vital role in human nutrition and health, farm income generation, and foreign currency earnings through export and foreign direct investment (Ayana *et al*., 2014). Processed products such as tomato paste and tomato juice are produced for export to Somalia, Djibouti and Saudi Arabia, making a significant contribution to the national economy (Baredo, 2012). Currently tomato is planted in 5,235.19 ha of land and produced 27,774.54 ton; with the productivity of about 5.305 t ha^{-1} (CSA, 2018). The highest production of tomato in Ethiopia was observed in 2016. The production was decreased unexpectedly in 2017 and 2018 (Fig. 1).

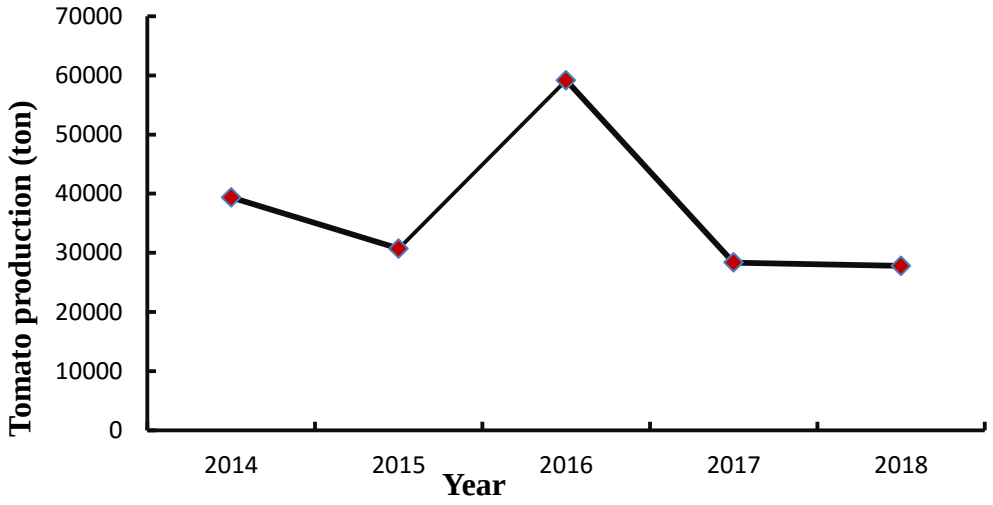

Figure 1. Tomato production trend in Ethiopia from 2014-2018 (CSA, 2014-2018).

2.2. Global constraints of tomato production

Tomato is amongst the commodities in horticultural expansion and development in the world. Tomato industry is one of the most advanced, globalized and innovative industries(Ayandiji and Adeniyi, 2011). The nutritive and economic value of tomato put it on world agenda in international horticultural forums (Ayandiji and Adeniyi, 2011). Tomato plays an important role in meeting domestic and nutritional food requirements, creation of employment, generation of income and foreign exchange earnings. The tomato production in world increased from year to year especially from 2008 to 2017 (Fig. 2). However, tomato industry has faced a number of constraints in several producing areas. It is perishable and easily affected by both biotic and abiotic factors such as pests and diseases and drought, markets, input supply and soil nutrients respectively (Ambecha, *et al*., 2007; Anang *et al*., 2013). Study conducted by Ravi *et al*. (2018) in India has showed high cost of quality seed, lack of labour, costly irrigation, costly equipments, lack of capital, lack of knowledge about insect, pest and diseases, lack of knowledge about seed treatment and high price of labour to the production constraints.

Cutworm (*Agrotis* spp.), whiteflies (*Aleurodicus dispersus* Russell and *Bemisia tabaci* Gennadius)**,** and root rot disease caused by *Phytophthora nicotianae* are the main problems

which reduce tomato production by attacking tomato seedling and farmers cannot disinfect the soil and use poor agronomic practices for the production of tomatoes in Eritrea (Asgedom *et al*., 2011). Pest pressure is predominant and the responsible agents in tomato production in Burkina Faso and other countries like Benin are mainly: whiteflies (*B. tabaci* Gennadius), caterpillars (*Helicoverpa armigera* Hübner) and tomato leafminer (*Tuta absoluta* Meyrick) (Chougourou *et al*., 2012; Ouattara *et al*., 2017; Son *et al*., 2017). About 85% of tomatoes producing smallholder farmers in South Africa are also challenged by disease and pest (Maliwichi *et al*., 2014). The production of tomato in Kenya is threatened by *Fusarium oxysporum* f.sp. lycopersici, RKN (*Meloidogyne* spp.), Fusarium wilt-root knot nematode complex and tomato leaf miner (*Tuta absoluta*) among others. Yield losses due to Fusarium wilt - root knot nematode complex reaches 80-100% **(**Waceke *et al*., 2018).

PPNs are small roundworms that cause tremendous economic damage in agricultural crop production including tomato in the world. Currently, about 4100 species of PPNs have been described. Globally, their distribution varies greatly (Manjunatha *et al*., 2017). Among them, some are cosmopolitan, and some species restricted in particular geographical condition or some are highly host specific (Manjunatha *et al*., 2017). Damage caused by PPN have become of great in agricultural and economic importance resulting in an estimated annual loss of 14 % of world crop production (Nicol *et al*., 2011). *Meloidogyne* spp. and *Pratylenchus* spp. are the two most important groups (Jone *et al*., 2013) and can infect, feed on and reproduce on an astonishing range of crops. *Pratylenchus, Meloidogyne, Paratylenchus, Criconemoides, Heterodera*, *Helicotylenchus* and *Hoplolaimus* spp. were associated with vegetable crops in Vermont (Bao and Neher, 2011). *Meloidogyne, Scutellonema* and *Helicotylenchus* were economic important nematodes in central and northern Tanzania where, *Meloidogyne* was the most predominant nematode across all tomato production areas (Missanga and Rubanza, 2018). RKN is widespread nematodes and has been reported from all the countries (Rathou, 2006). It is a serious and economically the most important pathogen of cultivated crops around the world (Trifonova *et al*., 2009). The incidence of root knot disease on tomato in Aligarh (India) showed that the tomatoes in all localities were infected with RKN (Esfahani, 2009). Tariq-Khan *et al*. (2017) assessed the prevalence of RKN, it was found in 64 % of the fields in Muzaffarabad, 30 % of the fields in Hattian Bala and 24 % of the fields in Neelum in

Pakistani administration. Janati *et al*. (2018) observed the occurrence of RKNs in vegetable crop fields; it was prevalent in all surveyed provinces of Souss region (South of Morocco).

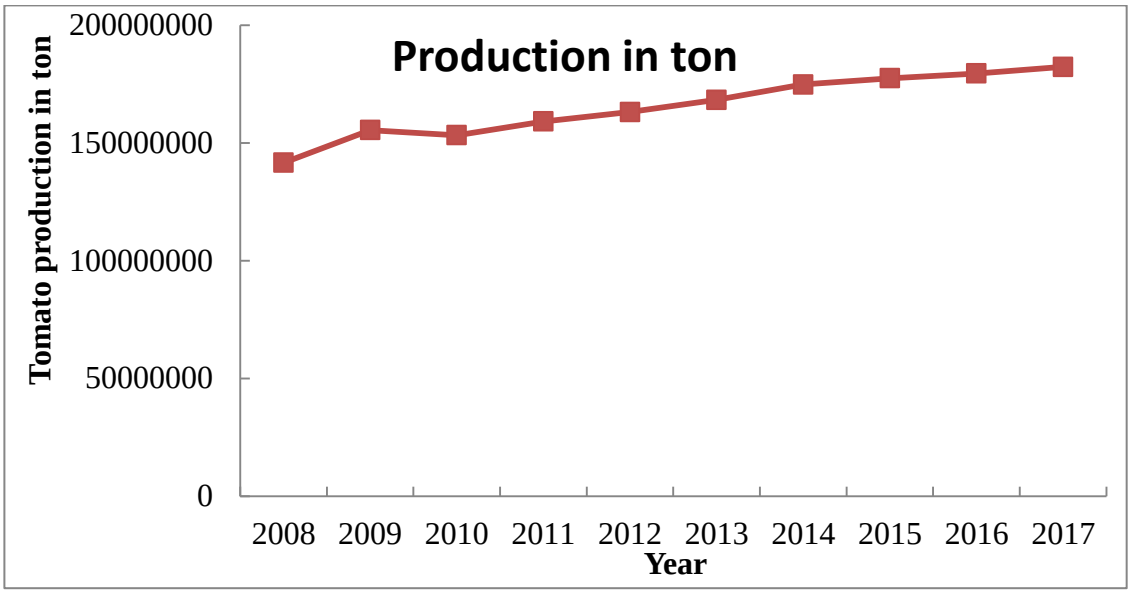

Figure 2. World tomato production trend from 2008–2017. Source: FAOSTAT. Accessed 11/8/2019. Available at http://www.factfish.com/statistic-country/world/tomatoes%2C%20production%20quantity.

2.3. Constraints of tomato production in Ethiopia

Tomato production in Ethiopia is low as compared to other producing countries. The productivity of tomato in Ethiopia is 8 MT ha^{-1} which is low compared to average yields of 51, 41, 36, 34 and 21 MT ha^{-1} in America, Europe, Asia, the entire world and Kenya, respectively (FAOSTAT, 2014). Several production constraints were identified for this low level of productivity. Inappropriate agronomic practices, high incidence of diseases and insect pests are among others the major constraints of tomato production in Ethiopia (Gemechis *et al*., 2012). Tomato production inefficiencies are manifested mainly by poor agronomical practice especially on nutrient management, irrigation, staking, pruning, weeding, pest and disease management and harvesting. In Ethiopia, loss of tomato has been caused by shortage of inputs, disease, pest, poor agronomic practices, drought, infrastructures and poor adoption of new technologies (Ambecha *et al*., 2007). Emana *et al*. (2017) conducted survey on postharvest losses of tomato at Bora and Dugda districts, their result revealed that more than

16 % of respondent told the loss of tomato was encountered by high incidence of diseases, insect pest and mechanical injuries.

Diseases are major constraints that limit production of tomato in Ethiopia. Early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), fruit spot (*Xanthomonas campestris* pv. vesicatoria), Septoria leaf spot (*Septoria lycopersici*), powdery mildew (*Leveillula taurica*), bacterial wilt (*Ralstonia solanacearum* or *Clavibacter michiganense* subsp. michganense), tomato leaf curl (Tobacco virus 16 or Nicotiana virus 10) and plant-parasitic nematodes (genera: *Pratylenchus*, *Meloidogyne*, *Helicotylenchus* and *Longidorus*) are the major and economically important tomato diseases in Ethiopia (Tesfaye and Habtu, 1986; Sakhuja *et al*., 2004; Seid *et al*., 2015). Late blight is a very severe disease in most tomato growing regions, including the Gamo Gofa Zone (Gudero *et al*., 2017).

PPNs have caused the major burdens to tomato production in Ethiopia. Among them *Helicotylenchus* spp., *Heterodera* spp., *M. incognita, M. ethiopica*, *Pratylenchus* spp. and *Tylenchus* spp. were identified by O'Bannon in 1975 from vegetable crops as cited by Abebe *et al*. (2015). *Meloidogyne* spp. were found to be the most dominant and widely distributed nematodes on tomato, pepper, onion, snap bean, cabbage, beetroot, carrot and potato in Ethiopia. Mandefro and Mekete (2002) assessed the wide distribution of RKNs in the Western (Bako, Ambo and Guder), Southern (Butajira and Alaba) and Central (Koka, Meki, Ziway, Melgaewondo, Melkassa, Upper Awash, Melkasedi and Melkawerer) parts of Ethiopia and they found on tomato, pepper, onion, snap bean, cabbage, beetroot, carrot and potato. Wondirad and Mekete (2002) have also reported that RKN is a serious pest of tomato in central and eastern parts of Ethiopia. The most common species were *M. incognita* followed by *M. ethiopica* and *M. javanica* (Mandefro and Mekete, 2002). It is a dominant PPNs group that threat tomato production in Rift Valley, Upper Awash and East Hararghie areas of Ethiopia (Seid *et al*., 2017, 2019).

2.4. Identification of *Meloidogyne* species

The accurate identification of *Meloidogyne* species is essential for implementing management strategies (Coyne *et al*., 2009). Methods based on the morphology of adults, isozymes phenotypes and DNA analysis can be used for the diagnosis of RKN (Cunha *et al*., 2018).

Traditionally, RKN species are identified by the analysis of the perineal patterns and esterase phenotypes. Perineal pattern remains as one of valuable character for the identification of RKN species. Identification of *Meloidogyne* spp. using perineal pattern morphology was reported by several authors (Eisenback *et al*., 1981; Ferris, 1999; Carneiro *et al*., 2016). For instance, Hunt and Handoo (2009) used perineal pattern morphology of adult female for identification of *Meloidogyne* spp. they identified seven economical important species of RKNs (Fig. 3). Aydinli and Mennan (2016) also identified *Meloidogyne arenaria* (Fig. 4), *M. incognita* and *M. javanica* using perineal pattern characteristic. Perineal pattern studied by Hasan and Abood (2018) revealed that two species of RKNs, *M. javanica* and *M. incognita*, were identified with 73.33% and 20% respectively.

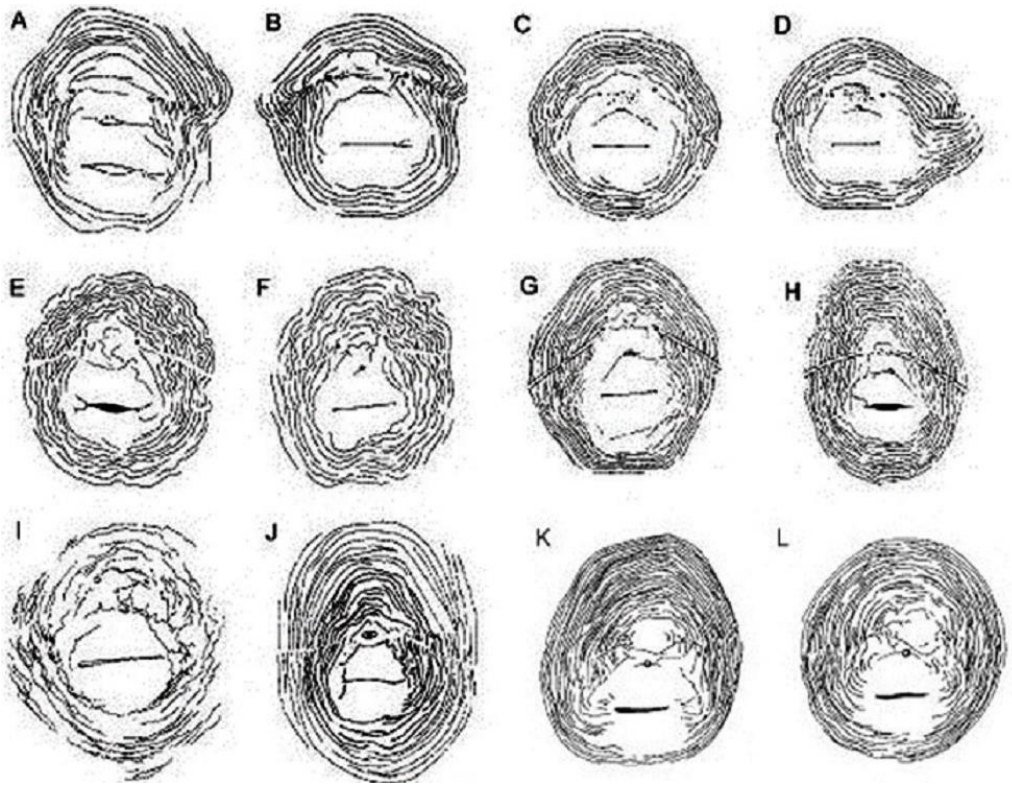

Figure 3. Comparison of perineal patterns for major RKN species. A, B: *M. arenaria*; C, D: *M. hapla*; E, F: *M. incognita*; G, H: *M. javanica*; I: *M. acronea*; J: *M. chitwoodi*; K, L: *M. enterolobii* (Hunt and Handoo, 2009).

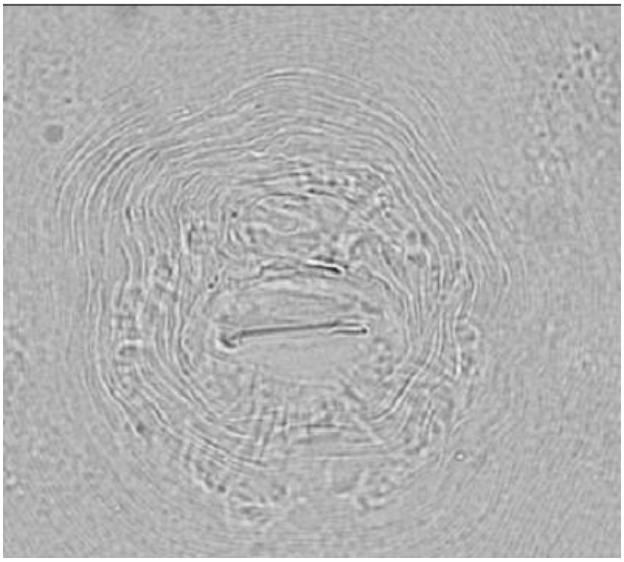

Figure 4. Perineal pattern of *M. arenaria* from the Middle Black Sea Region of Turkey (Source: Aydinli and Mennan, 2016).

The traditional method of RKN diagnosis was creating confusion due to overlapping of certain character among relative species. Various molecular approaches have been designed for accurate identification of *Meloidogyne* species. This is primarily because DNA-based methods are rapid and reliable compared to morphological or biochemical methods (Powers *et al*., 2005). It relies on the occurrence of polymorphisms in DNA sequences among groups of nematodes, especially in nuclear ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA). Diagrams of rDNA and mtDNA of some RKN species can be found in García and Sánchez-Puerta (2015). For identification and phylogenetic analysis of PPNs, the genes 18S, 28S, 5.8S and the spacer regions (internal transcribed spacer - ITS, external transcribed spacer – ETS and intergenic spacer- IGS) have been the most studied rDNA regions, while the gene cytochrome c oxidase subunits I (COI, CO1 or COX 1) and II (COII, COII or COX 2) have been the main targets of mtDNA (Roberts *et al*., 2016). Molecular method of *Meloidogyne* spp. identification is the popular one. Aydinli and Mennan (2016) confirmed morphologically identified species including *M. arenaria, M. incognita, M. javanica* and *M. ethiopica* by DNA analysis.

2.5. Taxonomy of *Meloidogyne* species

The Root knot nematodes, *Meloidogyne* spp. are among the nematodes in the family Heteroderidae and order Tylenchida. This genus comprises more than 100 species, with some species having several races (Hallmann and Meressa, 2018). It includes some of the most widespread and economically damaging nematodes, like *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, *M. chitwoodi* and *M. enterolobii*. RKNs occur throughout the world with some species such as *M. incognita*, *M. javanica*, *M. arenaria* being primarily distributed in tropical and sub-tropical climates while others like *M. hapla*, *M. chitwoodi* and *M. fallax* are well adapted to temperate or cool climates (Trudgill and Blok, 2001).

2.6. Life cycle of *Meloidogyne* species

The life cycle of most *Meloidogyne* spp. can take three to six weeks depending on environmental conditions such as temperature and moisture (Castagnone-Sereno *et al*., 2013). The infective second stage juveniles move in the soil and penetrate the root tips of the host plant using their stylet and feed cytosolic nutrients from roots (Fosu-Nyarko and Jone, 2016). They migrate intercellularly inside the root and initiate feeding sites (Karssen, 2002). The nematodes release an enzyme to form multinucleated feeding cells. These cells are served as supplier of nutrients to growing nematode (Gheysen *et al*., 2006). The juveniles feed on the host and become adult after three molts. In most case the male RKN develop and migrate out of the root when faced with adverse condition like inadequate food supply while females remain with their head in the root tissue and deposit up to 1000 eggs into a gelatinous matrix, which is protruding to the posterior end on the root surface (Abdou, 2014). The gelatinous matrix protects eggs from adverse environmental condition and from other microbial attack. Embryogenesis takes place inside the egg and after first molt; second stage juveniles begin to hatch (Abad *et al*., 2008). Infective J2 emerge from the eggs into the soil. Attracted by root exudates, they move towards neighboring roots, penetrate the root tip and migrate inside the vascular cylinder until they induce the differentiation of root cells into giant feeding cells (Fig. 5).

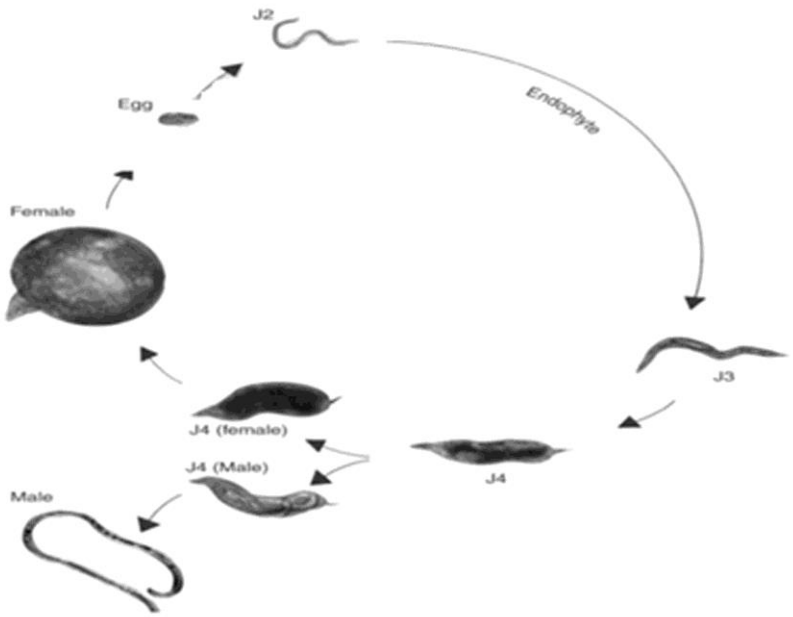

Figure 5. Life cycle of root-knot nematodes (modified from Abad *et al*., 2008).

2.7. Symptoms of damage

Symptoms caused by RKNs are often confused with nutrient deficiency including leaf yellowing, defoliation, stunted growth and wilting, which collectively reduce plant vigor and cause yield losses in mass or quality. Severe infections of the host plant results in chlorosis, yellowing and wilting of leaves. There may be premature dropping of fruits and flowers, and malformed fruits and the host plant may also show excessive wilting in early stage during the periods of mild temperature and moisture stress (Mulrooney, 2012).

RKN shows typical symptoms below ground in the root system, characterized by the formation of galls (Fig. 6). When plants are severely infected by *Meloidogyne* species, the normal root system is reduced to a limited number of severely galled roots with a completely disorganized vascular system. Rootlets are almost completely absent. The roots are seriously hampered in their main functions of uptake and transport of water and nutrients (Bala, 1984). Gall formation cause distortion and give unhealthy appearance to the root that limit the fruit production. They alter tissue at feeding site also disrupt the vascular tissue hampering the upward transport of water and dissolved nutrients by the xylem and translocation of photosynthesis to the other region of the plant by phloem (Hajra *et al*., 2009). Nutrient and

water uptake are substantially reduced due to damaged root system resulting in weak and low yielding of plants (Abad *et al*., 2003).

Figure 6. Underground symptom caused by *M. arenaria* on tomato

2.8. Survival and means of dissemination of Root knot nematodes

RKNs survive in soils as eggs and juveniles. The duration of survival of *Meloidogyne* spp. in the soil depends on the species, soil aeration and other factors (Taylor and Sasser, 1978). The effect of temperature on the survival of quarantine species of RKNs was examined by Baiye and Wesemel (2016). At lower temperatures egg hatching was delayed, this might be interpreted as eggs being in diapause in response to adverse condition (Khan *et al*., 2014) or eggs might not be viable as a result of extreme temperature above their normal tolerance level. Reproduction and survival of RKN is affected by temperature (Karssen *et al*., 2006). Optimum temperature requirement for the reproduction and survival of three species *M. arenaria, M. javanica and M. incognita* are in the range of 25°C to 30°C (Taylor and Sasser, 1978) whereas for *M. chitwoodi, M. hapla, M. naasi* and other temperate *Meloidogyne* species hatching can occur at temperatures below 10°C (Moens *et al*., 2009).

Dispersal of the PPN is largely restricted to the movement of soil (McNeill *et al*., 2006), infected plants and planting materials. Dissemination of root knot nematode among fields and between production area is through irrigation water, vegetative plant parts and soils infested with egg and juveniles adhering to farm implements, animals and man (Mai and Abawi, 1987).

2.9. Host ranges of Root knot nematodes

RKNs cause important crop losses in temperate, subtropical and tropical climates (Perry *et al.*, 2009). They are polyphagous pests causing severe damages on a wide range of crop plants and is particularly damaging to vegetable crops including tomato in tropical and subtropical agriculture (Ngele and Kalu, 2015). Over 100 *Meloidogyne* spp. attacking more than 3000 species of plants has been described (Abad *et al*., 2003; Adams *et al*., 2009). Many crops grown such as vegetables particularly tomato, okra, cucumber, carrot, lettuce, potato and pepper are susceptible to RKN. According to Tadele and Mengistu (2000), several vegetables (particularly tomato) damaged by *M. incognita* has been observed in the eastern part of Ethiopia. *Meloidogyne* spp. was predominant nematodes that infest tomato in Rift Valley, Upper Awash and East Hararghe areas of Ethiopia (Seid *et al*., 2017; 2019).

2.10. Economic Importance of *Meloidogyne* species

Tomato suffers huge qualitative and quantitative losses due to biological stresses present in the ecosystem. Among the various pests and diseases affecting tomato, PPNs pose a major threat (Nicol *et al*, 2011). *Meloidogyne* spp. causes an estimated annual loss of $157 billion globally (Abad *et al*., 2008). However, in most cases, the impact of *Meloidogyne* spp. is grossly underestimated. This is more in Africa than anywhere else in the world. RKN is one of the most damaging groups of PPNs and these nematodes are pests of almost all major crops (Gill and Mcsorley 2011). In addition, Karajeh *et al*. (2008) stated that about 5 % of the world crop production is destroyed by *Meloidogyne* species annually. More than 3,000 plant species have been designated as hosts to RKN, and most cultivated crops are attacked by at least one RKN species (Abad *et al*., 2003). The RKNs appears as stunted growth coupled with severe

deficiency symptoms of some nutritional elements, substantially reduced nutrient and water uptake, yield and product quality (Strajnar *et al*. 2011).

Based on the level of nematode populations, *Meloidogyne* spp. can cause high levels of crop loss during growth, increase the cost of production through increased fertilizer application and control programmes, and also significantly reduce post-harvest yields. Surveys carried out by Tariq-Khan *et al*. (2017) revealed that it was found in 64 % of the fields in Muzaffarabad, 30 % of the fields in Hattian Bala and 24 % of the fields in Neelum.

2.10.1. Damage and yield losses of tomato due to *Meloidogyne* species

RKNs cause severe damage to the roots of tomato. Symptoms are more prevalent with tropical species compared to temperate ones. *Meloidogyne* species are ranked as the first among top ten nematodes (Jones *et al*., 2013). Tomato cultivars have different degree of susceptibility towards different *Meloidogyne* spp. Damage and yield loss studies conducted so far have shown a considerable difference in degree of susceptibility among tomato cultivars. Moreover, different populations of the same species of *Meloidogyne* even exhibit different degree of pathogenicity on specific tomato cultivar Seid *et al*., 2015. Several studies reported the damage potential of different *Meloidogyne* spp. on various tomato cultivars under pot, micro plot and field experiment condition. In north eastern Spain, an initial population density in soil of 4750 juveniles 250 $cm^{-3}$of *M. javanica* caused a 61% yield reduction in tomato (Verdejo-Lucas *et al*., 1994). *Meloidogyne* spp cause more than 50% losses to tomatoes (Natarajan *et al*., 2006). Nevertheless, much more percentage has been documented in different regions, depending on population level, species, frequency of infestation and crop species. *Meloidogyne* spp. caused up to 80% yield losses in processing tomato growing areas in western Anatolia (Kaskavalci, 2007).

2.11. Management of *Meloidogyne* species

With different methods of management, nematode population is kept minimum to reduce economic losses and considers the whole system of care and treatment of crop pests while control refers to specific acts designed to reduce the number of nematode (Hooper and Evans,

1993). Different management option that are commonly used for PPNs are applicable for *Meloidogyne* spp. on tomato as described below.

2.11.1. Cultural management

In cultural management practices crop rotation, fallowing, soil solarization, organic amendment, destruction of residual of crop roots and cover crops become favorable practice to the farmers. According to Mweke *et al*. (2008), intercropping crops that are poor hosts or antagonistic to nematodes and using trap crops in a rotation program reduce the initial nematode population by allowing the subsequent crop to establish before the nematode population reaches to damaging levels. Cover crops can be grown outside the normal agricultural growing season. With the presence of cover crops, nematodes cannot migrate to another field if a cover crop is not a host to the nematodes because nematodes can move only a very short distance on their own (Gill and Mcsorley, 2011). A few examples of cover crops are cowpea (*Vignaunguic ulata*), sorghum-sudan grass (*Sorghum bicolor*× S. sudanense), sunn hemp *(Crotalaria juncea*) and marigolds (*Tagetes* spp.) (Gill and Mcsorley, 2011).

Soil solarization reduces RKN damage in which the soil is covered with plastic film for at least 2 weeks and this killed the egg of the nematode, thus reducing the population of RKN (Tisserat, 2006). According to Noling (2009), the most successful use of soil solarization takes place in heavier (loamy to clay soils) rather than sandy soils. Soils with good water holding capacity enhance the heat transfer to deeper soil horizons. Therefore, soil depth affected the number of RKN killed in the roots.

Several study showed that organic amendments of soil are an alternative method of nematode control (Renčo *et al*., 2007). Organic amendments and green manure are potential alternatives to the harmful chemical control means currently used against PPN and have been found to reduce plant feeding nematodes and increase tomato yields (Hassan *et al*., 2010; Mulrooy, 2012). Shiferaw *et al*. (2014) determine the influence of poultry litter and rapeseed cake application against *M. incognita* infestation in tomato. Their result of the study showed that applications of poultry at 5 to 15 ton/ha in combination with rapeseed cake at 200 kg/ha remarkably suppressed RKN infestation. Organic amendments are used to manage the effect

of PPNs on crop production. Organic amendment is not only increase the fertility status of the soil but also increase the microbial diversity and reduces the density of RKNs (Ahmed and Siddiqui, 2009). Application of organic manure influences soil nematode community structure, diversity and even the activity of nematodes (Liang *et al*., 2009). Application of organic materials to soil can cause a change in soil microflora and microfauna including nematodes (Renčo, 2013). It increases the abundance of fungivores, bacterivores and predator (Tabarant *et al*., 2011) but reduce the abundance of PPNs (Korthals *et al*., 2014). For instance, very high application rates (50-100%) of composts in pots reduced root galling and numbers of J2 in soil and roots (Nico *et al*., 2004). Many other reports proved that compost application improved growth of infected plants and reduced nematode population (Cayuela *et al*., 2008). Application of compost reduced the root galling and the final population on sun flower (Moselhy, 2009). Roldi *et al.* (2013) have found that the egg number of *M. incognita* in tomato plants was reduced from 4517.8 to 353.6 and from 5857.8 to 251.4 when bokashi was applied to soil in the concentration of 20 g per 2 l pot. Bokashi and crambe meal amendment reduced the number of eggs/g of root and promoted plant growth (Dias-Arieira *et al*., 2015).

2.11.2. Host plant resistance

Resistant varieties are considered to be efficient methods for root knot nematode control (Ferraz and Mendes, 1992). The basis of using resistant cultivars to control *Meloidogyne* spp. relies on knowing exactly which species is being targeted. Resistance of various crops to *Meloidogyne* spp. infection is important because a resistant crop can allow little or no *Meloidogyne* spp. reproduction, thus providing a better way of controlling nematodes in the field (Norshie *et al*., 2011).

Resistance to *M. incognita* was first noticed in wild relative of cultivated tomato of USDA accession 128657 of *Lycopersicon peruvianum*, and consequently it was found to be introduced by *Mi* gene to domesticated tomato, *L. esculentum* (Liharska, 1998). The resistance in tomato cultivars against *Meloidogyne incognita*, *M. javanica* and *M. arenaria* was controlled by *Mi* gene, which is located near the centromere of chromosome 6. However, the *Mi* resistance gene in tomato conferred resistant is associated with a hypersensitive response in RKN infected tissues (Liharska, 1998). The Mi gene mediates a hypersensitivity

response that stops giant cell formation which is a precondition for RKN development (Starr *et al*., 2013).

In tomato, several independent single dominant R genes have been identified and mapped in different chromosomes, which are designated as Mi-HT, Mi-1, Mi-2, Mi-3, Mi-4, Mi-5, Mi-6, Mi-7, Mi-8 and Mi-9. Those resistance genes are commercially used in tomato breeding programs in controlling three main RKN species, *M. incognita*, *M. javanica* and *M. arenaria* (Rashid *et al*., 2017). Currently, all available tomato cultivars carry the single dominant R gene Mi (Williamson and Roberts 2009). The effectiveness of the Mi gene varies with the RKN species and population, tomato cultivar, and environmental conditions, like soil temperature (Seid *et al*., 2015). Jaiteh *et al*. (2012) evaluate 33 tomato genotypes for resistance to RKN. Their result revealed that out of 33 genotypes screened, Tomato Mongal T-11 and Tomato Beef Master were found to be highly resistant to *Meloidogyne* spp. compared to other genotypes. Seid *et al.* (2017) tested 23 tomato cultivars and 10 breeding lines to aggressive *M. incognita* and *M. javanica* populations in which the materials showed different response to nematode infection. In order to achieve promising results with the use of resistant cultivars, there is need to constantly carry out accurate species identification and surveillance. It is also important to educate growers on the importance of containing resistance-breaking *Meloidogyne spp*. such as *M. enterolobii* to areas where they have been detected (Onkendi *et al*., 2014).

2.11.3. Biological control

One beneficial alternative to nematicides that is gaining popularity in nematode control is the biological control, predominantly utilizing the microorganism groups like the fungi and bacteria already present in the soil biota (Crawford and Clardy, 2011).

Bacterial strains such as *Pseudomonas fluorescens* and *Bacillus subtilis* significantly affected the reproductive factor of *M. javanica* and reduced the reproductive factor from 112.15 to 24.94 and 24.96 on tomato respectively (Sohrabi *et al*., 2018). Species of Bacillus Gartner interrupts the nematode life cycle by producing toxic metabolites which restrict their mobility and hinder the hatching and juvenile penetration into plant roots (Kavitha *et al*., 2007).

Pochonia chlamydosporia parasitizes females and eggs of RKNs, the latter being the most vulnerable stage of this pathogen life cycle. *P. chlamydosporia* caused a decline in the number of *M. javanica* galls and eggs, in tomato plants by 40% and 72.83%, respectively (Dallemole-Giaretta *et al*., 2014). Radwan *et al*. (2012) reported that the *Trichoderma* isolates showed positive effects by minimizing the degree of damage caused by *M. arenaria* and *M. javanica*, when the fungus was applied at an early point in time to the soil.

The obligate endoparasitic bacteria *Pasteuria penetrans* effectively parasitized *M .incognita* in rotations that included tomato, eggplant and common beans or cabbage (Amer-Zareen *et al*., 2004). In addition, some studies have also shown another biological strategy where endophytes such as *Fusarium oxysporum* (FO162) can induce systemic resistance against *Meloidogyne* spp. in some crops such as tomato (Walters, 2009). Colonization of roots by *F. oxysporum* (FO162) leads to the accumulation of root exudates in tomato roots which have a repelling effect on *M. incognita* (Mohamed, 2010). Belair *et al*. (2011) investigated in a glasshouse bioassay that a combined soil treatment with *Streptomyces* and chitin reduced *M. hapla* populations and galls on tomato. Biocontrol agents alone rarely provide adequate management and should be integrated with other management methods such as crop rotation trap crops resistant cultivar and antagonistic plants, either to promote the establishment of biocontrol agents or to reduce the nematode population in the soil (Viaene *et al*., 2013).

2.11.4. Botanical control

The plant extract from roots, leaves, seeds and whole parts are an alternative method of root knot nematode control. For instance, Korayem and Hasabo (1994) reported that the exposure of juveniles of root knot nematode to standard solutions of bulb extract of *Allium sativum* killed nematode within 24 hour after exposure. The root extracts of *chromolaena odorata* and *Azadirachta indica* exihibited 100% inhibition of egg hatch and juvenile mortality of root knot nematodes (Adegbite and Adesiyan, 2005). Hasabo and Noweer (2005) also indicated that aqueous extracts of basil leaves (*Ocimum basilium),* marigold leaves (*Tagetes* spp.), neem seed (*Azadirachta indica*) and china berry leaves (*Melia azedarach*) all affected the survival of root knot nematode juveniles in the soil.

2.11.5. Chemical control

Chemical methods of control involve the application of different inorganic formulations to kill or interfere with the reproduction of *Meloidogyne* spp. in infested soils. Nematicides containing active ingredients of methyl bromide, Aldicarb (Temik) and other harmful compounds have been banned in various parts of the world. Other nematicides which are known to control various *Meloidogyne spp*. include fenamiphos, oxamyl, 1, 3 dichloropropene (1, 3-D), dazomet and metam-sodium (Onkendia *et al*., 2014). Nematicides reduce high populations of various *Meloidogyne* spp. in the soil, but once symptoms have developed, they are incapable of completely eliminating those *Meloidogyne* species already in plant tissue (Sirias, 2011). Study conduct by Soltani *et al*. (2013) revealed that Rugby toxin with concentration of 8 ppm had the best effect in controlling the root-knot nematode of *M. javanica* (with reduction of 79/24) followed by Temik, Oxamyl, and Enzone. All tested toxins reduced the population of nematode from 50% (Enzone toxin) to 80% (Rugby).

3. MATERIALS and METHODS

3.1. Description of study area

3.1.1. Survey area

The survey was conducted at two districts: Dedo and Karsa district located in Jimma Zone of Oromia regional state (Fig. 7). Karsa is located at about 318 km from Addis Abeba and 28 km East from Jimma town (7^o 42'- 7^o 43' N latitude and 36^0 05'-37^o42' E longitude) at an altitude of 1740 masl and four kebeles were selected for sampling such as Kitmile, Babo, Grima and Bulbula. The average annual maximum and minimum air temperatures are 28.8 ^{0}C and 11.8 ^{0}C, respectively. Dedo is located at 7^0 13'-7^0 39' N latitude and 36^0 43'– 37^0 12' E longitude and is about 366 km far from Addis Abeba and 22 km south of Jimma town from this district Offole, Demasertha, Korjo and kollobo kebeles were selected for sampling purposefully.

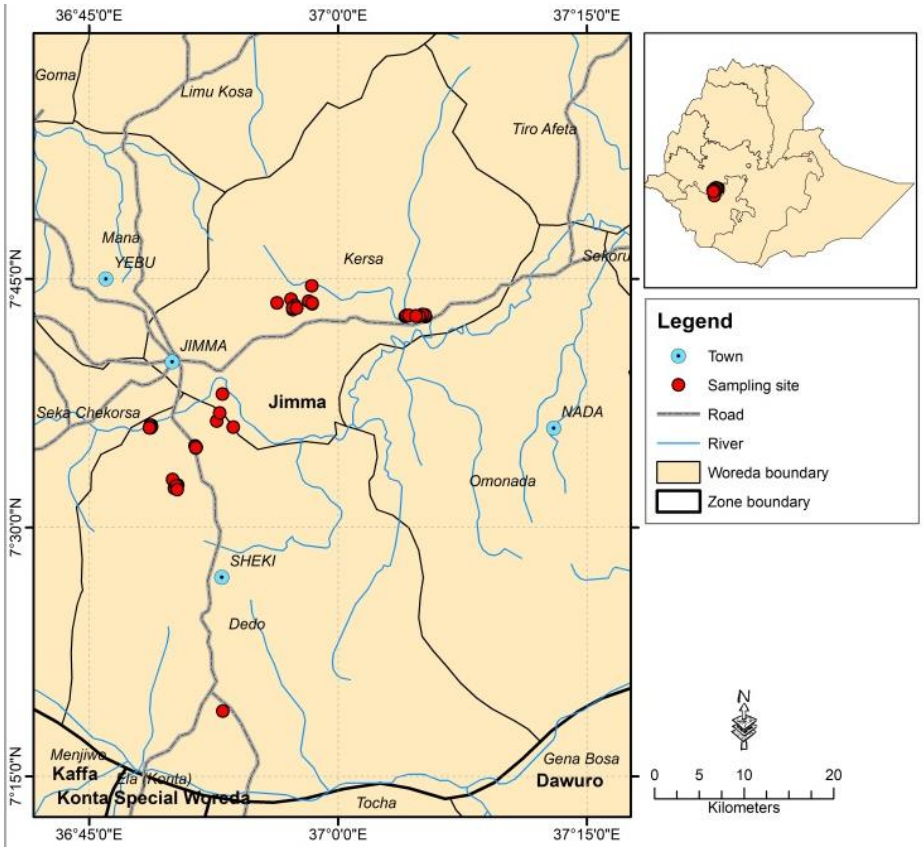

Figure 7. Geographical location of the sampling sites

3.1.2. Soil and root sampling

A survey was conducted to assess the prevalence of PPNs associated with tomato in selected districts. Roots of tomato plant and soil samples were collected from eight tomato producing kebeles which were selected based on purposeful sampling technique due to accessibility to irrigation source and potential tomato producing area. Moreover, five fields per kebele were selected randomly for sampling. Soil samples of about 300 g consisting of 17 soil cores were taken from the rhizosphere of 17 tomato plants from the top 5-25 cm depth along three W shaped sample walks or cross section method (Wiesel *et al*., 2015). The cores were combined to form a single composite sample. Hence, a total of 40 composite soil samples were collected from the study area.

About 50 g of adventitious roots were collected from each selected plants based on method described by Talwana *et al*. (2008). The samples were sealed in plastic bags and packed in wet fiber sac and transported to the PDDL of JUCAVM for extraction and further characterization of the nematodes. For each sampling field, cropping history, soil pH and altitude were recorded using GPS.

3.1.3. Extraction of Phytonematodes from the fields

The soil sample collected from tomato field was thoroughly mixed and aliquot of 100 ml soil from the composite soil sample was used for nematode extraction, using a modified Baermann tray method (Hooper, 1986). PPN in aliquots of 1ml of the extracted nematode suspension was counted in a counting slide under a compound microscope (A.KRUSS Optronic GmbH, Hamburg) and the population density expressed as the number of nematodes in 100 ml of soil.

Cysts were extracted from 100 g dried soil using the sieving and flotation method (Shepherd, 1986) due to the fact that those cysts contain air bubbles and therefore float on water. The soil samples were completely dried at room temperature. Counting and separation of cysts from soil debris and other organic materials retained on the filter paper were carried out using a stereomicroscope.

3.1.4. Identification of Phytonematodes

Nematodes were identified morphologically to genus level using a compound microscope at 10x to 100x magnification as described by Hooper (1990). Also, identification guide of nematodes and the interactive keys for nematode genus identification of the University of Davis http://plpnemweb.ucdavis.edu/nemaplex/_vti_bin/shtml.dll/index.htm and the University of Nebraska http://nematode.unl.edu/key/nemakey.htm were used and nematodes were identified based on their specific key features as described in table 1.

Table 1. Diagnostic key features for Plant Parasitic nematodes

Key Feature	**Description**
Cephalic setae	Indistinct or absent
Stylet	Present
Stylet knob	Knobbed or Flanged
Valvate median esophageal bulb	Present or absent
Stylet length	Less than 50 micron or greater than 80 micron
Vulva position	Mid body or at lower third bod
Labium	Offset or flattened amalgamated
Cuticle	Annulated or not
Cuticular sheath	Present or absent
Female body	White without eggs or brown with eggs

Source: http://nematode.unl.edu/key/nemakey.htm

3.2. *Meloidogyne* isolation and pure culture establishment

Soil sample was collected from a *Meloidogyne* infested plot from JUCAVM campus. Ten tomato seedlings of three weeks old were planted on the 2 l pot filled with the infested soil and allowed to grow. After two months, thirty single eggmasses were collected and allowed to hatch individually. Hatched J2 of each eggmass was individually inoculated to tomato seedling planted in 200 ml transparent plastic pot filled with equal volume of sterilized sand and field soil. Two months later, tomatoes that developed typical root galls were transferred into 1 l pot filled with sterilized sand and field soil (1:1 v/v). The pure culture of *Meloidogyne* spp were raised and maintained on Moneymaker variety which is susceptible to RKNs (Seid

et al., 2017).

3.3. Identification of *Meloidogyne* spp.

3.3.1. Morphological identification

The species of root knot nematode was identified based on perennial pattern. Here, 15- 20 matured females were teased out from large galls on the roots of tomato plant after removing eggmass and females were transferred to 45% lactic acid on a Petri dish for 10-15 min using fine-pointed forceps. On the stage of a stereomicroscope, speared at the neck end with a very sharp fine needle. The head and neck regions of the nematode were excised until remaining the posterior end with a surgical blade and the inner tissue removed carefully by brushing with a flexible bristle. The cuticle was transferred to a drop of glycerol on a clean glass slide. Perineal patterns of JUCAVM isolates were mounted on permanent slides and examined under compound microscope to study their characteristics (Eisenback *et al*., 1981; Shurtleff and Averre, 2000).

3.3.2. Preparation of second stage Juveniles

Eggmasses were handpicked by forceps from pure culture maintained roots of tomato and placed on eppendorff tube allowed to hatch at room temperature. Three to five hatched juveniles from each tube was transferred to new eppendorff tube containing 1 ml of water. The juveniles were killed using hot water and two drop of 97 % ethanol was added to preserve the nematodes and labelled (Coyne *et al*., 2018). Then, the tube containing nematode suspension was sent to Belgium for molecular analysis.

3.3.3. Molecular analysis

3.3.3.1. DNA extraction

All molecular works were performed in the Research Unit Nematology Lab, Gent University. Total DNA was extracted from a single second stage juvenile (J2). Individual J2 was handpicked using needle and placed into 5µl double deionized water in 200 µl PCR tubes. The tubes were centrifuged for 5 s and left open at room temperature until the water gets fully

evaporated. Then, 10 µl worm lysis buffer (Waeyenberge *et al*., 2000) containing 2 µl 20 mg ml^{-1} Proteinase K was added to the nematode and thoroughly stirred with sterilized pipet tips and vortexed. The lysate was incubated for 3 hr. at 60 ^{o}C followed by 10 min incubation at 95 ^{o}C and centrifuged for 3 min and stored at -20 °C until needed for PCR.

3.3.3.2. PCR assay

The primer set used to amplify the NADH dehydrogenase subunit 5 gene of the mitochondria were malF (5'-GGATAGAGCCRACG TATCTG-3') and 1006R (5' GTTCGATTAGTCTTTC GCCCCT-3') as described by Holterman *et al*. (2008). The PCR mix (25 µl) contained 1 µl DNA template, 2.5 µ2 mM dNTPs, 2.5 µl 25 mM $MgCl_2$, 0.6 µl 10 µM of each primer, 5 µl 5× Go Taq® buffer and 0.5 u/ µl of Go Taq® DNA polymerase (Fisher Scientific Inc., Schwerte, Germany). The PCR reaction was set for heating at 95°C for 5 min followed by first 5 cycles of amplification at 94°C for 30s, 45°C for 30s, and 72°C for 1 min; and a second 35 cycles of amplification for 30s 94°C, 30s 54°C, 30s 72°C, with a final incubation for 5 min at 72°C.

All PCR reactions were run in Applied Biosystem® Thermal cycler (Applied Biosystems, Foster City, CA, USA). A 5 µl of the amplified products with 1 µl lading dye were separated on 1.0% agarose gels in 0.5× TBE buffer at 80 V 34 Am for 85 min, stained with gel red, and visualized at UV-light. 1 kb plus DNA marker was used.

3.3.3.3. Sequencing

The PCR product was sequenced directly (without cloning) in two directions. Prior to sequencing, PCR product was purified using the Wizard® SV Gel and PCR Clean-Up System according to the manufacturer's instruction for PCR-product purification. 5 µl of the PCR products and 5 µl of 10 pmole µl-1 of the respective forward primers were mixed. Sequencing was performed at the Macrogene sequencing facility service (Amsterdam, The Netherlands).

3.3.3.4. Phylogenetic analysis

The newly obtained new sequence together with other related published sequences from GenBank were used to reconstruct the phylogenetic trees. *Rotylenchus reniformis* was chosen as an outgroup taxon. Raw sequence obtained was first edited in Finch TV Version 1.4.0 software (2006) (Geospiza Inc.) to remove ambiguous nucleotide sequences before BLAST was performed for sequence similarity search in GenBank NCBI database (Altschul *et al*., 1990). Our sequences and those from GenBank were aligned using ClustalX Version 2.0 (Larkin *et al*., 2007) and trimmed to equal length in MEGA7 (Tamura *et al*., 2011). Phylogenetic analysis was carried out with Maximum likelihood using heuristics searches with Nearest-Neighbour Interchange (NNI) branch swapping filter. The support for each branch was estimated using a bootstrap method using heuristics search and 1000 replicates in MEGA7.

3.4. Greenhouse experiment

3.4.1. Preparation of inoculum

For inoculation, the eggmasses from heavily infected roots of tomato on which a pure culture of *Meloidogyne* species maintained were extracted following the method described by Hussey and Barker (1973). The roots were rinsed gently with tape water to remove adhering material and then chopped in to small piece as method described in 3.4.6. After every 24 h, fresh water added on hatched-out juveniles and stored in refrigerator at 5^{o}C until used for inoculation. For enumeration, the nematode suspension was thoroughly air bellowed to make homogenous distribution of nematodes before taking 1ml of suspension immediately into a counting dish. An average of three counts was taken to determine the density of nematodes in the suspension. The suspension was concentrated to required volume for inoculation.

3.4.2. Planting material and growing condition

Tomato varieties were obtained from the Melkassa agricultural research center. The growth media was prepared in 1:1 v/v pot filled with sand and field soil. The mix was dry sterilized in

oven at 111 ^{o}C for 30 minutes. Sterilised soil mix was used to fill each pot and each plant was grown in each of these 2 and 1 l capacity pots for the first and second experiment respectively. Tomato seeds were nursed in dry sterilized soil in a plastic tray. The seedlings were allowed to grown until the development of three to four leaves. Then two seedlings per pots were transplanted and regularly watered following the demand up to inoculation of nematodes. At the time of inoculation one seedling was removed. All agronomic practices (weeding, fertilization (0.6 g DAP per plant was applied), staking) were done uniformly as required until the termination of the experiment.

3.4.3. Experimental design

Two experiments were conducted in greenhouse at JUCAVM. Screening variety was carried out to test the reaction of fourteen tomato varieties (Table 3) against to *M. arenaria*. The experiment was arranged with RCBD which containing fourteen treatments. Each treatments were replicated three times.

Coffee husk amendment was carried out by 3x4 factorial arrangement fitted into RCBD with three block were used which comprising three tomato variety (Moneymaker, Miya and Melkashola) and four coffee husk level (0:4, 1:3, 1:1, and 3:1 v/v (CH: SM). Treatments were replicates three times. Where 0:4= only SM (non-amended), 1:3= growth media contained 25% coffee husk and 75% SM, 1:1= 50% coffee husk and 50% SM and 3:1= 75% coffee husk and 25% SM (Table 2).

Table 2. Treatment of combination of different proportion of coffee husk and varieties

Coffee husk proportion	**Varieties**		
	Moneymaker	**Miya**	**Melkashola**
0:4 (non-amended)	Moneymaker* 0:4	Miya* 0:4	Melkashola* 0:4
1:3 (CH: SM)	Moneymaker* 1:3	Miya* 1:3	Melkashola* 1:3
1:3 (CH: SM)	Moneymaker* 1:1	Miya* 1:1	Melkashola* 1:1
3:1 (CH: SM)	Moneymaker* 3:1	Miya* 3:1	Melkashola* 3:1

Table 3. Description of the tomato varieties used for the experiment

Varieties	Fruit shape	Maturity days	Purpose	Growth habit	Altitude	Unique characteristics
Moneymaker	Standard	80	Fresh	Indeterminate	700-2000	Early maturing and large fruit size
Roma VF	Roma	70-80	Fresh	Determinate	700-2000	
Fetan	Cylindrical	78-80	Fresh	Determinate	700-2000	Early maturing and concentrated fruit yield
Melkashola	Cylindrical	100-120	Processing	Determinate	700-2000	Globular fruit shape
Melkasalsa	Pear	100-110	Processing	Determinant	700-2000	Small fruit size, slightly cylindrical fruit shape
Metadel	Slightly flatten	75-80	Fresh	Semi-determinate	700-2000	Medium fruit size, slightly flatten fruit shape
Cochoro	Square	75-90	Processing	Semi-determinate	700-2000	Round fruit shape, green shoulder fruit color before mature
Bishola	Slightly Cylindrical	85-90	Fresh	Determinate	700-2000	Large fruit size, green shoulder fruit color before mature
Gelilal	Plum	60-80	Fresh	Determinate	700-2000	
Gelima	Square	75-80	Fresh	Determinate	700-2000	
Arp tomato d2	Cylindrical	75- 80	Fresh	Semi-determinate	700-2000	Large fruit size, green shoulder fruit color before mature
Chali	Round	110-120	Processing	Determinate	700-2000	Round fruit shape
Margilobe	Square	75-80	Fresh	Determinate	700-2000	
Miya	Plum	75-80	Fresh	Semi determinate	700-2000	High leaf coverage, hard skin and plum fruit shape

Source: Meseret *et al*. (2012)

3.4.4. Nematode inoculation

Tomato 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 which 1 J2 per g of dry soil was injected using pipette for the first experiment.

In coffee husk amended experiment, all seedlings in the experimental observations were injected with 2 J2 per g of soil media using pipette similarly as an experiment one. The holes were covered with the same growth media and gently watered after three day of inoculation in both experiments.

3.4.5. Nematode extraction from soil

Ten weeks after inoculation the greenhouse experiments were terminated and nematodes were extracted from triplicate 100 ml of soil per pot for both experiments. Soil was placed over a single layer tissue paper on plastic sieve of 250 µm pore mesh on an Oostenbrink dish. The setup was kept for 48 hours at room temperature without disturbance with a modified Baermann tray method (Hooper, 1986). Nematodes were collected from each dish on a 38 µm aperture stainless steel sieve into beaker. Suspension from each dish was collected and allowed to settle at room temperature. The volume of each suspension was standardized to 10 ml. Concentrated nematodes were stored in a refrigerator at 5^{o}C until nematode quantification. Each suspension was homogenized by blowing air through with a pipette. Aliquot of 1 ml of suspension was taken with a pipette into a counting slide and counting done with the aid of a compound microscope. The density of nematode was expressed as the number of nematodes per volume of soil per pot.

3.4.6. Nematode extraction from plant root

Nematodes were extracted from tomato root from both experiments as follows. Roots were washed gently free of adhering soil and chopped in to small pieces (1-2 cm long), placed on plastic bottle. 10% Sodium hypochlorite (NaOCl) solution was added to the bottle up to covering of root tissue and the constituents were agitated gently for 4 min (Hussey and Barker, 1973) to dislodge eggs from eggmass. Nematodes and eggs were collected on 38 µm-pore mesh sieves over 250 µm-pore mesh sieves and rinsed with tap water. Suspension was allowed to settle and then concentrated and stored at room temperature for three days for hasten hatching. J2 and eggs were counted using a counting slide with the aid of a compound microscope. Counting was done three times for each experimental observation.

3.5. Data collection

Data on soil pH, altitude, number of J2 and incidence of root knot were collected from surveyed areas. Root knot incidence was calculated using the following formula. Soil pH was determined using AD8000 pH meter (Alsókikötõ Sor 11, 6726 Szeged, Hungary). The

relationship between altitude with RKN density and soil pH with RKN density were determined using regression analysis.

RKI= $\frac{\text{total number of galled plant}}{\text{total number plant observed}}$* 100. Where, RKI= root knot incidence.

Shoot height, shoot dry and fresh weight, root length and fresh weight, number of eggmass and root gall per root system, gall index, final nematode population, and reproduction factor were collected from both experiments. Roots were stained using Phloxine B for 15- 20 min according to Holbrook *et al*. (1983) for nematode observation. All root systems were rated for galling using an index of 0 to 5, where 0= no galls,1= l to 2, 2= 3 to 10, 3= 11 to 30, 4= 31 to 100, and 5= >100 galls per root system (Taylor and Sasser, 1978). The varieties were rated as resistant, susceptible or tolerant as follows: (GI ≤ 2, RF ≤ 1) = resistant, (GI ≥ 2, RF > 1) = susceptible, (GI ≤ 2, RF > 1) = tolerant (Devran and Elekçioglu, 2004). Where GI= gall index and RF= reproduction factor.

3.6. Data analysis

The survey data was analyzed using SPSS 20. Data on population density (PD), Frequency of Occurrence (FO), and Prominence Value (PV) were calculated as described by De Waele *et al*. (1998).

- Frequency of occurrence (FO) = $\frac{n}{N} \times 100$

  Where, n = Number of positive samples and N = total number of samples.

- Population densities (PD) = $\frac{\text{number of nematodes}}{\text{total number of samples}}$
- Prominence value (PV) = PD* $\sqrt{(\frac{FO}{10})}$

Tomato growth parameter and nematode reproductive parameter data were subjected to ANOVA and means were separated using least significance difference tests at the P <0.05 to test significance level. Their relationship was determined by Pearson correlation analysis. All

statistical analyses were performed using SAS 9.3 version (SAS, 2013). For each treatment, the reproduction factor was calculated using the formula below. The number of nematodes per pot and per root system and the RF values were used in statistical analysis. Before statistical analysis, nematode counts were transformed using the log10 (x + 1) transformation to homogenize variance.

$RF = \frac{Pf}{Pi}$ where, RF= reproduction factor, Pf= total number of root knot nematodes extracted from the soil and entire root system and Pi= initial population of nematodes inoculated per pot (Ferris and Noling, 1987).

4. RESULTS AND DISCUSSION

4.1. Diversity of nematodes associated with tomato

4.1.1. Occurrence and distribution of nematodes associated with tomato in the field

This survey resulted in a total of nine genera of nematodes associated with tomato. These included *Meloidogyne, Pratylenchus, Helicotylenchus, Scutellonema, Rotylenchulus, Aphelenchus, Criconema, Paratylenchus* and cyst nematodes (*Globodera* spp) (Table 4 and Fig. 8). The most abundant PPN genera found were the root-knot nematodes followed by *Helicotylenchus* and *Pratylenchus*. The highest mean population density of RKN was encountered per 100 ml of soil from Babo kebele (230) followed by Dema sertha (116), while the lowest mean population was recorded at Kitmile and Grima (24). Maximum mean population of *Pratylenchus* was recovered at Babo (50) whereas the minimum was recorded at Dema Sertha kebele (6). The highest mean population of *Helicotylenchus* was noted at Kolobo (110) followed by Girma (82) whereas the lowest mean population was encountered at Bulbula kebele (4). The mean highest population of *Scutellonema* was found at Korijo (30) whereas the minimum was recovered from both Kitmile and Bulbula kebeles (4) (Table 4).

RKN was the most frequently encountered and widely distributed in all farmers' fields of the surveyed kebeles with FO of 100 %. *Pratylenchus, Helicotylenchus and Scutellonema* were frequently occurred at all kebele with FO of 100 %. Whereas, *Rotylenchulus*, cyst nematodes *Aphelenchus, Criconema*, and *Paratylenchus* less frequently occurred with FO of 62.5%, 50%, 37.5%, 25%, and 12.5% respectively. Among a total individual nematode recorded from Karsa and Dedo district root knot nematodes are the most frequent genera. This might be due to their endoparasitic nature, ability to attack a wide range of crops and their short life cycle which enables them to reproduce fast and form multiple generations within a short time (Manzanilla-Lopez and Sterr, 2009).

The highest prominence value was for that of RKN followed by *Helicotylenchus, Pratylenchus, Scutellonema, Rotylenchulus, Aphelenchus*, cyst nematode, *Criconema* and *Paratylenchus* with the value 230.85, 118.59, 73.52, 39.53, 38.5, 18, 8.94, 5.17 and 2.24,

respectively (Table 3). Over all, RKN was the most prominent nematode genera in the study area followed by *Helicotylenchus* and *Pratylenchus* whereas *Criconema and Paratylenchus* were less frequent with low prominence value (Table 4).

A number of factors are contributed for the abundance of prevalent nematode in the study areas including soil type and pH, cropping pattern, climatic condition, agronomic practice employed and particular cropping sequence (David, 1985). The farmer's agronomic practices might have contributed to the distribution and abundance of PPNs in the study area. It was observed that farmer use water from a nearby river to irrigate their tomato field. This practice might be responsible for introduction and distribution of a wide range of PPNs in tomato field. Afolami *et al*. (2014) reported that the practice of furrow irrigation has a significant role in nematode distributions.

PPNs might be less important under more extensive and varied growing systems typical of shifting cultivation and multiple intercrop farming systems in subsistence agriculture, as well as in widely spaced rotations of some commercial farming systems (Li, 2016). However, nematodes are very important in more intensive production systems, for example, in protected cultivation where mono cropping or continuous cropping is practiced. It is noted that damage intensity usually increases slowly with time in the multiple intercropping system, as compared with the rapid increase in damage encountered in large scale vegetable production where monoculture or continuous cropping is practiced (Li, 2016).

RKN are among the most widespread nematodes and have been reported from all the countries of the globe (Rathou *et al*., 2006). However, differences have been seen in their distribution in the study area. These variations in RKN distribution might be ascribed to many environmental and edaphic factors (Sasser and Carter, 1985). The soil pH of the study sites ranged from 6.31 to 7.15 (Table 3), which was nearest to neutral pH and was ideal for the reproduction of nematodes. Soils with higher sand content and near neutral pH in lower altitude in eastern Ethiopia were found to be suitable to inhabit more nematode population (Tadele, 1998). Soil type and soil pH have also been shown to influence nematode distribution and it may also influence the types of crops grown, thereby affecting nematode

distribution, population build-up and damage intensity. Soil pH influences nematode population and it varies significantly (Asif *et al*., 2015).

Several studies have investigated the presence of PPN associated with economically important crops including tomato in Ethiopia. Survey conducted by Mandefro and Mekete (2002) revealed that *Meloidogyne* spp. to be the most dominant and widely distributed on tomato fields. Numerous species of PPNs belonging to 15 genera are reported to be associated with cereals, pulses and oil crops in Ethiopia (Abebe *et al.*, 2015*).* Survey conducted by Bao and Neher (2011) on vegetable field indicated that *Pratylenchus, Meloidogyne, Paratylenchus, Criconemoides, Heterodera, Helicotylenchus* and *Hoplolaimus* spp. were found in Vermont. *Meloidogyne* species were the predominant nematode across tomato production areas in central and northern Tanzania (Missanga and Rubanza, 2018). *Meloidogyne incognita*, *M. javanica, M. arenaria* and *M. hapla* were occur alone or in mixed populations in major tomato growing localities in Rift Valley, Upper Awash and Eastern Hararghie areas of Ethiopia but *M. arenaria* was not prevalent in localities of Eastern Hararghie (Seid *et al*., 2019).

Table 4. Frequency of occurrence (%), population density (%) and prominence value of PPN associated with tomato in Dedo and Karsa district.

Sampling Kebele	Altitude	pH	Mean number of nematodes per 100 ml soil								
			Meloidogyne	***Pratylenchus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Rotylenchulus***	***Aphelenchus***	***Paratylenchus***	**Cyst nematodes**
Kitmile	1693	6.71	24	44	12	4	4	-	-	-	4
Grima	1663	6.31	24	12	82	10	2	2	-	-	-
Bulbula	1758	6.75	38	8	4	4	-	-	2	-	2
Babo	1765	6.93	230	50	20	16	-	5	-	-	-
Offole	1749	6.88	70	26	10	10	2	-	2	-	2
Kolobo	1736	6.74	50	26	110	20	-	46	24	2	8
Dema sertha	1811	7.15	116	6	12	6	-	4	4	-	-
Korijo	1754	6.84	32	14	50	30	-	20	4	-	-
Frequency of occurrence (FO)			100	100	100	100	37.5	62.5	62.5	12.5	50
Mean population density/100 ml soil			73	23.25	37.5	12.5	2.67	15.4	7.2	2	4
Maximum population density			230	50	110	30	4	46	24	2	8
Prominence value (PV)			230.85	73.52	118.59	39.53	5.17	38.5	18	2.24	8.94

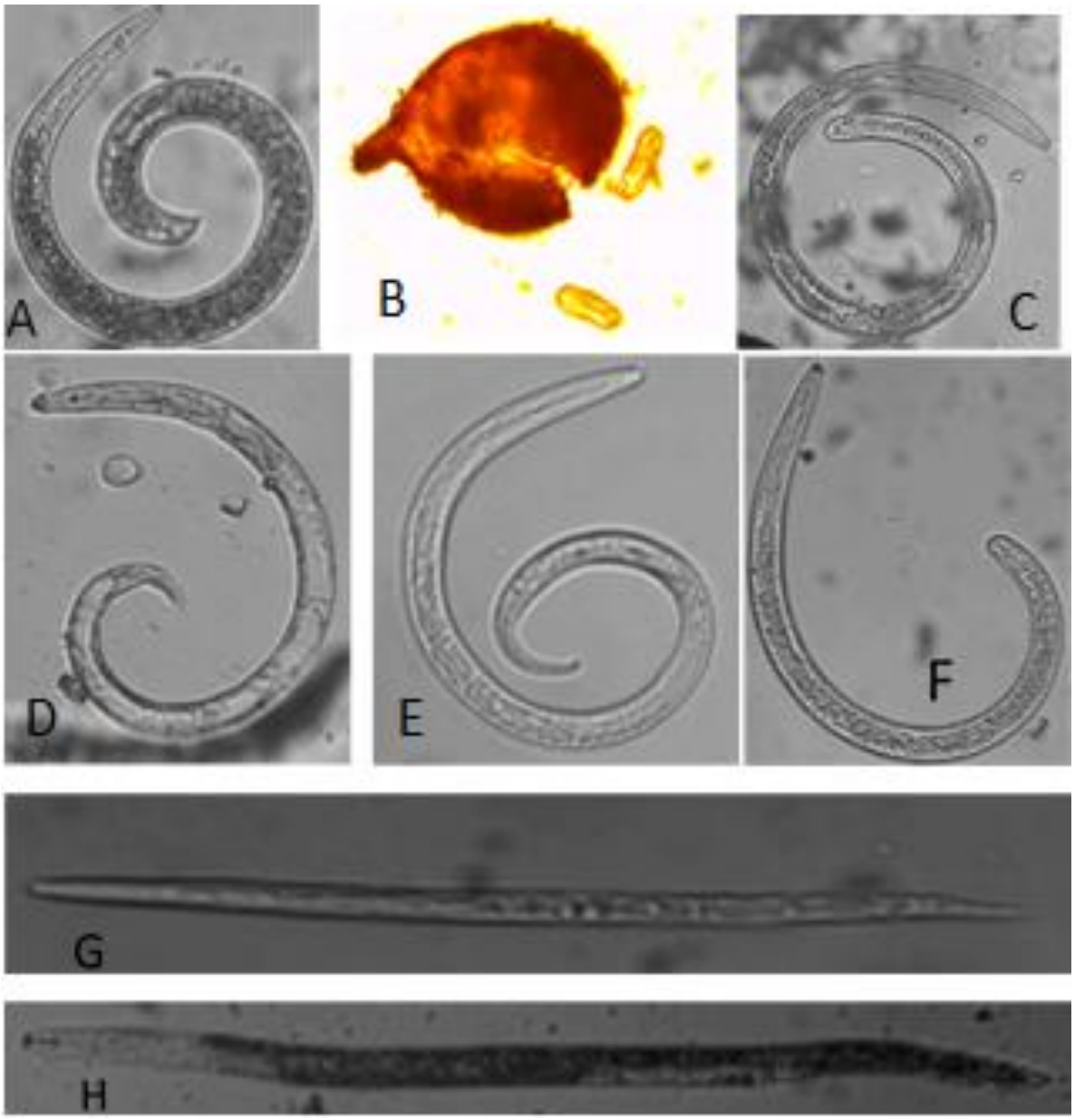

Figure 8. Photograph of PPNs associated with tomato in study area.(A) *Helicotylenchus* spp., (B) Cyst nematode, (C and F) *Scutellonema* spp., (D and E) *Rotylenchulus* spp., (G) *M. arenaria* J2 and (H) *Pratylenchus* spp. (Magnification: 10X).

4.1.2. Identification of *Meloidogyne* species

From perineal pattern morphology it was confirmed that the species identified was *M. arenaria* (Fig. 9). Perineal patterns of this nematode had low dorsal arch slightly indented near lateral fields to form rounded shoulders. Lateral lines were not distinct, dorsal and ventral striae connected with an angle and forked. In some perineal patterns of this isolate,

there was slight wing formation in one or two lateral lines. The striae of this isolates were smooth and slightly wavy. The mean vulva slit length was 20.41± 1.68 µm at distance from anus with 14.78 ± 1.77 µm. Anus to tail revealed to be 18.68± 4.0 µm. Identification of *Meloidogyne* spp. using perineal pattern morphology was reported by Ferris (1999) and Carneiro *et al*. (2016). The current perineal pattern study was similar with identification made by Aydinli and Mennan (2016).

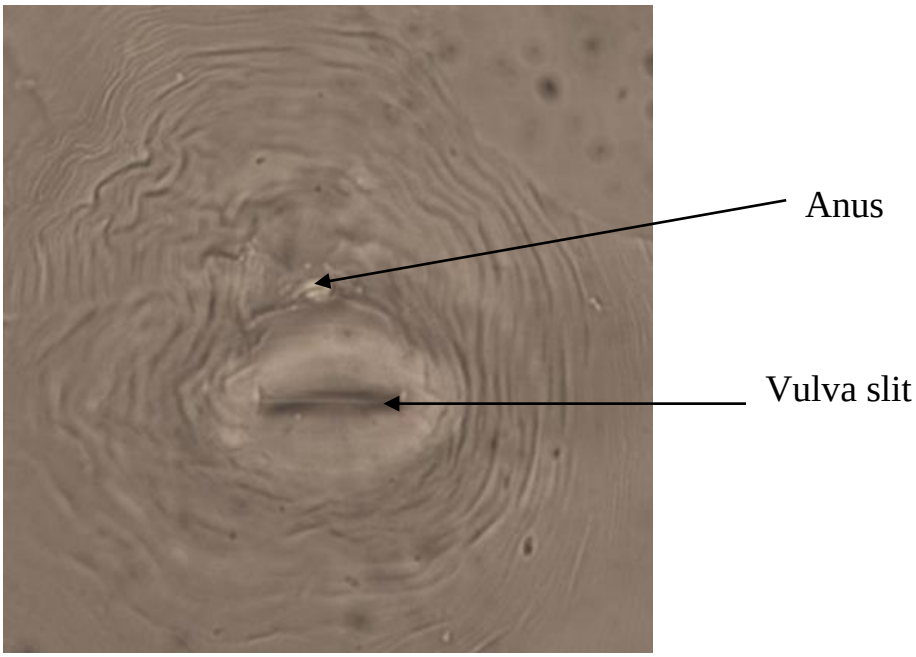

Figure 9. Perineal pattern of female *M. arenaria* isolate (Magnification: 100X)

The morphological identification was also confirmed by molecular analysis using mitochondrial DNA analysis. This isolate showed 99.82 % sequence similarity with *M. arenaria* isolate (KP202350) from USA. The mitochondrial DNA is one of the most useful targets that have been used in identifying various *Meloidogyne* spps (Blok *et al*., 2002). The low level of recombination that is associated with the mtDNA coupled with high rates of evolution also provides a unique region that has been utilized for phylogenetic studies and studying species variation in different *Meloidogyne* species (Blok and Powers, 2009). Maximum Likelihood Phylogenetic analysis also further confirms the identity of our isolate as *M. arenaria* which formed a cluster on same branch with those previously identified and characterized isolates from elsewhere in the world (Fig. 10).

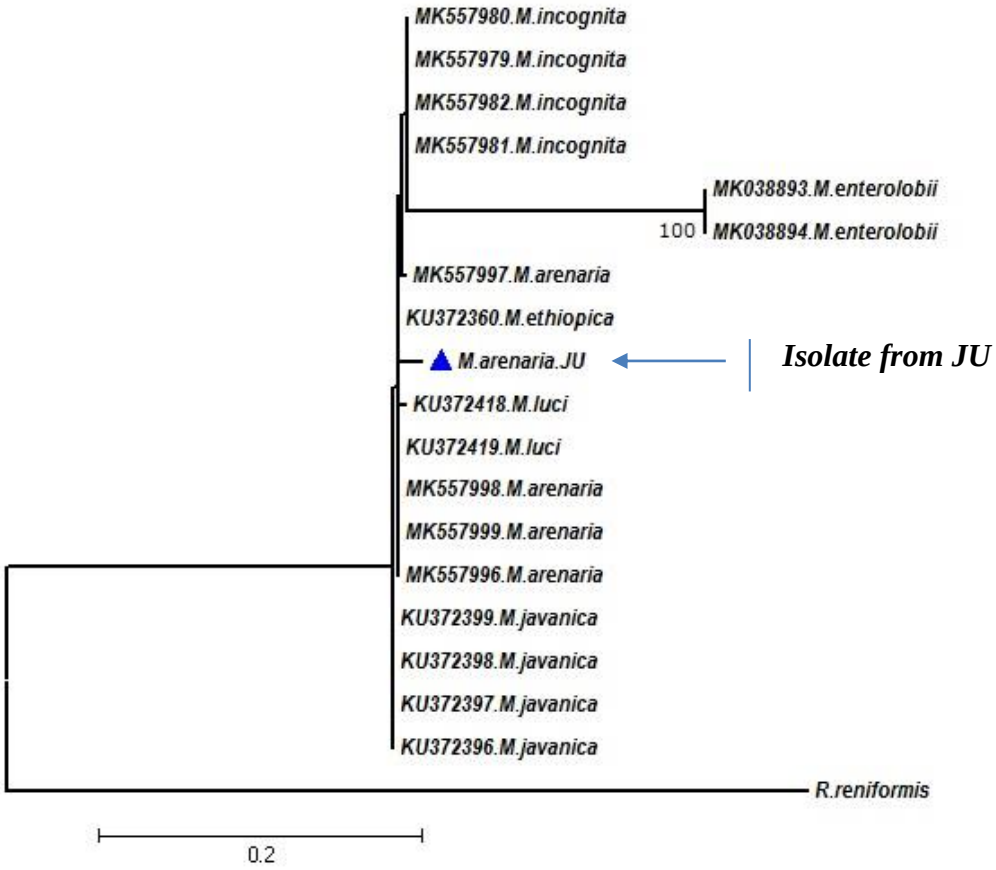

Figure 10. Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model

4.1.3. Incidence of root knot and effect of intercropping system and cropping pattern on RKN population density in the study areas

The result showed that root knot nematodes were prevalent in all the surveyed kebeles. All forty sampled fields were infested with *Meloidogyne* spps. Disease incidence varied in entire kebeles and ranged from 47.99 to 80.95 %. The highest disease incidence was noted at Babo kebele followed by Dema sertha and lowest at both Kitmile and Girma (Fig. 11). In the current study, severe stunting and extensive root galling of tomato was observed (Appendix 8f and 9c). The abundance of this nematode may be due to completing multiple generations within short period of time, high reproduction rate and ability to attack wide range of host and several other factors. Wondirad and Mekete (2002) reported that RKN is a serious pest of tomato in central and eastern parts of Ethiopia; it was also dominant pest of tomato in Rift

Valley, Upper Awash and East Hararghie area of Ethiopia (Seid *et al*., 2017). Survey conducted by Seid *et al*. (2019) revealed that RKNs were prevalent in 23 localities out of 40 with disease incidence ranging from 50- 100 % in Ethiopia. The incidence of root knot disease on tomato showed that the tomatoes in all localities were infested with RKN in India (Esfahani, 2009).

The current study revealed that cropping system and cropping pattern had significant influences on RKN population density. The mean highest population density was recorded on mono-cropped and continuously planted tomato fields, while the lowest density was noted in tomato intercropped with tuber crops and rotated with sorghum (Fig. 12 and 13). Some of the farmers intercrop tomato with other *Solanaceae* crop like potato and pepper while 87.5% of farmers used mono cropping and 70% of farmers rotate tomato with maize, sorghum and other crops (yam, enset and khat) (Fig. 12 and 13). Moreover, 95% of the farmer's field was irrigated based on furrow irrigation system. In the intercropped fields, reduction of nematode population was observed as compared to mono-cropping fields. This agronomic practice could enhance the distribution and prevalence of PPNs in the study area. Damage caused by nematodes was reduced in tomato planted after sweet corn or in sweet corn with *Tagetes patula* and *Sorghum bicolor*. Nematode populations decreased when tomato planted with sweet corn alone or sweet corn under sown with *Tagetes* spp., sorghum, asparagus or garlic as compared to tomato monoculture (Otipa *et al*., 2003). The author reported that, sorghum, sweet corn, *Capsicum* and peanuts were suppressive to RKNs under greenhouse and field conditions. In mono-cropped and continuously cropped fields the damage was higher than multiple intercropped and rotated fields (Li, *et al*., 2016).

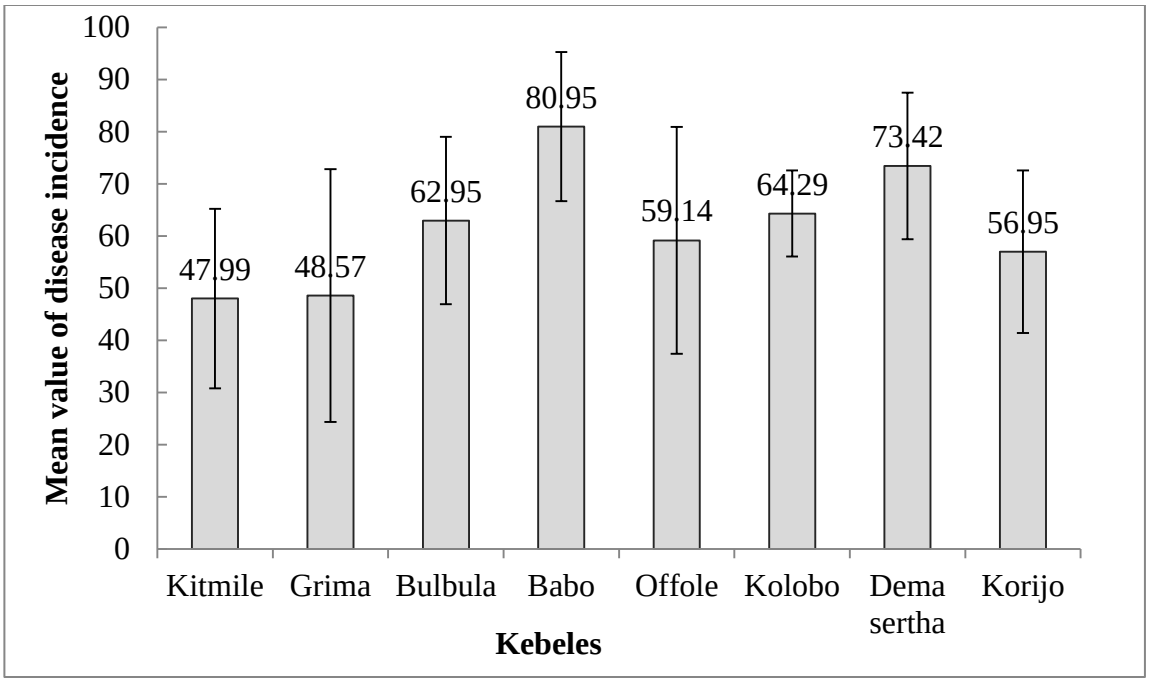

Figure 11. Mean value of disease incidence recorded from surveyed Kebeles. Data are means of five replicates. Vertical error bars represent standard errors.

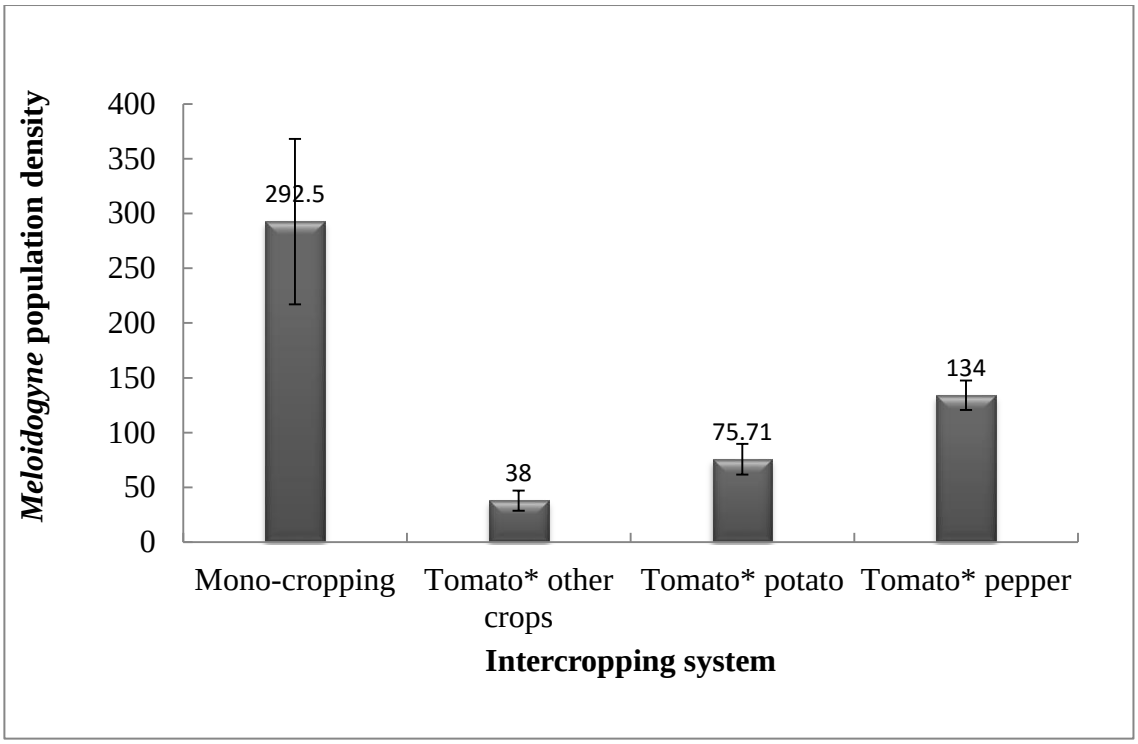

Figure 12. Effect of intercropping system on RKN population density in Dedo and Karsa districts. Vertical error bars represent standard errors.

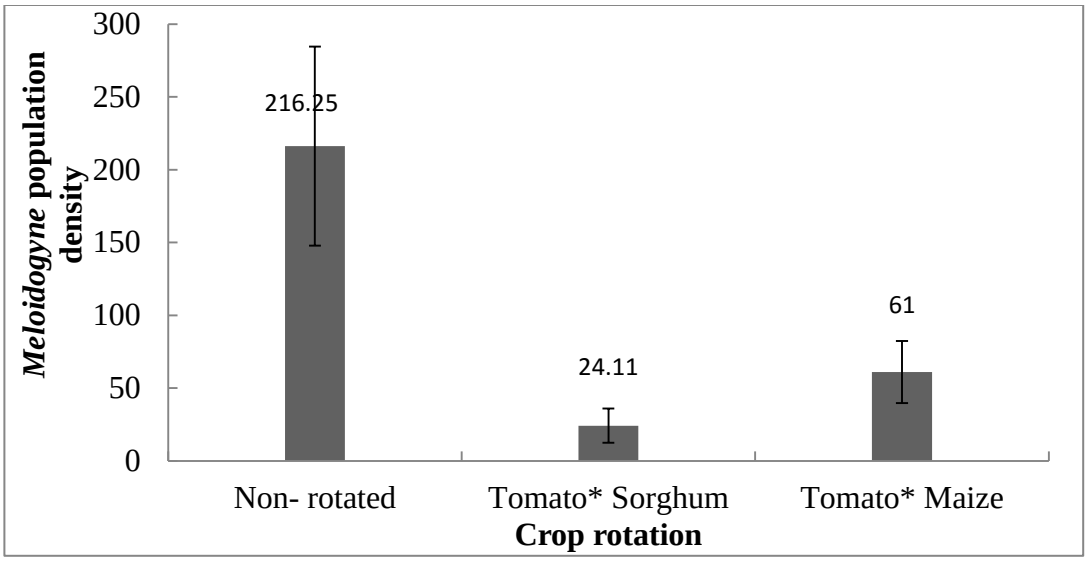

Figure 13. Effect of crop rotation on RKN population density in Dedo and Karsa districts. Vertical error bars represent standard errors.

4.1.4. Regression analysis for RKN population and soil pH and altitude

The RKN population density is significantly influenced by soil pH and altitude at p= 0.0099 and p= 0.0096 respectively. The regression analysis showed that variation of RKN population density is being explained 69 % by soil pH and 70 % by altitude (R^2= 0.69 and 0.70) respectively. This indicated that the variation of nematode population in the study area was caused 69 % by soil pH and 70 % by altitude. The result showed that for every additional 1 unit in soil pH and 1 m in altitude, the expected density of RKN population increases by 160.66 and 0.6124 on average respectively, holding all other variable constant (Fig. 14 and 15). In the current study, as the altitude increases the number of second stage Juveniles increases in soil. Study held by Meressa *et al*. (2014a) revealed that *Meloidogyne* and *Rotylenchulus* were prevalent in all altitudinal ranges during their sampling seasons. Increasing soil pH from 6.5 to 7.2 increase the density of RKNs in soil but the pH of the soil has no direct effect on the density of RKNs because RKNs population increased when the pH was in the range of 5-7 and as the pH goes more than 7 nematode populations was decreased (Asif *et al*., 2015). *Meloidogyne* species survive and reproduce at pH levels ranging from 4.0 to 8.0 (Ferris and Van Gundy, 1979). This study was in accordance with the report of Tadele (1998) and Ferris and Van Gundy (1979).

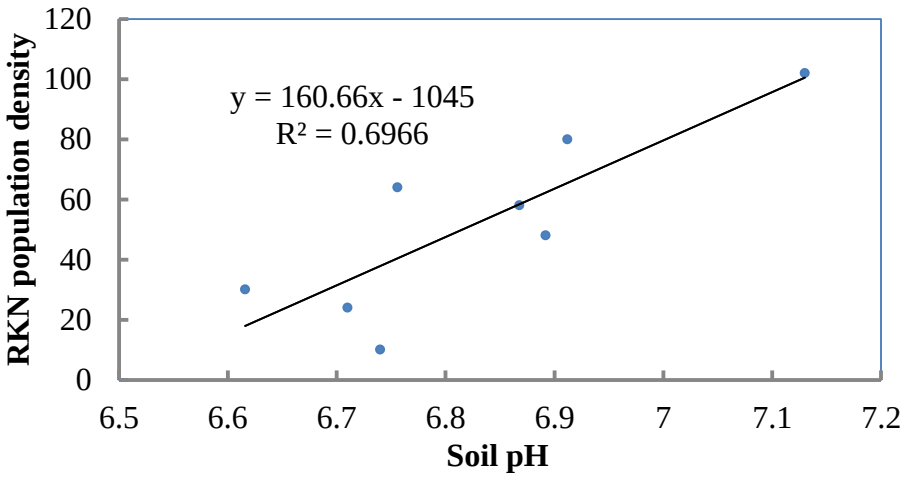

Figure 14. Scatterplot for RKN population density versus soil pH.

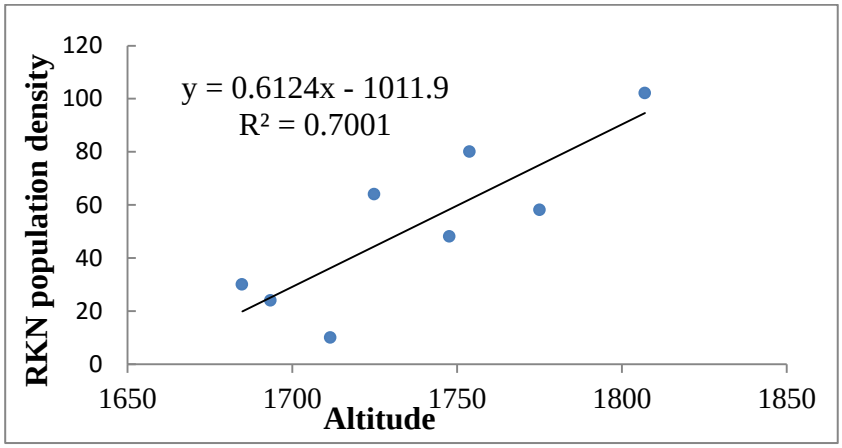

Figure 15. Scatterplot for RKN population density versus altitude.

4.2. Evaluation of tomato varieties for the reaction to *Meloidogyne arenaria*

4.2.1. Effect of *Meloidogyne arenaria* on growth of tomato varieties

There was significant variation (p= 0.01) in mean shoot height among nematode infected tomato varieties. The mean maximum shoot height was recorded from variety Melkashola (65.43 cm) and Gelila (64.33 cm) whereas the mean minimum was noted at Gelilema (42.33 cm) (Table 5).

The result clearly indicated that there was significant (p= 0.05) difference of mean fresh shoot weight in tomato varieties. The highest mean fresh shoot weight was recorded on Melkashola (91.03 g), Metadel (87.0 g) and Roma VF (87.90 g) and the lowest mean weight was noted at Gelilema (54.61 g), Moneymaker (57.96 g), and Mariglobe (57.14 g) varieties (Table 5).

The current result indicated that dry shoot weight was significantly (p= 0.05) different among the varieties. The mean maximum dry shoot weight was recorded on Melkashola (12.88 g) whereas the mean minimum was noted on Gelilema (9.27 g) which was not significantly different from Moneymaker and Margilobe (Table 5).

The decrease in plant height and shoot weight observed on the thirteen varieties might be due to inoculation of *M. arenaria* attributed to the damage caused by the nematode to the plants. Galled roots lead to modification in absorption of water and nutrient from soil and their translocation to foliage resulting in foliage chlorosis (Appendix. 8c) and stunting of vegetative growth (Bala,1984) because arrested root system could not be able to fully explore the soil for water and nutrients (Clark *et al*., 2003). Sasanelli *et al*. (1992) found out cabbage plants attacked by *M. incognita* showed stunting and yellowing within two weeks of infestation. Haider *et al*. (2003) inoculation of 100 J2 of *M. incognita* per plant caused a significant reduction in growth of French bean and pea. The RKN infestation adversely affected the plant growth showing that the nematode infestation was the limiting factor. Singh and Khurma (2007) reported heavily infested plant with RKNs exhibited stunted growth and in some cases the plant die before reaching maturity. Under heavy nematode infestation, transplanted crop may fail to develop and get stunted to have poor stand (Noling, 2009). *M. javanica* infection significantly reduced plant height of both verified nematode resistant and susceptible tomato genotypes (Banora and Almaghrabi, 2019).

In the current study, inoculation of *M. arenaria* showed significant reduction in shoot weight of 13 tomato varieties as compared to Melkashola. This might be due to interference of nematodes on the plant growth and development (Ganaie and Khan, 2011). *Meloidogyne* spp. have led to the malfunctioning of the plant roots that caused decreased absorption of key nutrients required for the plant growth; this attributed to the reduction of root volume and surface area hence inhibiting the shoot weight (Hussey and Boerma, 1989). The nematodes

could have modified the host mineral composition and created wounds that allow various pathogens to gain entry to the plants (Ganaie and Khan, 2011). The reduction of shoot dry weight of susceptible control variety (Moneymaker) was similar to both Melkasalsa and Gelilema varieties. The metabolic activities of an economic plant are reflected in its dry weight, growth and yield. The reduction in biomass of the heavily galled plants indicated a decrease in metabolic activities. The growth of tomato and pepper were curtailed by *M. incognita* that reduced the fresh weight of both crops (Mekete *et al*., 2003). Nematode inoculation significantly reduced foliar dry weight of the susceptible cultivars, but not the resistant cultivars (Corbett *et al*., 2011). Inoculation of *M. Javanica* on tomato cultivar brought about a reduction in the fresh shoot weight of nematode inoculated tomato cultivars (Mwangi *et al*., 2017). *M. javanica* infection significantly reduced fresh and dry shoot weights of all tomato genotypes except Fayrouz genotype (Banora and Almaghrabi, 2019).

Mean value of root fresh weight showed that there was a highly significant difference among the varieties at p= 0.05. The mean maximum fresh root weight was noted at Moneymaker (susceptible control) (47.3 g) whereas the mean minimum was recorded on Melkashola variety (22.92 g) (Table 5).

The maximum root fresh weight was recorded at Moneymaker (susceptible control) variety of tomato this might be due to the higher number and size of galls formed on their roots and diseased root tissues were heavier than the healthy tissues. Because the infective second stage juvenile penetrates through the root and migrates to a site near the vascular tissue (Williamson and Hussey, 1996) to get the feeding site and complete its life cycle until it becomes an adult. Adult females spend most of their active life time within plant roots feeding on host cells and they form giant cells. In the current study, the increased root weight on Moneymaker variety may be due to intensive galling on plant roots (Mai and Abawi, 1987). Roots of susceptible genotypes were found to be more favourable to RKN galling. More eggs were developed on susceptible genotype roots compared with the less susceptible or resistant genotypes. Root galling on tomato varied with different genotypes (Jaiteh *et al*., 2012), similar result was observed in this study (Table 5).

Table 5. The effect of *Meloidogyne arenaria* on growth parameter of tomato varieties

Tomato Var.	Shoot height	Shoot FW**	Shoot DW**	Root FW**
Moneymaker	54.0±1.7^{e}	57.9±4.0^{f}	9.4±1.3^{cde}	47.3±1.2^{a}
Roma VF	55.7±1.5^{de}	87.9±2.7^{ab}	11.3±1.1^{abc}	30.7±1.5^{d}
Fetan	48.0±4.0^{g}	65.4±1.1^{e}	11.1±1.1^{bc}	41.0±2.4^{bc}
Melkashola	65.3±1.5^{a}	91.0±3.1^{a}	12.9±0.6 ^{a}	22.9±1.0e
Melkasalsa	61.0±2.6^{bc}	66.0±2.2^{e}	10.6±1.6^{bcd}	41.2±1.2^{bc}
Metadel	49.3±2.1^{fg}	87.0±1.1^{ab}	12.2±1.2 ^{ab}	33.6±0.8^{d}
Cochoro	54.0±1.0^{e}	73.4±2.2^{c}	12.1±0.9^{ab}	39.7±0.7^{c}
Bishola	57.7±0.6^{cd}	84.1±1.2^{b}	11.6±1.0^{ab}	30.4±1.5^{d}
Gelila	64.3±2.5^{ab}	67.7±2.2^{de}	10.5±2.2^{bcd}	39.7±1.6^{c}
Gelilema	42.3±2.5^{h}	54.6±2.0^{f}	9.3±1.1^{de}	41.6±1.0^{abc}
Arp d2	49.0±0.1^{g}	67.0±2.5^{ed}	10.9±0.9^{bcd}	29.5±2.4^{d}
Chali	49.3±1.5^{fg}	72.3±1.2^{cd}	10.8±0.2^{bcd}	41.6±0.5^{abc}
Margilobe	52.7±2.1^{ef}	57.1±3.7^{f}	9.7±0.3^{cde}	42.9±1.9^{abc}
Miya	47.0±2.6^{g}	72.2±3.3^{cd}	11.5±0.9^{ab}	41.3±1.0^{bc}
Cv	4.0	4.5	9.6	9.0
Lsd	3.6	5.4	1.7	5.7

*Means with the same letter in the column are not significantly different at probability of $P \leq 0.05$ using fisher Lsd test. **FW= fresh weight, DW= dry weight

4.2.2. Response of tomato varieties to the development of galls and eggmass of *Meloidogyne arenaria*

There was a significant difference (p= 0.05) in number of eggmass and root gall development among the varieties. The mean maximum number of eggmass and root gall was recorded from Moneymaker variety followed by Gelilema variety whereas the minimum was recorded from Melkashola variety (Fig. 16).

Highly susceptible genotypes support nematode reproduction as shown by high gall numbers and eggmasses present while in resistance genotypes limited numbers of juveniles were develop to maturity and lay eggs (Kamran *et al*., 2012). Khan (1994) reported that the development of galls increased significantly in susceptible genotypes compared to resistant genotypes. Nematode reproduction, as measured by the number of eggs per gram of root was significantly lower on the resistant tomato cultivar Motelle (2,800 eggs/g) than on the susceptibl e cultivar Moneymaker (85,260 eggs/g) (Corbett *et al*., 2011). The compatible reaction of tomato genotypes to *M. incognita* infection might be due to lack of resistant genes so genotypes, unable to stop the penetration, development and reproduction (Sujatha *et al*., 2017). The current finding indicated that the Moneymaker and other twelve varieties had high number of eggmass and root gall development than Melkashola variety. The study indicated

that varieties had different response to *M. arenaria* infection. RKN eggs developed poorly on resistance accession compared to susceptible accession (Cousins and Walker, 1998). In a field study, it was observed that abundant gall and eggmass were developed on the roots of susceptible cultivar (Sorribas *et al*., 2005). *M. incognita* populations 'Babile' and 'Jittu' and *M. Javanica* populations 'Jittu'and 'Koka' were highly aggressive on susceptible cultivars Moneymaker as shown by the high number of egg masses formation (Seid *et al*., 2017). All the tested plant parameters were negatively affected by both populations of *M. incognita*. The Jittu *M. incognita* population had greater effect on the majority of tomato parameters than Babile population from Ethiopia (Seid *et al.*, 2019).

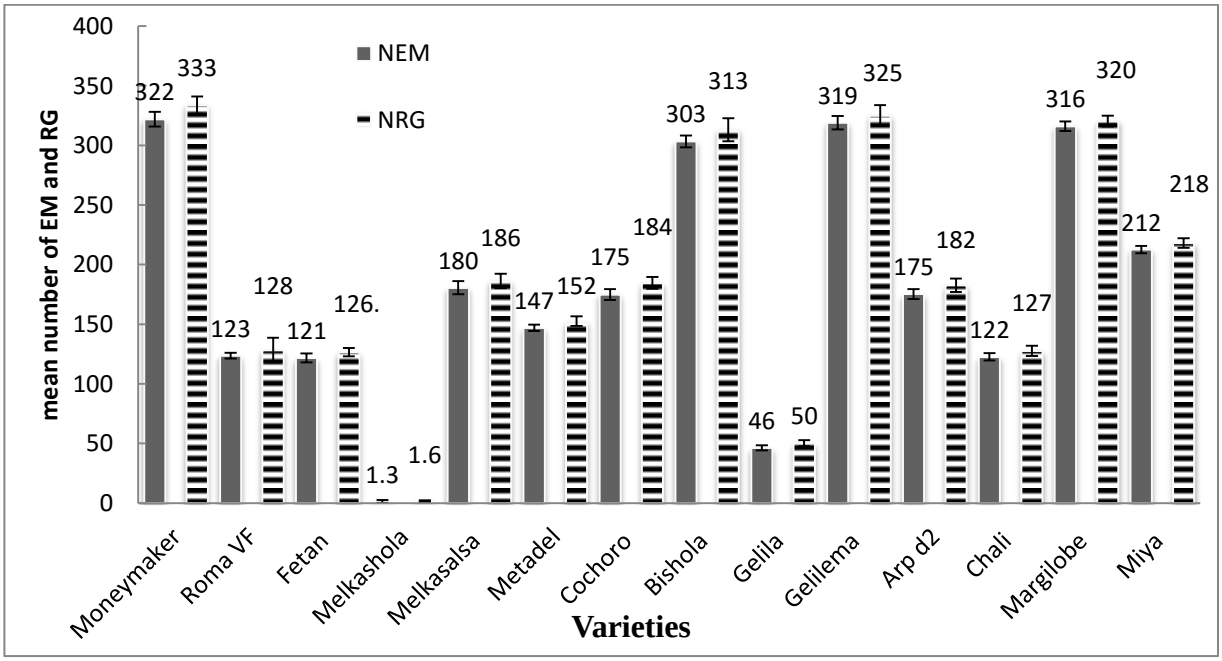

Figure 16. Mean number of root gall and eggmass counted per root system of tested tomato varieties. Data are means of three replicates. Vertical error bars represent standard errors of differences of means. NEM= number of eggmass, NRG= number of root gall, EM= eggmass and RG= root gall.

4.2.3. Effect of tomato varieties on gall index, final population density and reproduction factor of *Meloidogyne arenaria* and their host status

The study revealed that there was a significant difference in root gall index among the varieties at p= 0.05. The mean highest root gall index was noticed in twelve varieties. Variety Gelila had 4 root gall index and the mean lowest root gall index was recorded on variety Melkashola (1.33). ANOVA indicated that there was a significant difference of final

nematode population among fourteen varieties at p= 0.05. The mean highest final nematode population was recorded at variety Moneymaker (205127 J2 per root) followed by Gelilema variety (171663 J2 per root system) whereas the lowest was noted at Melkashola variety (271 J2 per root system) which was lower than initially inoculated population density (Table 6).

A significant difference (p= 0.05) was observed in reproduction factor of nematode in tomato varieties. The mean highest reproduction factor was observed on tomato variety Moneymaker (102.56) followed by Gelilema (85.83) whereas the mean lowest was recorded on Melkashola variety (0.14) (Table 6). The results show that *M. arenaria* developed and reproduced in all varieties with different rates except on Melkashola variety.

The current result indicated that tomato varieties had different response to *M. arenaria* infestation; this might be the genetic variability among the varieties (Jacquet *et* al., 2005; Castagnone- Sereno, 2006). Out of the 14 varieties 13 varieties supported the development and reproduction of *M. arenaria* with different rates and they were found to be susceptible while Melkashola variety restricted their development and reproduction and was found to be resistant. Nematode resistance in host plant was manifested by low rate of nematode reproduction and low nematode population density than susceptible varieties (Khan, 1994). The final nematode population was highest in Moneymaker variety than others but twelve varieties had relatively high nematode population than Melkashola variety. These varieties may lack resistance gene, repelling agent and barriers that restricts the penetration and reproduction potential of *M. arenaria* (Jaubert *et al*., 2002). Karssen and Moens (2006) reported that highly susceptible host plants allowed the juvenile to enter the host, reached maturity and produce many eggs, while the resistance plants limited their development and do not allow them to reproduce. Based on reproduction factors, Assila, CLN-2366B, Chochoro, Tisey and Moneymaker varieties were found to be a good hosts for Jittu and Babila populations of *M. incognita* (Seid *et al*., 2019).

Resistance in tomato varieties to RKN has the same genetic origins and controlled by a dominant gene, Mi gene (Seid *et al*., 2015). It was effective against three RKN species such as *M. incognita*, *M. arenaria* and *M. javanica*. In this study, without considering, Mi genes in view, the reactions of 14 tomato varieties were evaluated against *M. arenaria* in which, the

different characters, such as the reproduction factors, number of root gall and eggmass and gall index were taken into account as several reports mentioned (Khan and Khan, 1991; Jaiteh *et al*., 2012; Esafahani *et al*., 2012). The results also suggest that the variety Melkashola was resistant to *M. arenaria*; maybe this variety contains a high phenolic, flavonoid and organic acids content as one of the chemical characteristics of tomato cultivars resistant to RKNs (Flores *et al*., 2017). The findings in this work give some evidence to suggest that there could be resistant gene in this variety.

Mechanism of resistance in plant could be the production of toxins from root exudates, the lack of attractant or the hatching factor in the exudates, barrier for penetration or failure of nematode to develop within plant tissues, the production of lignin and synthesis of phytoalexins (Favery *et al*., 2001; Jaubert *et al*., 2002). The presence of ascorbic acid in plant also provides resistance against several pathogens including nematodes. Low level of ascorbic acid in tomato cultivars was associated with their susceptibility to *M. incognita* attack (Brueske, 1980). The root cells of resistant plants react against nematodes through increase in NADPH oxidase activity. The production of superoxidase in plant cells directly or indirectly may cause the death in hypersensitive cells and as a subsequent to these reactions, establish resistance in the plants. Studies have indicated that, respiratory resistant cyanide and superoxidase induce the phytoalexin synthesis, and then establish resistance in plant during infection process (Favery *et al*., 2001).

Nematode infection stimulates the formation of root galls which interferes with plant water supply (Waller *et al*., 2002). The high root gall index was made the varieties as good host for *M. arenaria*. Host plants have varying degrees of susceptibility to RKNs. The susceptibility of plants to RKNs depends on the ability of J2s to penetrate the roots of the plant and form galls and eggmass on the root (Karssen and Moens, 2006). The level of susceptibility of tomato to *Meloidogyne* spp. is measured by the tomato genotype (Jacquet *et* al., 2005; Castagnone-Sereno, 2006). However, susceptibility of host plant is not only depending on its genotype but on how many nematodes affect it. Fassuliotis, (1979) reported that the presence of root gall on tomato is ideal for the measuring the host status of tomato.

Table 6. The effect of *Meloidogyne arenaria* on final nematode population, reproduction factor and host status of tomato varieties

Tomato Var.	RGI**	FP**	RF**	Host status
Moneymaker	5.00[a]	205127 (5.31)[a]	102.5 (2.02)[a]	Susceptible
Roma VF	5.00[a]	58896 (4.77)[h]	29.45 (1.48)[i]	Susceptible
Fetan	5.00[a]	136918 (5.14)[e]	68.45 (1.84)[f]	Susceptible
Melkashola	1.33[c]	271 (2.43)[m]	0.14(0.06)[n]	**Resistance**
Melkasalsa	5.00[a]	149425(5.17)[d]	74.71 (1.88)[d]	Susceptible
Metadel	5.00[a]	69700(4.84)[g]	34.85 (1.55)[h]	Susceptible
Cochoro	5.00[a]	25536 (4.41)[l]	12.77 (1.14)[m]	Susceptible
Bishola	5.00[a]	121374 (5.08)[f]	60.68 (1.79)[g]	Susceptible
Gelila	4.00[b]	39775 (4.6)[j]	19.88 (1.32)[k]	Susceptible
Gelilema	5.00[a]	171663 (5.23)[b]	85.83 (1.94)[b]	Susceptible
Arp d2	5.00[a]	33570 (4.53)[k]	16.78 (1.25)[l]	Susceptible
Chali	5.00[a]	44554 (4.64)[i]	22.27 (1.37)[j]	Susceptible
Margilobe	5.00[a]	158623 (5.19)[c]	79.31 (1.91)[c]	Susceptible
Miya	5.00[a]	138781 (5.14)[e]	69.39(1.85)[e]	Susceptible
Cv	8.74	1.93	1.93	
Lsd	0.68	3136.9	1.56	

*Means with the same letter in column are not significantly different at $p \leq 0.05$. Figures in parentheses are transformed by log10(x+1). **RGI= root gall index, FP= final nematode population and RF= nematode reproduction factor.

4.2.4. Correlation between growth character of tomato and nematode reproductive parameters

The correlation analysis indicated that shoot height has highly and moderately positive relation with shoot fresh weight and shoot dry weight (r= 0.47, p= 0.002 and r= 0.17, p= 0.25) respectively. It has a strong negative relation with number of eggmass and root gall and reproduction factor at p= 0.001 and moderate negative relation with root fresh weight and final nematode population. Shoot fresh weight has a strong positive relation with shoot dry weight (r= 0.554, p= 0.001) and has strong negative relation with nematode reproduction parameters. The result of correlation analysis also shown that shoot dry weight was negatively correlated with nematode reproduction parameter. Root fresh weight had a negative correlation with growth parameters of tomato whereas a positive correlation with nematode reproduction parameters. The number of root galls has a strong positive correlation with number of eggmass (r= 0.99, p= 0.001), reproduction factor (r= 0.788, p= 0.001) and it has a moderate relationship with final nematode population (r= 0.359, p= 0.02). The results also

showed that final nematode population density had moderate positive correlation with reproduction factor (r= 0.359, p= 0.02) (Table 7).

Table 7. Correlation between growth character of tomato and nematode reproduction parameters for fourteen tomato varieties

	#SH	#SFW	#SDW	#RFW	#NEM	#NRG	#FP	#RF
SH	1							
SFW	.472**	1						
	0.002							
SDW	0.179	.554**	1					
	0.257	0						
RFW	-0.216	-.546**	-0.049	1				
	0.17	0	0.76					
NEM	-.569**	-.542**	-0.254	0.275	1			
	0	0	0.104	0.078				
NRG	-.571**	-.540**	-0.248	0.274	0.999**	1		
	0	0	0.113	0.079	0			
FP	-0.108	-.307*	-0.243	.363*	0.248	0.242	1	
	0.495	0.048	0.121	0.018	0.113	0.123		
RF	-.507**	-.626**	-0.229	.540**	.792**	.788**	.359*	1
	0.001	0	0.145	0	0	0	0.02	

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).
#SH= Shoot height, SFW= Shoot fresh weight, SDW= Shoot dry weight, RFW= Root fresh weight, NEM = number of eggmass, NRG= number of root gall, FP= final nematode population and RF= reproduction factor

4.3. Management of *Meloidogyne arenaria* using decomposed coffee husk application

4.3.1. Effect of coffee husk amendment and variety on growth parameter of tomato

Shoot height was significantly influenced by coffee husk level. It was increased with increasing of level of coffee husk application. The interaction had also a significant influence on shoot height (p= 0.05). The mean maximum shoot height was observed from a treatment combination of Melkashola with 3:1 (CH: SM), while the mean minimum was noted from a combination of Moneymaker with non-amended treatment (Table 8).

Shoot fresh weight was significantly influenced by coffee husk level and variety. The interaction had a significant influence on shoot fresh weight at p= 0.05. Shoot fresh weight was observed to be high in combination of Melkashola with 3:1, while the mean minimum

was noted at combination of Moneymaker with non-amended control and Miya with non-amended control (Table 8).

Root length and root fresh weight were significantly influenced by variety and coffee husk level (Table 9). The interaction had no significant effect on root length and root fresh weight at $p< 0.05$. The mean maximum root length and weight was observed at Melkashola variety while the mean minimum was recovered from Moneymaker variety. There was no significantly different in root weight between Moneymaker and Miya varieties. Root length and weight increased with increasing of coffee husk level. The mean maximum root length and weight was recorded at 3:1 ratio of CH to SM, while the mean minimum was noted at non-amended control. There was no significant difference in root length between 3:1 and 1:1 (CH: SM) (Table 9).

The current study revealed that the reduction of shoot height and weight in control treatment might be the influence of nematode infestation and growth media in which sandy soils enable easy movement of nematodes thus increasing their pathogenicity (Cadet and Spaull, 2005). Sandy soils have lower water holding capacity so that roots found there are restricted in growth, this might be coupled with destruction by nematodes makes their impact even worse. Ogwulumba *et al*. (2009) reported poultry manure amendment significantly increased plant height as compared to untreated treatments. Similarly, Hassan *et al*. (2010) also showed that amending soil with rice husk, saw dust and refuse dump increased plant dry weight, plant shoot height and root length as compared to non-amended control. The author reported that as the rate of application increases, the growth parameter of tomato as well increased. A significant enhancement was noted at highest rate application in these three organic amendments. Shiferaw *et al*. (2017) reported that the highest plant height was noted at combination of highest poultry manure (20 ton/ha) and rapeseed cake (200 kg/ha) amended treatments than lower amount amended one.

In the current study enhancement of shoot height and weight was observed in combination of variety with coffee husk amended treatments as compared to control this might be change in plant physiology, improvement of soil structure, porosity, increases infiltration and permeability of soil, improve water holding capacity, supply significant amount of organic

matter, improve CEC, stabilize soil pH, provides humus, vitamins, hormones and plant enzymes (Ahmad *et al*., 2009; Rashad *et al*., 2010), in which increased resistance against nematode development in the roots and organic material may stimulate root development. Wachira *et al*. (2009) reported that addition of organic carbon to the soils in form of manure leads to an increase in the number of free-living and predatory nematodes thus a decrease in PPNs.

Root length and weight were increased with increasing of coffee husk level due to rich source of nutrient elements which are taken up more rapidly and the effect of nematode damage is suppressed due to the presence of organic matter, nutrients, caffeine, tannins and phenolic compounds in coffee husk (Franca and Oliveira, 2009). The author reported that CH contains 9 % of phenolic compound. Tannins and phenolic compounds released from some plant residues may be toxic to nematodes and affect soil microbial populations by increasing saprophytic fungi (Kokalis-Burelle *et al*., 1994). Park *et al*. (2005) showed that compost contains chitinase producing bacteria that suppress nematodes in tomato plants and reduce their population. Plants grown in soil that is high organic matter is less damaged by nematode than plant grown in soil with less organic matter (Efthimiadou *et al*., 2009).

The study showed that root length and weight was highest at Melkashola variety as compared to Moneymaker and Miya. This might be caused by the genetic response of varieties to the nematode (Jacquet *et* al., 2005; Castagnone-Sereno, 2006); in the former experiment it was observed that Melkashola was resistant to *M. arenaria*. The highest root weight was observed at highest proportion coffee husk amended treatment than others which is in contrary with the finding of Shiferaw *et al*. (2017) who reported that the highest root fresh weight was obtained from control treatment as compared to poultry manure amended treatments when tomato was infested with *M. incognita* but Meyer *et al*. (2011) reported that the highest root weight of cacao plants was noted at poultry litter compost extract mixed treatments as compared to control treatments.

Table 8. The interaction effect of coffee husk proportion and varieties on shoot height and fresh weight of tomato

Treatments	**Mean value for Parameters**	
Interaction	SH** in cm	SFW** in g
Moneymaker *3:1 (CH: SM)	50.0±1.0^{b}	28.9±0.78^{bc}
Miya *3:1 (CH: SM)	52.8±5.25^{ab}	32.1±1.80^{a}
Melkashola *3:1 (CH: SM)	59.0±2.65^{a}	32.5±1.82^{a}
Melkashola *1:1 (CH: SM)	50.0±3.46^{b}	31.3±0.51^{ab}
Miya *1:1 (CH: SM)	49.3±4.51^{b}	28.6±0.76^{c}
Melkashola *1:3 (CH: SM)	49.0±2.0^{b}	27.2±0.36^{c}
Miya *1:3 (CH: SM)	46.3±6.03^{bc}	24.2±0.84^{d}
Melkashola *0:4 (CH: SM)	46.0±6.0^{bcd}	16.5±2.15^{e}
Moneymaker *1:1 (CH: SM)	45.7±7.23^{bcd}	23.4±0.86^{d}
Moneymaker *1:3 (CH: SM)	41.7±4.04^{cd}	18.6±1.4^{e}
Miya *0:4 (CH: SM)	40.3±1.53^{cd}	12.8±0.63^{f}
Moneymaker *0:4 (CH: SM)	39.0±1.73^{d}	11.6±3.30^{f}
Lsd	7.19	2.55
Cv	8.99	6.33

* Means with the same letter in column are not significantly different at $p \leq 0.05$. **SH= shoot height, SFW= shoot fresh weight

Table 9. Effect of varieties and coffee husk proportion on root length and weight of tomato

Treatments	**Mean value for Parameters**	
Varieties	RL** in cm	RFW** in g
Moneymaker	15.20^{c}	20.52^{b}
Miya	16.88^{b}	20.75^{b}
Melkashola	18.93^{a}	23.63^{a}
Lsd	1.016	1.027
Cv	7.06	5.61
Coffee husk level		
0:4 (control, only SM)	13.056^{c}	15.76^{d}
1:3 (CH: SM)	16.044^{b}	21.42^{c}
1:1 (CH: SM)	19.03^{a}	23.52^{b}
3:1 (CH: SM)	19.88^{a}	25.82^{a}
Lsd	1.173	1.1862
Cv	7.06	5.61

*Means with the same letter in column are not significantly different at $p \leq 0.05$. **RL= root length and RFW= root fresh weight.

4.3.2. The interaction effect of coffee husk and variety on nematode reproductive parameter on tomato

The effect of variety and coffee husk amendment had a significant influence on eggmass production ($p<0.05$). The interaction effect on mean number of eggmass was also found to be significant, the mean maximum number of eggmass per 5 g of root was noted on combination of Moneymaker with non-amended control (102.00) followed by Miya with non-amended control (0:4) (61.00) while the mean minimum was recorded on combination of Melkashola amended with 3:1 (CH: SM) (0.0) (Table 10).

The study revealed that both varieties and coffee husk level had significant influence on root gall formations. The interaction effect on root gall formation is found to be significant ($p<0.05$). The mean maximum number of root gall per 5 g of root was recorded on treatment combination of Moneymaker with non-amended control (248.00) followed by Moneymaker amended with 1:3 (CH: SM) (188.33) while the mean minimum was recorded on combination of Melkashola amended with 3:1 (CH: SM) (0.00) (Table 10).

The current study revealed that final nematode population and reproduction factor were significantly influenced by variety and coffee husk level. The interaction had a significant influence on both nematode reproductive parameters. The final nematode population density was observed to be higher in treatment combination of Moneymaker with non-amended control, which was not significantly different from treatment combination of Miya with non-amended control while lower population was recorded from combination of Melkashola with 3:1 (CH:SM) (Table 10). The mean maximum reproduction factor was recorded at a combination of Moneymaker with non-amended control, which was not significantly different from combination of Miya with control while the mean minimum was noted at a combination of Melkashola with 3:1 (CH: SM) (Table 10).

The result of the study demonstrated that coffee husk amended treatment of combinations were significantly reduced the number of eggmass and root gall per 5 g of root, final nematode population and reproduction factor of *M. arenaria* as compared to non-amended treatments. Application of organic manure influences soil nematode community structure,

diversity and activity (Liang *et al*., 2009). Organic amendment increases the abundance of fungivores, bacterivores and predator (Tabarant *et al*., 2011) but reduce the abundance of plant parasites including PPNs (Korthals *et al*., 2014). Coffee husk is composed of 58-85% of carbohydrates, 8-11% of proteins, 0.5-3% of lipids, 3-7% of minerals, and minor amounts of bioactive compounds, such as caffeine 1%, chlorogenic acid 2.5% and tannins 5-9% are also present in this decomposed residue (Cruz, 2014; Bondesson, 2015). It is also a suitable substrate for the microbial growth and enzyme production, due to its high amount of fermentable sugars (Bondesson, 2015). In this study it was observed that as increasing the proportion of coffee husk proportion in treatment of combinations nematode reproduction parameter reduced significantly. This might be the releasing of nitrogen compounds, organic acids, or other compounds that may have adverse effects on nematodes (Oka, 2010). Decomposed coffee husk may exert antagonistic effect on *M. arenaria* due to the existence of these toxic compounds.

Several studies confirm that soil organic amendments are an alternative method of nematode control (Renčo *et al*., 2007). Moselhy (2009) reported that compost significantly reduced the root galling and the final population on sun flower. For instance, very high application rates (50-100%) of composts in pots reduced both root knot nematode galling and numbers of J2 in soil and roots (Nico *et al*., 2004). Other reports proved that compost application improved growth of infected plants and reduced nematode population (Cayuela *et al*., 2008). Dias-Arieira *et al*. (2015) reported that bokashi and crambe meal amendment reduced the number of eggs/g of root and promoted plant growth.

The reduction of eggmass development, root galling, final nematode population and reproduction factor in treatment combination of Melkashola with 0:4, 1:3, 1:1 and 3:1 (CH to SM) may be due to induction of resistance (Siddiqui and shaukat, 2004) may be variety contains a high phenolic content (Flores *et al*., 2017). The reduction in number of root gall and eggmass, final nematode population and reproduction factor in CH amended treatment might be resulted due to releasing of phenolic compounds (Kokalis-Burelle *et al*., 1994). Use of organic amendment not only enhance soil fertility, but also increase microbial diversity and reduce population density of root knot nematode (Ahmed and Siddiqui, 2009).

Nico *et al*. (2004) reported amending the potting mixture with dry grape marc reduced the root galling by 24.4% and 25.6% and final populations by 34.2 % and 34.7 % of *M. incognita* race 1 and *M. javanica* on tomato respectively. The author stated that the increasing of rate of amendment reduced the root galling caused by *Meloidogyne incognita* race 1 by 40.8 % and the final nematode population by 81.9 %. Similarly, Ogwulumba *et al*. (2010) showed that soil amended with grass ash and rice husk ash at the range of 10-20 ton/ha significantly reduced population density of *Meloidogyne* spp in tomato in Nigeria. In other study in Nigeria, amending soil with rice husk, saw dust and refuse dump reduce the final nematode population, number of gall and eggmass per 10 gram of root on tomato as compared to non-amended control (Hassan *et al*., 2010). The author described as the rate of application increases, the nematode reproductive parameters were decreased. Abdeldaym *et al*. (2014) stated that the root galling, nematode population density and the reproduction rate of *M. incognita* on melon were significantly reduced in all amended plots in comparison to control.

Table 10. The interaction effect of variety and coffee husk level amendment on number of eggmass and root gall, final nematode population and reproduction factor

Treatments	**Mean value for nematode reproductive parameters**			
Interaction	**NEM****	**NRG ****	**FP****	**RF****
Moneymaker *0:4	102.00^{a}	248.00^{a}	69933 (4.84)^{a}	34.97 (1.5)^{a}
Miya *0:4	61.00^{b}	183.67^{b}	61533 (4.78)^{a}	30.77 (1.5)^{a}
Moneymaker *1:3	44.33^{c}	188.33^{b}	23467 (4.37)^{b}	11.73 (1.1)^{b}
Miya *1:3	41.67^{c}	120.67^{c}	21900 (4.34)^{b}	10.95 (1.1)^{b}
Moneymaker *1:1	5.67^{d}	73.00^{d}	5527 (3.74)^{c}	2.76 (0.57)^{c}
Miya *1:1	4.00^{de}	58.67^{e}	3092 (3.49)^{c}	1.55 (0.41)^{d}
Moneymaker *3:1	3.67^{de}	23.33^{f}	873 (2.94)^{d}	0.44 (0.15)^{e}
Miya *3:1	2.33^{de}	19.33^{f}	775 (2.88)^{d}	0.38 (0.14)^{e}
Melkashola *0:4	1.33^{e}	1.67^{g}	653 (2.801)^{d}	0.327 (0.12)^{e}
Melkashola *1:3	0.00^{e}	0.67^{g}	553 (2.72)^{de}	0.27 (0.1)^{ef}
Melkashola *1:1	0.00^{e}	0.00^{g}	310 (2.48)^{e}	0.155 (0.06)^{fg}
Melkashola *3:1	0.00^{e}	0.00^{g}	70 (1.68)^{f}	0.035(0.01)^{g}
Lsd	4.27	8.73	6853.1 (0.31)	3.43 (0.05)
Cv	11.43	6.77	5.29	5.89

*Means with the same letter in column are not significantly different at $p \leq 0.05$. Figures in parentheses are transformed by log (x+1). **NEM= number of eggmass, NRG= number of root gall, RGI= root gall index, FP= final nematode population, RF= reproduction factory.

5. SUMMARY AND CONCLUSION

Tomato is an important vegetable crop in the world and produced widely in our country, Ethiopia. Its production and productivity was declined by biotic and abiotic factors. PPNs are the major constraints to tomato production. *Meloidogyne* species are predominant phytoparasitic nematode which poses the major threat in production of tomato.

A nematological survey was carried out at Karsa and Dedo district. A total of nine nematodes genera were detected namely *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Scutellonema*, *Rotylenchulus*, *Aphelenchus*, *Criconema*, *Paratylenchus* and cyst nematodes. RKNs are the most frequent and prominent genera in the study area. Cyst nematodes (*Globodera spp.)* and *Aphelenchus spp.* were detected and reported here for the first time from Ethiopia and *Meloidogyne* spp isolated from JUCAVM was characterized and identified both morphologically and molecularly, it was confirmed as *Meloidogyne arenaria*. The farmer's agronomic practices have an influence on the distribution and abundance of PPNs in the study area.

In general, the study revealed that significant differences were noticed among the varieties against the *M. arenaria*. Moneymaker, Margilobe, Gelilema, Roma VF, Fetan, Melkasalsa, Metadel, Cochoro, Bishola, APR d2 tomato, Chali, Miya and Gelila were found to be susceptible by supporting high rate of nematode reproduction while, Melkashola were found to be resistance and reduce the reproduction potential of *M. arenaria*. So that Melkashola is promising materials to be used as resistant variety. Cultivation of resistance variety to root knot nematode will be a profitable alternative for the production of healthy, toxic free tomato to the consumers.

The result clearly suggested that combination of coffee husk and variety drastically decreased nematode population in the soil as well as in the root. Coffee husk amendment was found to be effective to manage *M. arenaria*; moreover application of high proportion of coffee husk in treatment of combination gave better result. Therefore, both Melkashola variety and coffee husk application are an alternative management option to reduce root knot disease inflicted by *M. arenaria*. The severe infections on tomato plants and growth impairment observed in fields

during sampling, requires immediate attention and implementation of feasible control strategies. Therefore, further study needed to test cross compatibility between *M. arenaria* and resistance variety and further testing of the varieties across several locations under field condition will be important to determine the durability of resistance. And also further study will be needed to investigate and identify the candidate gene found in Melkashola variety that made variety resistant to *M. arenaria* infestation. Moreover, several attempts in CH amendments are needed to confirm actual rates and timing of amendments under field condition before any recommendation to the farmers can be made.

6. REFERENCES

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. *Molecular plant pathology*, ***4*(4)**, pp.217-224.

Abad, P., Gouzy, J., Aury, J.M., Castagnone-Sereno, P., Danchin, E.G., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., Blok, V.C. and Caillaud, M.C., 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature biotechnology*, ***26*(8)**, p.909.

Abdeldaym, E.A., Erriquens, F., Sasanelli, N., Ceglie, F.G., Zaccone, C., Miano, T. and Cocozza, C., 2014. Effects of several amendments on organic melon growth and production, Meloidogyne incognita population and soil properties. *Scientia Horticulturae*, ***180***, pp.156-160.

Abd-Elgawad, M.M.M., 2014. Plant-parasitic nematode threats to global food security. In *Journal of Nematology*. ***46*(2)**, pp. 130-130. PO Box 311, Marceline, MO 64658 USA: SOC Nematologists.

Abdou, M.A.M., 2014. Root-knot nematodes: abundance in organic farming, differentiation among populations, microbes attached to juveniles in soil, and bacterial antagonists. *Dissertationen aus dem Julius Kühn-Institut*, p.148.

Abebe, E., Mekete, T., Seid, A., Meressa, B.H., Wondafrash, M., Addis, T., Getaneh, G. and Abate, B.A., 2015. Research on plant-parasitic and entomopathogenic nematodes in Ethiopia: a review of current state and future direction. *Nematology*, ***17*(7)**, pp.741-759.

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

Afolami, S.O., Solomon, S. and Daramola, F., 2014. Biodiversity of plant-parasitic nematodes of sugarcane in Bacita, Nigeria. *Journal of Entomology and Nematology*, *6*(6), pp.71-79.

Ahmad, F. and Siddiqui, M.A., 2009. Management of root-knot nematode, Meloidogyne incognita in tomato. *Pak J Nematol*, ***27*(2)**, pp.369-373.

Ahmad, N., Hassan, F. and Belford R., 2009. Effects of soil compaction in the sub-humid cropping environment in Pakistan on uptake of N P K and grain yield in wheat (*Triticum aesitivum*) II, Alleviation. *Field Crops Res* ***110***:61-68.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J., 1990. Basic local alignment search tool. *Journal of molecular biology*, ***215*(3)**, pp.403-410.

Ambecha, O.G., Struik, P.C. and Emana, B., 2007. Tomato production in Ethiopia: constraints and opportunities. http://www.tropentag.de/2012/abstracts/full/659.pdf.

Amer-Zareen, Z.M.J., Abid, M., Gowen, S.R. and Kerry, B.R., 2004. Management of root knot nematode (*Meloidogyne javanica*) by biocontrol agents in two crop rotations. *Int. J. Biol. Biotechnol*, *1*, pp.67-73.

Anang, B.T., Zulkarnain, Z.A. and Yusif, S., 2013. Production constraints and measures to enhance the competitiveness of the tomato industry in Wenchi Municipal District of Ghana. *American journal of Experimental agriculture*, ***3(4)***, p.824.

Asgedom, S., Struik, P.C., Heuvelink, E. and Araia, W., 2011. Opportunities and constraints of tomato production in Eritrea. *African Journal of Agricultural Research*, ***6*(4)**, pp.956-67.

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

Ayana, A., Afari-Sefa, V., Emana, B., Dinssa, F.F., Balemi, F. and Temesgen, M., 2014. Analysis of vegetable seed systems and implications for vegetable development in the humid tropics of Ethiopia. *International Journal of Agriculture and Forestry*, ***4*(4)**, pp.325-337.

Ayandiji, A., Adeniyi, O.R. and Omidiji, D., 2011. Determinant postharvest losses among tomato farmers in Imeko-Afon local government area of Ogun State, Nigeria. *Global Journal of Science Frontier Research*, ***11*(5)**, pp.23-27.

Baiye, M.B. and Wesemael, W.M.L., 2016. *Survival of the quarantine root-knot nematodes M. chitwoodi and M. fallax in waste products of the processing industry* (Doctoral dissertation, MSc thesis University of Ghent.

Banora, M.Y. and Almaghrabi, O.A.A., 2019. Differential response of some nematode-resistant and susceptible tomato genotypes to Meloidogyne javanica infection. *Journal of Plant Protection Research*, ***59*(1)**.

Bao, Y. and Neher, D.A., 2011. Survey of lesion and northern root-knot nematodes associated with vegetables in Vermont. *Nematropica*, ***41*(1)**, pp.100-108.

Baredo, Y., 2012. Gamo Gofa zone diagnosis and planning document, Livestock and Irrigation Value Chains for Ethiopian Smallholders (LIVES) project. *Addis Ababa, Ethiopia*.

Barker, K.R. and Koenning, S.R., 1998. Developing sustainable systems for nematode management. *Annual review of phytopathology*, ***36*(1)**, pp.165-205.

Bauchet, G. and Causse, M., 2010. Genetic diversity in tomato (Solanum lycopersicum) and its wild relatives.

Been, T., Schomaker, C. and Norshie, P., 2011. Estimation of partial resistance in potato genotypes against Meloidogyne chitwoodi. *Nematology*, *13*(4), pp.477-489.

Bélair, G., Dauphinais, N. and Jobin, G., 2011. Soil amendments with Streptomyces lydicus WYEC108 and chitin against the northern rootknot nematode, Meloidogyne hapla Chitwood, on tomato. *Russian Journal of Nematology*, ***19*(1).**

Belay, B., Sakhuja, P.K. and Tefera, T., 2013. Integrated management of root-knot nematode (Meloidogyne incognita) for tomato production and productivity. *Ethiopian Journal of Science and Technology*, ***6*(2)**, pp.79-91.

Bhowmik, d., Sampath Kumar, k.p., Paswan, s., Srivastava, s., 2012: Tomato − A natural medicine and its health benefits. *J. Pharmacognosy Phytochem*. ***1***, 24-36.

Blok, V. C., and Powers, Thomas O., 2009. Biochemical and molecular identification. In R. N Perry, M. Moens, & J. L. Starr (eds.), Root-knot nematodes (pp. 98-118). *CAB International*. Nosworthy Way. Wallingford, Oxfordshire OX10 8DE, UK.

Bond, J.P., McGawley, E.C. and Hoy, J.W., 2000. Distribution of plant-parasitic nematodes on sugarcane in Louisiana and efficacy of nematicides. *J. of Nematology*, ***32*(4)**, p.493.

Bondesson, E. 2015. A nutritional analysis on the by-product coffee husk and its potential utilization in food production. Bachelor thesis. Faculty of natural Resources and Agricultural Sciences, Uppsala.

Brueske, C.H., 1980. Phenylalanine ammonia lyase activity in tomato roots infected and resistant to the root-knot nematode, *M. incognita*. *Physiological Plant Path.* ***16*** **(3)**, pp. 409-414.

Cadet, P. and Spaull, V.W., 2005. Nematode Parasites of Sugarcane. In: Nematodes in Subtropical and Tropical Agriculture. Luc, M., Sikora, R.A. and Bridge, J. (Eds.), pp 645-674. *CAB International*, Wallingford, UK.

Capel, C., Yuste-Lisbona, F.J., López-Casado, G., Angosto, T., Heredia, A., Cuartero, J., Fernández-Muñoz, R., Lozano, R. and Capel, J., 2017. QTL mapping of fruit mineral contents provides new chances for molecular breeding of tomato nutritional traits. *Theoretical and applied genetics*, ***130*(5)**, pp.903-913.

Carneiro, R.M.D.G., Monteiro, J.M.S., Silva, U.C. and Gomes, G., 2016. Gênero *Meloidogyne*: diagnose através de eletroforese de isoenzimas e marcadores SCAR. *Diagnose de fitonematoides*.

Castagnone-Sereno, P., 2006. Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. *Heredity*, ***96***, 282–289.

Castagnone-Sereno, P., Danchin, E.G.J., Perfus-Barbeoch, L., and Abad, P., 2013. Diversity and evolution of root-knot nematodes, genus *Meloidogyne*: new insights from the genomic era. *Annual Review of Phytopathology*. ***51***, 203–220.

Cayuela, M.L., Millner, P.D., Meyer, S.L.F. and Roig, A., 2008. Potential of olive mill waste and compost as biobased pesticides against weeds, fungi, and nematodes. *Science of the total environment*, ***399*****(1-3),** pp.11-18.

Choudourou, D.C., Agbaka, A., Adjakpa, J.B., Koutchika, R.E. and Adjalian, E.J.N., 2012. Inventaire préliminaire de l'entomofaune des champs de tomates (Lycopersicon esculentum Mill) dans la Commune de Djakotomey au Bénin. *International Journal of Biological and Chemical Sciences*, ***6*****(4),** pp.1798-1804.

Clark, L.J., Whalley, W.R. and Barraclough, P.B., 2003. How do roots penetrate strong soil?. In *Roots: The Dynamic Interface Between Plants and the Earth* (pp. 93-104). Springer, Dordrecht.

Corbett, B.P., Jia, L., Sayler, R.J., Arevalo-Soliz, L.M. and Goggin, F., 2011. The effects of root-knot nematode infection and mi-mediated nematode resistance in tomato on plant fitness. *Journal of nematology*, ***43*****(2)**, p.82.

Cousins, P. and Walker, M.A., 1998. Improved techniques for evaluating root-knot nematode resistance in Vitis rootstocks. In *VII International Symposium on Grapevine Genetics and Breeding* ***528*** (pp. 575-577).

Coyne, D.L., Fourie, H.H. and Moens, M., 2009. 19 Current and Future Management Strategies in Resource-poor Farming. *Root-knot nematodes*, p.444.

Coyne, D.L., Nicol, J.M. and Claudius-Cole, B. 2018. Practical plant nematology: a field and laboratory guide. 3rd edition, *International Institute of Tropical Agriculture* (*IITA*), Ibadan, Nigeria

Crawford, J.M. and Clardy, J. 2011. Bacterial symbionts and natural products. *Chemical Communications*, Cambridge, ***47 (27)***:7559-7566.

Cruz, R., 2014. Coffee by-products: Sustainable Agro-Industrial Recovery and Impact on Vegetables Quality: dissertation thesis. Universidade de Porto.

CSA, 2014. Agricultural sample survey. Volume I. Report on area and production of major crops (private peasant holdings, meher season) Statistical bulletin 532. Addis Abeba, Ethiopia.

CSA, 2015. Agricultural sample survey. Volume I. Report on area and production of major crops (private peasant holdings, meher season) Statistical bulletin 278. Addis Abeba, Ethiopia.

CSA, 2016. Agricultural sample survey. Volume I. Report on area and production of major crops (private peasant holdings, meher season) Statistical bulletin 584. Addis Abeba, Ethiopia.

CSA, 2017. Agricultural sample survey. Volume I. Report on area and production of major crops (private peasant holdings, meher season) Statistical bulletin 584. Addis Abeba, Ethiopia.

CSA, 2018. Agricultural sample survey. Volume I. Report on area and production of major crops (private peasant holdings, meher season) Statistical bulletin 586. Addis Abeba, Ethiopia.

Cunha, T.G.D., Visôtto, L.E., Lopes, E.A., Oliveira, C.M.G. and God, P.I.V.G., 2018. Diagnostic methods for identification of RKNs species from Brazil. *Ciência Rural*, ***48*****(2)**.

Dallemole-Giaretta, R., Freitas, L.G.D., Xavier, D.M., Zooca, R.J.F., Ferraz, S. and Lopes, E.A., 2014. Soil amendment with substrate containing mycelium and conidia of *Pochonia chlamydosporia* for the management of *M. javanica*. *Ciência Rural*, ***44(4)***: p.629-633.

Davide, R.G., 1985. Summary report on the current status, progress and needs for Meloidogyne research in region VI.

De Waele, D, Mcdonald AH, Jordaan EM, Orion D, Van Den Berg E, Loots, GC. 1998. Plant-parasitic nematodes associated with maize and pearl millet in Namibia. *African Plant Protection.* **4**:113-117.

Devran, Z. and Elekçioğlu, İ.H., 2004. The Screening of F_2 Plants for the Root-Knot Nematode Resistance Gene, Mi by PCR in Tomato. *Turkish Journal of Agriculture and Forestry*, ***28*****(4)**, pp.253-257.

Dias-Arieira, C.R., Mattei, D., Puerari, H.H. and Ribeiro, R.C., 2015. Use of organic amendments in the management of root-knot nematode in lettuce. *Horticultura Brasileira*, ***33*****(4)**, pp.488-492.

Efthimiadou, A., Bilalis, D., Karkanis, A., Froud-Williams, B. and Eleftherochorinos, I., 2009. Effects of cultural system (organic and conventional) on growth, photosynthesis and yield components of sweet corn (Zea mays L.) under semi-arid environment. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, ***37*****(2)**, pp.104-111.

EHDA, 2012. Ethiopian Horticulture Sector Statistical Bulletin. Issue 01, Addis A. Ethiopia. Available at http://ehda.gov.et/ Downloads/Statistical Bulle-tin% 282012% 29.

Eisenback, J.D., Hirschmann, H., Sasser, J.N. and Triantaphyllou, A.C., 1981. *A guide to the four most common species of root-knot nematodes [Meloidogyne species] with a pictorial key* (No. REP-7253. CIMMYT.).

Emana, B. and Gebremedhin, H., 2007. Constraints and opportunities of horticulture production and marketing in eastern Ethiopia. *Dry land coordination group (DCG) report*, ***46***.

Emana, B., Afari-Sefa, V., Nenguwo, N., Ayana, A., Kebede, D. and Mohammed, H., 2017. Characterization of pre-and postharvest losses of tomato supply chain in Ethiopia. *Agriculture & Food Security*, ***6*****(1)**, p.3.

Emana, B., Ayana, A., Balemi, T. and Temesgen, M., 2014. Scoping study on vegetables seed systems and policy in Ethiopia. *Addis Ababa, Ethiopia*.

Esfahani, M.N., 2009. Distribution and identification of root-knot nematode species in tomato fields. *Mycopath*, ***7*****(1)**, pp.45-49.

Esfahani, M.N., Ahmadi, A.R. and Shirazi, K., 2012. Susceptibility Assessments of Tomato Genotypes to Root-Knot Nematodes, Meloidogyne javanica. *Journal of Ornamental and Horticultural plants*, ***2*****(2)**, pp.113-121.

FAO, 2014. Area and production statistics of tomato. http://faostat.fao.org

FAO, 2017. FAOSTAT Database. Available online at: http://www.fao.org/home/en/ (Date of access: 12.10.2018.

FAOSTAT., 2014. Food and Agriculture Organization statistical database. *Retrieved Aug*.

Fassuliotis, G. 1979. Plant breeding for root-knot resistance. pp. 425-453. In: Lambert, F. and Taylor, C.E. (Eds.). Root-knot nematodes (*Meloidogyne* species) systematics, biology and control. *Academic Press*. New York.

Favery, B., Ryan, E., Foreman, J., Linstead, P., Boudonck, K., Shaw, P., Steer, M. and Dolan, L. 2001. KOJAK is a cellulose synthase-like gene required for root hair cell morphogenesis in Arabidopsis. Genes Dev. ***15***: 79-89.

Fentik, D.A., 2017. Review on genetics and breeding of tomato (Lycopersicon esculentum Mill.). *Adv Crop Sci Tech*, ***5*****(5)**, p.306.doi:10.4172/2329-8863.1000306

Ferraz, S. and Mendes, M.L. 1992. The root knot nematode in tomato. *Eur J Plant Pathol*. Infor Agropecu. ***16***: 43-45.

Ferris, H. and Noling, J.W. 1987. Analysis and prediction as a basis for management decisions. *Principles and practice of nematode control in crops/edited by RH Brown and BR Kerry*.

Ferris, H. and Van Gundy, S.D., 1979. *Meloidogyne* ecology and host interrelationships. In: Lamberti, F. and Taylor, C.E. (eds) Root-knot Nematodes (*Meloidogyne* species). Systematics, Biology and Control. Academic Press, London, pp. 205–230.

Ferris, H. N., 1999. Available at: http://plpnemweb.ucdavis.edu/nemaplex/ Taxamnus/ G076mnu.htm>. Accessed: Aug.11, 2019.

Flores, p., Sánchez, E., Fenoli, j., Heltin, p., 2017. Genotypic variability of carotenoids in traditional tomato cultivars. Food Res. Int. ***100***, pp.510-516.

Fosu-Nyarko, R. and Jones, M.G.K., 2016. Advances in understanding the molecular mechanisms of root lesion nematode host interactions. *Annu. Rev. Phytopathol*. ***8***. pp. 253–278.

Franca, A.S. and Oliveira, L.S., 2009. Coffee processing solid wastes: current uses and future perspectives. *Agricultural wastes*, ***9***, pp.155-189.

Ganaie, M. A. and Khan, T. A., 2011. Studies on the interactive effect of *M. incognita* and *fusarium solani* on *Lycopersicon esculentum*, mill. *Intern. J. of Botany* ***7*****(2)**: 205 - 208.

García, L.E. and Sánchez-Puerta, M.V., 2015. Comparative and evolutionary analyses of Meloidogyne spp. based on mitochondrial genome sequences. *PloS one*, ***10*****(3)**, p.e0121142.

Gemechis, A. O., Struik, P., and Emana, B., 2012. Tomato production in Ethiopia: constraints and opportunities (Abstract). http://dpg.phytomedizin.org/fileadmin/tagungen/07_

Gheysen, G., Jones, J., Perry, R. and Moens, M. 2006. Molecular aspects of plant-nematode interactions. In Perry, R.N. and Moens, M. (eds). *Plant nematology*, pp. 234-254.

Gill, H.K. and McSorley, R.O.B.E.R.T. 2011. Effect of different inorganic/synthetic mulches on weed suppression during soil solarization. *Proc Fla State Hortic*, ***124***:310-313.

Gudero, G., Hussien, T., Dejene, M. and Biazin, B., 2017. Integrated Management of Tomato Late Blight [*Phytophthora infestans* (Mont.) de Bary] Through Host Plant Resistance and Reduced Frequency of Fungicide in Arbaminch Areas, Southern Ethiopia.

Haider, M.G., Dev, L.K. and Nath, R.P., 2003. Comparative pathogenicity of root knot nematode, *M. incognita* on different pulse crops. *Indian Journal of Nematology*, ***33***: pp.152-153.

Hajra, N., Firoza, K. and Shahina, F., 2009. Effects of VAM and nematode interaction on some biochemical parameters of sunflower. *Pakistan Journal of Nematology*, ***27*****(2)**, pp.193-201.

Hallmann, J. and Meressa, B.H., 2018. 10 Nematode Parasites of Vegetables. *Nematodes in Subtropical and Tropical Agriculture*, p.346.

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

Hasan, S.I. and Abood, I.D., 2018. Identification of *Meloidogyne* spp. According to Morphological characteristics, PCR- SCAR Marker and 18S rDNA region in Iraq. *Journal of Agriculture and Veterinary Science*. ***11*****(1)**, Pp**.** 50-56.

Hassan, M.A., Chindo, P.S., Marley, P.S. and Alegbejo, M.D., 2010. Management of root knot nematodes (*Meloidogyne* spp.) on tomato (*Lycopersicon lycopersicum*) using organic wastes in Zaria, Nigeria. *Plant Protection Science*, ***46*(1)**, pp.34-38.

Holbrook, C.C., Knauft, D.A. and Dickson, D.W., 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria*. *Plant Disease*, ***67*(9)**, pp.957-958.

Holterman, M., Rybarczyk, K., Van den Elsen, S., Van Megen, H., Mooyman, P., Santiago, R.P., Bongers, T.O.M., Bakker, J. and Helder, J., 2008. A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources*, ***8*(1)**, pp.23-34.

Hooper, D.J. and Evans, K., 1993. Extraction, identification and control of nematodes.

Hooper, D.J., 1986. Extraction of Free Living Stages From Soil, 5–30. *Labarotory Methods for Work with Plant and Soil Nematodes.(Ed. Southey, JF). Her Majesty's Stationery Office, London*.

Hunt, D.J. and Handoo, Z.A., 2009. Taxonomy, identification and principal species. *Root-knot nematodes*, ***1***, pp.55-88.

Hussey, R.S, and Barker, K.R. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. *Plant Disease Reporter*. ***57***:1025-28.

Hussey, R.S. and Boerma, H.R., 1989. Tolerance in maturity groups of soybean cultivars to *Heterodera glycines. Supplement to Journal of Nematology*, ***21***: pp.686-692.

Jacquet, M., Bongiovanni, M., Martinez, M., Verschave, P., Wajnberg, E. and Castagnone-Sereno, P., 2005. Variation in resistance to the root-knot nematode Meloidogyne incognita in tomato genotypes bearing the Mi gene. *Plant Pathology*, ***54*(2)**, pp.93-99.

Jaiteh, F., Kwoseh, C. and Akromah, R. 2012. Evaluation of tomato genotypes for resistance to root-knot nematodes. *African Crop Science Journal*. ***20*(1)**: 41 – 49.

Janati S., Houari A., Wifaya A., Essarioui A., Mimouni A., Hormatallah A., Sbaghi M. , Dababat A. A. and Mokrini F., 2018. Occurrence of the Root Knot Nematode species in Vegetable Crops in Souss Region of Morocco. *Plant Pathol. J.* ***34*(4)**: 308-315.

Jaubert, S., Ledger, T.N., Piotte, C., Abad, P., and Rosso, M.N. 2002. Direct identification of stylet secreted proteins from root-knot nematodes by a proteomic approach. *Molecular and Biochemical Parasitology*, ***121***, 205-211.

Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M.L., 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol. Plant Pathology.****14***, 946-961.

Ka¸skavalci, G., 2007. Effect of soil solarization and organic amendment treatments for controlling *M. incognita* in tomato cultivars in Western Anatolia. *Turkish Journal of Agriculture and Forestry*: ***31***, 159-167.

Kamran, M., Anwar, S.A., Javed, N., Khan, S.A., ul Haq, I. and Ullah, I., 2012. Field evaluation of tomato genotypes for resistance to Meloidogyne incognita. *Pakistan J. of Zool.*, ***44*****(5)**.

Karajeh, M., 2008. Interaction of root-knot nematode (*M. Javanica*) and tomato as affected by hydrogen peroxide. *J Plant Prot Res*. ***48*****(2)**:2.

Karssen, G., 2002b. The plant-parasitic nematode genus Meloidogyne Göldi, 1892(Tylenchida) in Europe. *Brill Academic Pub*, 160 pp.

Karssen, G., Moens, M. and Perry, R., 2006. Root-knot nematodes. In Perry RN, and Moens M. (eds) Plant nematology. CABI Publishing Wallingford, UK, pp 59–90.

Karuri, H.W., Olago, D., Neilson, R., Mararo, E. and Villinger, J., 2017. A survey of root knot nematodes and resistance to Meloidogyne incognita in sweet potato varieties from Kenyan fields. *Crop protection*, ***92***, pp.114-121.

Kaushik, S. K., Tomar, D. S. and Dixit, A. K. 2011. Genetics of fruit yield and it's contributing characters in tomato (*Solanum lycopersicom*). *J. Agr.Biotec.Sust.dev`t*, ***3*****(10)**: 209-213.

Kavitha, J., Jonathan, E.I. and Umamaheswari, R., 2007. Field application of Pseudomonas fluorescens, *B. subtilis* and *Trichoderma viride* for the control of *M. incognita* (Kofoid and White) Chitwood on sugar beet. *Journal of Biological Control*, ***21*****(2)**:211-215.

Khan, A., Wesemael, W. M., Moes, M., 2014. Influence of temperature on the development of the temperate root-knot nematodes Meloidogyne chitwoodi and M. fallax. *Russian journal of nematology*: ***22*****(1)**, 1-9.

Khan, M.R. 1994. Nematology in developing countries; India-IMP, Region VIII. pp. 379-398. In: Carter, C.C. and Sasser, J.N. (Eds.). An advanced treatise on M*eloidogyne* vol. ***1***: Biology and control. Co-publication of Department of Plant Pathology North Carolina State University and the USAID, Raleigh, North Carolina, USA.

Kokalis-Burelle, N., Rodríguez-Kábana, R., Weaver, C.F. and King, P.S., 1994. Evaluation of powdered pine bark for control of *Meloidogyne arenaria* and *Heterodera glycines* on soybean. *Plant and Soil*, ***162*****(2)**, pp.163-168.

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

Korthals, G.W., Thoden, T.C., Van Den Berg, W. and Visser, J.H.M., 2014. Long-term effects of eight soil health treatments to control plant-parasitic nematodes and *Verticillium dahliae* in agro-ecosystems. *Applied soil ecology*, ***76***, pp.112-123.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R. and Thompson, J.D., 2007. Clustal W and Clustal X version 2.0. *bioinformatics*, ***23*****(21)**, pp.2947-2948.

Lemma, D., 2002. Research experience and production prospects. Ethiopian Agricultural Research Organization (EARO), Addis Ababa, Ethiopia, pp 20-28.

Li, X., Lewis, E.E., Liu, Q., Li, H., Bai, C. and Wang, Y., 2016. Effects of long-term continuous cropping on soil nematode community and soil condition associated with replant problem in strawberry habitat. *Scientific reports*, ***6***, p.30466.

Liang, W., Lou, Y., Li, Q., Zhong, S., Zhang, X. and Wang, J., 2009. Nematode faunal response to long-term application of nitrogen fertilizer and organic manure in Northeast China. *Soil Biology and Biochemistry*, ***41*****(5)**, pp.883-890.

Liharska, T.B., 1998. Genetic and molecular analysis of the tomato root-knot nematode resistance locus Mi-1. Liharska.

Mae, J.H. 1985. Varietal trial on tomato. Training Report of the 3rd Regional Training Programme in Vegetable Production and Research. TOP?AVRDC, Kasetsart University, Kamphaeng Saen, Nakhon Pathom.

Mai, W.F. and Abawi, G.S., 1987. Interactions among root-knot nematodes and Fusarium wilt fungi on host plants. *Annual Review of Phytopathology*, ***25*****(1)**, pp.317-338.

Maliwichi, L.L, Pfumayaramba, T.K.and Katlego, T. 2014. An Analysis of Constraints That Affect Smallholder Farmers in the Production of Tomatoes in Ga-Mphahlele, Lepelle Nkumbi Municipality, Limpopo Province, South Africa. *J Hum Ecol.* **47(3)**: 269-274.

Mandefro, W. and Mekete, T. 2002. Root-knot nematode on vegetable crops in Central and Western Ethiopia. *Pest Management Journal of Ethiopia*.***6***:37-44.

Manjunatha, T. G., Rai, A.b. and Singh, B., 2017. Root knot nematode: A treat to vegetable production and its management. IIVR technical Bulletin No. 76, IIVR, Varanasi, pp.32.

Manzanilla-López, R.H. and Starr, J.L., 2009. 10 Interactions with Other Pathogens. *Root-knot nematodes*, p.223.

McNeill, M.R., Phillips, C.B., Bell, N.L. and Proffitt, J.R., 2006. Potential spread of pests in New Zealand through commercial transport of nursery plants. *New Zealand Plant Protection*, ***59***, pp.75-79.

Mekete, T., Mandefro, M. and Greco, N., 2003. Relationship between initial population densities of Meloidogyne javanica and damage to pepper and tomato in Ethiopia. *Nematologia mediterranea*, ***31*****(2)**.

Meressa, B.H., Dehne, H.W. and Hallmann, J. 2014a. Nematodes of cut-flowers in Ethiopia. *International Journal of Nematology*. ***24***:1-10.

Meseret, D., Ali, M. and Kassahun, B., 2012. Evaluation of Tomato (*Lycopersicon esculentum* L.) Genotypes for yield and yield component. The *African Journal of Plant Science and Biotechnology* pp 45-49.

Meyer, S.L. F., Samuel, B. O., Chitwood D. J., Bryan, T.V. and Millner, P.D., 2011. Poultry litter compost for suppression of root-knot nematode on cacao plants. *International J. of Nematology*. ***21(2)*** pp. 153-162.

Missanga, J.S. and Rubanza, C.D., 2018. Distribution and Identification of Nematodes in Tomato Farmers' Fields in the Selected Semi-Arid Climates of Central and Northern Tanzania. *Journal of Advances in Microbiology*, pp.1-8.

Moens, M., Perry, R. N. and Starr, J. L., 2009. Meloidogyne species a diverse group of novel and important plant parasites. In Perry, RN, Moens, M and Starr, JL. (eds). Root-knot nematodes CABI International, Cambrige, MA (USA), pp. 1-17.

Mohamed, S., 2010. Biological, Chemical and Molecular Studies on the Systemic Induced Resistance in Tomato against Meloidogyne incognita Caused by the Endophytic *Fusarium oxysporum*, Fo162. Bonn, Germany: Institute of Crop Science and Resource Conservation, University of Bonn, PhD thesis.

Moselhy, M.A., 2009. Bioconversion of Rice Straw and Certain Agro-Indu.l Wastes to Fertilizers for Organic Farming Systems. Ph.D. Thesis. Fac, Agric, Cairo Univ, Egypt, 221 p.

Mulrooney, R., 2012. Nematode control suggestions for vegetables. University of Delaware. Fact sheet number p -01v.

Mwangi, M.W., Kimenju, J.W., Narla, R.D., Muiru, W.M. and Kariuki, G.M., 2017. Evaluation of selected tomato cultivars reaction to infestation with *Meloidogyne javanica* in greenhouse conditions.

Mweke, A.N., Kimenju, J.W., Seif, A.A., Mutitu, E.W. and Mutua, G.K., 2008. Potential of sequential cropping in the management of root knot nematodes in okra.*Asian Journal of Plant Sciences*, ***7*****(4)**.

Nagaraju, N., Karemegam, N. and Kadalmani, B., 2010. Eco-friendly management of root-knot nematode *Meloidogyne incognita* using organic amendments on tomato. *International Journal of Research Pharmacological Science*, ***1***: 530-532.

Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S. and Dhakshnamoorthy, G., 2006. Cold aqueous extract of African marigold, *Tagetes erecta* for control tomato root knot nematode, *Meloidogyne incognita*. *Crop Protection*, ***25***: 1210-1213.

Ngele, K.K. and Kalu, U.N., 2015. Studies on different species of nematodes attacking vegetable crops grown in Afikpo North L.G.A, Nigeria. *Direct Research Journal of Agriculture and Food Science*; ***3*****(4)**:88-92.

Nico, A.I., Jiménez-Díaz, R.M. and Castillo, P., 2004. Control of root-knot nematodes by composted agro-industrial wastes in potting mixtures. *Crop protection*, ***23*****(7)**, pp.581-587.

Nicol, J.M., Turner, S.J., Coyne, D.L., Den Nijs, L., Hockland, S. and Maafi, Z.T., 2011. Current nematode threats to world agriculture. In *Genomics and molecular genetics of plant-nematode interactions* (pp. 21-43). Springer, Dordrecht.

Noling, J.W., 1999. *Nematode management in tomatoes, peppers and eggplant*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Ogwulumba, S.I., Ugwuoke, K.I. and Ogbuji, R.O., 2010. Studies on *Meloidogyne javanica* infestation on roma tomato (*Lycopersicon esculentum* Mill) under different soil amendments. *African journal of Biotechnology*, ***9*****(22)**, pp.3280-3283.

Oka, Y., 2010. Mechanisms of nematode suppression by organic soil amendments—a review. *Applied Soil Ecology*, ***44*****(2),** pp.101-115.

Onkendia, E.M., Kariukib, G.M., Maraisc, M. and Molelekia, L.N., 2014. The threat of root knot nematodes (*Meloidogyne* spp.) in Africa: a review. *Plant Pathology*: **63**,727–737.

Otipa, M. J., Kimenju, J. W., Mutitu, E.W. and Karanja, N. K., 2003. Potential rotation crops and cropping cycles for root-knot (*Meloidogyne* spp.) nematode control in tomato. African Crop science Conference Proceedings, ***6***. Pp.191-197.

Ouattara, A., Tiendrébéogo, F., Lefeuvre, P., Claverie, S., Hoareau, M., Traoré, E.V., Barro, N., Traoré, O. and Lett, J.M., 2017. Tomato leaf curl Burkina Faso virus: a novel tomato-infecting monopartite begomovirus from Burkina Faso. *Archives of virology*, ***162*****(5),** pp.1427-1429.

Park, R.D., Kim, K.Y., Kim, Y.W., De Jin, R., Krishnan, H. and Suh, J.W., 2005. Effect of chitin compost and broth on biological control of Meloidogyne incognita on tomato (*L. esculentum* Mill.). *Nematology*, ***7*****(1)**, pp.125-132.

Perry N.R., Moens M., Star L.J., 2009. Root-knot nematodes. CABI, p 483.

Powers, T. O., Mullin, P. G., Harris, T. S., Sutton, L. A., and Higgins, R. S., 2005. Incorporating molecular identification of *Meloidogyne* spp. into a large-scale regional nematode survey. *Journal of Nematology*, ***37***, 226-235.

Radwan, M.A., Farrag, S.A.A., Abu-Elamayem, M.M. and Ahmed, N.S. 2012. Biological control of the RKN, *M. incognita* on tomato using bioproducts of microbial origin. *Applied Soil Ecology*, ***56***:58-62.

Rashad, F.M., Saleh, W.D. and Moselhy, M.A., 2010. Bioconversion of rice straw and certain agro-industrial wastes to amendments for organic farming systems: 1. Composting, quality, stability and maturity indices. *Bioresource Technology*, ***101*****(15)**, pp.5952-5960.

Rashid, M.H., Al-Mamun, M.H. and Uddin, M.N., 2017. How durable is root knot nematode resistance in tomato?. *Plant Breeding and Biotechnology*, ***5*****(3)**, pp.143-162.

Rathour, K. S., Jola, P. and Sudershan, G., 2006. Community structure of nematodes in Champawat district of Uttaranchal, *India. Indian J. Nematology*, ***36***, 89–93.

Ravi K.K., Sharma N.P. and Vijay K.B., 2018. Profitability of Tomato Production in some Selected Areas in Panna District of Madhya Pradesh. *Int. J. Curr. Microbiol. App. Sci*. ISSN: 2319-7692 Special Issue-6 pp. 2117-2124.

Renčo, M., 2013. Organic amendments of soil as useful tools of nematodes control. *Helminthologia*, ***50*****(1),** pp.3-14.

Renčo, M., D'addabbo, T., Sasanelli, N., Papajová, I., 2007. The effect of five compost of different origin on the survival and reproduction of *G. rostochiensis*. *Nematology*, ***9*****(4)**, pp. 537–43.

Renčo, M., Sasanelli, N. and Šalamún, P., 2009. The effect of two compost soil amendments, based on municipal green and penicillin production wastes, on nematodes. *Helminthologia*, ***46*****(3)**, pp.190-197.

Roberts D., Oliveira C.M.G., NEILSON R. and Blok V., 2016. Molecular diagnostic of nematodes. Pp. 281-324.

Roldi, M., Dias-Arieira, C.R., Severino, J.J., de Melo Santana, S., Santo Dadazio, T., Marini, P.M. and Mattei, D., 2013. Use of organic amendment to control *M. incognita* on tomatoes. *Nematropica*. ***43***: 49-55.

Saavedra, T.M., Figueroa, G.A. and Cauih, J.G.D., 2017. Origin and evolution of tomato production *Lycopersicon esculentum* in México. *Ciência Rural*, ***47*****(3).**

Sakhuja, P.K., Amare Ayalew and Rananjia, K.R. 2004. Diseases and insect pests of agricultural and horticultural crops in Jijiga and Gode area of Somali region. A Status Report. College of Agriculture, Alemaya University. 35 pp.

SAS Institute, 2013. SAS/STAT. User's Guide version. 13.0 Cary, NC, USA, SAS Institute.

Sasanelli, N., D'addabbo, T., Convertini, G. and Ferri, D., 2002. Soil phytoparasitic nematodes suppression and changes of chemical properties determined by waste

residues from olive oil extraction. In *Proceedings of 12th ISCO Conference* (pp. 588-592).

Sasanelli, N., Di Vito, M. and Zaccheo, G., 1992. Population densities of *Meloidogyne incognita* and growth of cabbage in pots. *Nematologia Mediterranea*, ***20*(1)**, pp.21-23.

Sasser, J.N., Hartman, K.M. and Carter, C.C., 1987. *Summary of preliminary crop germplasm evaluations for resistance to root-knot nematodes* (No. QL391. N4 S25). NCSU Department of Plant Pathology and US Agency for International Development.

Seid, A., Fininsa, C., Mekete, T.M., Decraemer, W. and Wesemael, W.M.L., 2015. Tomato (*Solanum lycopersicum*) and root knot nematode (*Meloidogyne* spp.)- a century old battle. *Nematology*, ***17*(9):** pp. 995- 1009.

Seid, A., Fininsa, C., Mekete, T.M., Decraemer, W. and Wesemael, W.M.L., 2017. Resistance screening of breeding lines and commercial tomato cultivars for *Meloidogyne incognita* and *M. javanica* populations (Nematoda) from Ethiopia. *Euphytica*, ***213*(4)**, p.97.

Seid, A., Fininsa, C., Mekete, T.M., Decraemer, W. and Wesemael, W.M.L., 2019. Biodiversity of *Meloidogyne* spp. from major tomato growing areas of Ethiopia. *Eur J Plant Pathol*, ***154***: 513–528.

Seid, A., Fininsa, C., Mekete, T.M., Decraemer, W. and Wesemael, W.M.L., 2019. Damage potential of *Meloidogyne incognita* populations on selected tomato genotypes in Ethiopia. *Nematropica* ***49***:124-133

Shepherd, A.M. 1986. Extraction and estimation of cyst nematodes. In: Southey, J.F. (Ed). *Laboratory Methods for Work with Plant and Soil Nematodes*. London, UK: Her Majesty's Stationery Office, pp. 51–58.

Shiferaw, T., Dechassa, N. and Sakhuja, P.K. 2014. Influence of poultry litter and rapeseed cake on infestation of *Meloidogyne incognita* on tomato in Dire Dawa, eastern Ethiopia. *Pakistan Journal of Nematology*, ***32*(1)**: 67-72.

Shiferaw, T., Dechassa, N. and Sakhuja, P.K., 2017. Management of root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood in Tomato (*Lycopersicon esculentum* Mill. through poultry manure and rapeseed cake. *Journal of Horticulture and Forestry*, ***9*(7)**, pp.59-65.

Shurtleff, M.C. and Averre, C.W., 2000. Diagnosing plant diseases caused by nematodes. *American Phytopathological Society*. pp 21-49.

Siddiqui, I.A. and Shaukat, S.S., 2004. Systemic resistance in tomato induced by biocontrol bacteria against the root-knot nematode, Meloidogyne javanica is independent of salicylic acid production. *Journal of Phytopathology*, ***152*(1),** pp.48-54.

Sikora, R.A. and Fernandez, E., 2005. Nematode parasites of vegetables. *Nematodes in subtropical and tropical agriculture*, ***2***, pp.319-392.

Singh, S.K. and Khurma, R.K., 2007. Susceptibility of six tomato cultivars to *Meloidogyne incognita*. *The South Pacific Journal of Natural Sciences*, ***13***: pp.73-77

Sipes, B.S., Caswell Chen, E.P., Sarah, J.L. and Apt, W.J., 2005. Nematode parasites of pineapple.

Sirias, H.C.I. 2011. Root-knot Nematodes and Coffee in Nicaragua: Management Systems, Species Identification and Genetic Diversity, Plant Breeding. Uppsala, Sweden: Swedish University of Agricultural Sciences, PhD thesis.

Sohrabi, F., Sheikholeslami, M., Heydari, R., Rezaee, S. and Sharifi, R., 2018. Evaluation of four rhizobacteria on tomato growth and suppression of RKNs, *M. javanica* under greenhouse conditions, a pilot study. *Egyptian Journal of Biological Pest Control*, ***28*(1)**, p.56.

Soltani, T., Nejad, R.F., Ahmadi, A.R. and Fayazi, F., 2013. Chemical control of root-knot nematode (Meloidogyne javanica) on olive in the greenhouse conditions. *J. Plant Pathol. Microbiol*, ***4****:183*.

Son, D., Bonzi, S., Somda, I., Bawin, T., Boukraa, S., Verheggen, F., Francis, F., Legrève, A. and Schiffers, B., 2017. First record of Tuta absoluta (Meyrick, 1917)(Lepidoptera: Gelechiidae) in Burkina Faso. *African Entomology*, ***25*(1)**, pp.259-264.

Sorribas, F.J., Ornat, C., Verdejo-Lucas, S., Galeano, M. and Valero, J., 2005. Effectiveness and profitability of the Mi-resistant tomatoes to control root-knot nematodes. *European Journal of Plant Pathology*, ***111*(1)**, pp.29-38.

Starr J.L., McDonald A.H., Claudius-Cole A.O., 2013. Nematode Resistance in Crops. In: Perry RN, Moens M (eds) Plant nematology, 2nd edn. *CABI*, Wallingford, pp 411–36

Strajnar, P. and Sirca, S., 2011. The effect of some insecticides, natural compounds and tomato cv. Venezia with Mi gene on the nematode Meloidogyne ethiopica (Nematoda) reproduction. *Acta Agriculturae Slovenica*, ***97*(1)**, p.5.

Strajnar, P. Sirca, S. Knapic, M. and Urek, G. 2011. Effect of *Meloidogyne ethiopica* parasitism on water management and physiological stress.

Sujatha, R., Vethamoni, P.I., Manivannan, N. and Sivakumar, M., 2017. Screening of Tomato genotypes for root knot nematode (Meloidogyne incognita Kofoid and White. Chitwood). *International Journal of Current Microbiology and Applied. Sciences*, *6*(3), pp.1525-1533.

Sunil, K. Singh and Uma, R. Khurma, 2007. Susceptibility of six tomato cultivars to the root-knot nematode, *M. incognita*. *South Pacific Journal of Natural Science*, ***13***: 73-77.

Tabarant, P., Villenave, C., Risede, J.M., Roger-Estrade, J., Thuries, L. and Dorel, M., 2011. Effects of four organic amendments on banana parasitic nematodes and soil nematode communities. *Applied soil ecology*, ***49***, pp.59-67.

Tadele, T., 1998. Distribution of RKNS *Meloidogyne* spp. In some vegetable farms in Harerge, Eastern Ethiopia. MSc Thesis, Alemaya University of Agriculture, Ethiopia.

Talwana, H.L., M.M. Butseya and G. Tusiime. 2008. Occurrence of plant-parasitic nematodes and factors that enhance population build-up in cereal-based cropping systems in Uganda. *African Crop Science Journal*. ***16*****(2)**:119-131.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution*, ***28*****(10)**, pp.2731-2739.

Tariq-Khan, M., Munir, A., Mukhtar, T., Hallmann, J. and Heuer, H., 2017. Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. *Journal of Plant Diseases and Protection*, ***124*****(3)**, pp.201-212.

Tefera, T. and Huluka, M., 2000. Distribution of Meloidogyne incognita (root-knot nematode) in some vegetable fields in eastern Ethiopia. *Pest Management Journal of Ethiopia*.

Tesfaye T. and Habtu A., 1985. A review of vegetable disease research in Ethiopia. pp 495 – 518. In: Tsedeke Abate (ed.). Proceedings of the 1st Crop Protection Symposium. Addis Ababa, Ethiopia.

Tewodros, M. and Asfaw, K. 2013. Promotion and evaluation of improved technologies through participatory approach in South Ethiopia: Experience from hot pepper. *Unique Res. J. Agric. Sci.* ***1*****(4)**:57-62

Tisserat, N., 2006. RKN of tomato. Fact sheets tomato Extension plant pathology Kansas State University, Manhattan.

Trifonova, Z., Karadjova, J. and Georgieva, T., 2009. Fungal parasites of the root-knot nematodes Meloidogyne spp. in southern Bulgaria. *Estonian Journal of Ecology*, ***58*****(1)**.

Tropentag/Doku/ Tropentag_2012.pdf. Accessed 30 Septmeber 2016.

Trudgill, D.L. and Blok, V.C., 2001. Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annual review of phytopathology*, ***39*****(1)**, pp.53-77.

Van Eck, J., 2018. Genome editing and plant transformation of solanaceous food crops. *Current opinion in biotechnology*, ***49***, pp.35-41.

Verdejo Lucas, S., Sorribas, J. and Puigdomènech, P., 1994. *Perdidas de produccion en lechuga y tomate causadas por meloidogyne javanica en invernadero*. Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria, Madrid (España)..

Viaene, N., Coyne, D. and Davies, K.G., 2013. Biological and cultural management. In: Perry, R.N. & Moens, M. (Eds).Plant nematology, 2nd ed. Wallingford, UK, *CAB International*, pp. 383-410.

Wachira, P.M., Kimenju, J.W., Okoth, S.A. and Mibey, R.K., 2009. Stimulation of nematode-destroying fungi by organic amendments applied in management of plant parasitic nematode. *Asian Journal of Plant Sciences*, ***8*(2)**, p.153.

Waeyenberge, L., Ryss, A., Moens, M., Pinochet, J. and Vrain, T., 2000. Molecular characterisation of 18 Pratylenchus species using rDNA restriction fragment length polymorphism. *Nematology*, ***2*(2)**, pp.135-142.

Walters, D.R. 2009. Are plants in the field already induced? Implications for practical disease control. *Crop Protection.****28***: 459–565.

Wanjohi, W.J., Wafula, G.O. and Macharia, C.M., 2018. Integrated management of fusarrium wilt-root knot nematode complex on tomato in central highlands of Kenya.

Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M. and Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual review of phytopathology*, ***40*(1)**, pp.309-348.

Wesemael, W., Viaene, N. and Moens, M., 2011. Root-knot nematodes (*Meloidogyne* spp.) in Europe. *Nematology*, ***13*(1)**, pp.3-16.

Wiesel, L., Daniell, T.J., King, D. and Neilson, R., 2015. Determination of the optimal soil sample size to accurately characterise nematode communities in soil. *Soil Biology and Biochemistry*, ***80***, pp.89-91.

Williamson, V.M, and Roberts, P.A., 2009. Mechanisms and genetics of resistance. In: RN. Perry, M. Moens, JL. Starr (eds.). RKNs. CAB International, Wallingford, UK. p. 301-325.

Williamson, V.M. and Hussey, R.S., 1996. Nematode pathogenesis and resistance in plants. *The Plant Cell*, ***8*(10)**, p.1735.

Wondirad, Mandefro and Tesfamariam Mekete, 2002. RKNs on vegetable juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. *Nematology*. ***38***: 98-111.

7. APPENDIX

Appendix 1. ANOVA skeleton for the effect of Meloidogyne arenaria on growth parameter of tomato at probability level of 0.05

		Tomato growth parameters							
		SH*		**SFW***		**SDW***		**RFW***	
Source	**DF**	Mean square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F
Rep	2	3.0238^{Ns}	0.5258	29.65^{Ns}	0.0737	2.37^{Ns}	0.1288	16.52^{Ns}	0.2624
Var	13	135.93^{**}	<.0001	422.41^{**}	<.0001	3.81^{**}	0.0028	149.39^{**}	<.0001
Error	26	4.5879		10.267		1.068		11.72	
Total	41								
Cv		4.00		4.46		5.95		9.07	
Lsd		3.59		5.37		1.73		5.74	

*SH= shoot height, SFW= shoot fresh weight, SDW= shoot dry weight and RFW= root fresh weight. Ns= non-significant, **= highly significant

Appendix 2. ANOVA skeleton for the effect of Meloidogyne arenaria on root gall index, nematode reproduction parameter on tomato varieties at probability level of 0.05

		Nematode reproductive parameter					
		RGI*		**FP***		**RF***	
Source	**DF**	Mean square	**Pr > F**	Mean square	**Pr > F**	Mean square	**Pr > F**
Rep	2	0.167^{Ns}	0.3816	0.00000952^{Ns}	0.6860	0.2696^{Ns}	0.7368
Var	13	2.974^{**}	<.0001	1.5971777^{**}	<.0001	3141.4^{**}	<.0001
Error	26	0.167		3493321.981		0.872	
Total	41						
Cv		8.74		1.93		1.93	
Lsd		0.68		3136.9		1.56	

*RGI= root gall index, FP= final nematode population and RF= reproduction factor. Ns= non-significant, **= highly significant

Appendix 3. ANOVA skeleton for effect coffee husk application and variety on growth parameter of tomato inoculated with *Meloidogyne arenaria* at probability level of 0.05

		Tomato growth parameters							
		SH* **		**SFW*** **		**RL*** **		**RFW*** **	
Source	**DF**	Mean square	Pr > F	Mean Square	Pr > F	Mean square	Pr > F	Mean Square	Pr > F
Rep	2	38.674^{ns}	0.1170	0.736^{ns}	0.7424	0.591^{ns}	0.6683	0.022^{ns}	0.9854
Var	2	17.965^{ns}	0.3503	116.277**	<.0001	41.755**	<.0001	35.977**	<.0001
CH	3	234.93**	<.0001	515.73**	<.0001	86.66**	<.0001	166.978**	<.0001
var*CH	6	48.23*	0.0286	6.486*	0.0428	2.872^{ns}	0.1100	2.466^{ns}	0.1743
Error	22	16.33		2.438		1.44		1.472	
Total	35								
Cv		8.52		6.52		7.06		5.61	

***SH= shoot height, SFW= shoot fresh weight, RL= root length and RFW= root fresh weight. Ns= non-significant, *= significant, **= highly significant

Appendix 4. ANOVA skeleton for effect coffee husk application and variety on nematode reproduction parameter on tomato at probability level of 0.05

		Nematode reproductive parameter							
		NRG*		**NEM***		**FP***		**RF***	
Source	**DF**	Mean square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F
Rep	2	22.03^{ns}	0.4583	3.58^{ns}	0.5920	0.0063^{ns}	0.8364	0.0005^{ns}	0.6181
Var	2	56031.7**	<.0001	4698.58**	<.0001	9.046**	<.0001	2.19**	<.0001
CH	3	30817.6**	<.0001	5613.96**	<.0001	4.657**	<.0001	1.624**	<.0001
var*CH	6	8331.3**	<.0001	1626.32**	<.0001	0.169**	0.0028	0.325**	<.0001
Error	22	27.24		6.67		0.035		0.0012	
Total	35								
Cv		6.83		11.66		5.48		6.03	

*NRG= number of root gall, NEM= number of eggmass, FP= final nematode population and RF= reproduction factor. Ns= non-significant, **= highly significant

Appendix 5.Data sheet for plant parasitic nematode survey

Sample code_____

Date_____

PERSONAL INFORMATION

Name of respondent_____ Education_____

Profession_____

Age-------- Sex-------- Male ☐

Female ☐

LOCATION

Administrative region_____ Zone_____

District_____ Kebele_____

GPS INFORMATION

Waypoint Id (as inserted in GPS)

Altitude_____ Longitude_____ Latitude_____

FARM/FIELD:

Farm type_____

Topography_____

Soil type_____

Field size_____

FIELD HISTORY

Planting date _____ previous crop _____

Irrigated _____

Season Normal Late Early Dry Wet Warm Cool

Influence of weather factors (temperature, RH and rainfall) on the population of the disease

Yes ☐

No ☐

CROP

Variety_____ Growth stage _____

Plant density _____ General condition _____

CROPPING SYSTEM

Intercropping ☐

Mono cropping ☐

Crop rotation ☐

Sole cropping ☐

Appendix 6. Photographs taken while conducting the experiments under greenhouse and laboratory condition. (A and B) infested tomato seedling with J2, (C) above ground symptom of nematode damage, (D) eggmass on tomato roots shown by arrow, (E) female *M. arenaria* indicated by arrow and (F) root gall on susceptible tomato variety

Appendix 7. Photograph taken while conducting experiments under field, greenhouse and laboratory conditions. (A) Inoculated resistant variety comparatively showing health root, (B) organic amended plants exhibits health root (C) root and soil sampling in farmer fields (D) counting nematodes.