JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOGY

INVESTIGATION ON PLANT PARASITIC NEMATODES ASSOCIATED WITH COFFEE ROOT IN JIMMA TOWN, SOUTHWESTERN ETHIOPIA

BY: HANNA DAGNE

ADVISOR: MR. EBA ALEMAYEHU

CO- ADVISOR: MR. WASSIHUN WEDAJO

A RESEARCH PAPER FOR THE PREPARATION OF THE SENIOR PAPER TO BE SUBMITTED TO DEPARTMENT OF BIOLOGY FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR DEGREE OF BACHELOR OF SCIENCE IN BIOLOGY

OCTOBER, 2013

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APPROVED BY:

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ABSTRACT

Plant parasitic nematodes are ever-present and are incidental with plant growth and crop production. They are significant constraints to sustainable agriculture and can be difficult to control. The objectives of this study were to identify the species of parasitic nematodes from coffee root and also to determine the abundance of parasitic nematodes that inhabit coffee root form randomly selected coffee trees in Jimma town, Southwestern Ethiopia. Cross sectional study on the investigation of plant parasitic nematodes associated with coffee root was carried out. Six coffee plants were sampled for nematodes. Soil sample of 8 cores was collected. Nematodes were extracted from 100g soil by using 2 mm, 1 mm and 0.25 mm sieves. The 14 extracted nematodes were counted and identified to order level. Those extracts were found to belong to 2 orders; Dorylaimida predominated throughout the area (92.9%) and reached the highest population density of any nematode taxa with 13 nematodes/100 g soil, and followed by Mononchida (7.1%) with 1 nematode/100 g soil. No plant-parasitic nematodes were recovered. This research concluded that free-living nematodes are most abundant in coffee field soils. Finally, it was recommended that assessment on plant parasitic nematodes should be established through collection and relation with symptoms, and with expert help accurately identifying the species involved.

Keywords- *Coffee arabica, Coffee robusta,* Nematode

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CHAPTER ONE

1. INTRODUCTION

1.1 Background

Coffee is the sole most significant tropical product traded universally, accounting for nearly half of the total exports of tropical products. According to the International Coffee Organization (ICO), international imports of all kinds of coffee of all origins reached 6.1 million tons in 2008, up by an average 2.4 percent annually since 2000, when worldwide imports stood at 5.1 million tons. The EU is the world's chief importer of coffee, accounting for 66 percent of worldwide imports, or 4 million tons, in 2008, followed by the United States (24%, 1.5 million tons) and Japan (7%, 423, 602 tons) (FAO, 2009).

So far, 80 species of coffee have been described of which only two are of financial importance, *viz Coffee Arabica* (Arabica coffee) and *Coffee canephora* (Robusta coffee). The manufacture of *Coffee arabica* accounts for 75% of globe sell abroad and is produced in 60 countries, with the maximum production in South and Central America and in East and Central Africa (Campos & Villain, 2005).

Robusta coffee is mostly used in blend, but Arabica is the only coffee to be drunk on its own unblended, and this is the type grown and drunk in Ethiopia. In the Jimma district alone annual production is approximately 30,000 tons. Nationally, the country produces 200,000 tons a year, of which almost half is for domestic consumption, the highest in Africa (http://www.selamta.net).

Generally, at the present Arabica coffee production is expanding in Ethiopia, thereby increasing the farmers' profits.

Plant-parasitic nematodes are a major limiting factor in coffee producing areas worldwide. Meloidogyne (root-knot nematodes) and Pratylenchus (root lesion nematodes) are the predominant genera and are widely distributed in coffee plantations, causing great economic losses to both farmers and industry (Campos & Villain, 2005).

To obtain information on potentially harmful plant-parasitic nematodes with Coffee Arabica in soil, the research was conducted in a coffee plantation field of the Jimma town.

1.2 Statement of the problem

Today, coffee plantation is suffering from many parasitic nematodes. The study was conducted to answer the following questions.

1. What are the types of parasitic nematodes inhabit around a coffee root?
2. Which parasitic nematode is most prevalent?

1.3 Objective of the study

1.3.1 General objective

- To assess root associated parasitic nematodes from coffee plant.

1.3.2 Specific objectives

- To identify the species of parasitic nematodes that affect coffee root
- To determine the abundance of parasitic nematodes that inhabit coffee root

1.4 Significance of the study

Now a day, Ethiopia's coffee market is suffering from these parasites and various species of coffee plants are reducing time to time especially *C.arabica*. The significance of this research was to identify the major plant parasitic nematodes. The study provides base line for other researchers. It also contributes first-hand information for interested governmental and non-governmental organizations for detail and further investigation in the future.

1.5 Delimitation of the study

This study was limited coffee plantation site in Jimma University College of Agriculture and Veterinary Medicine Campus.

1.6 Limitation of the study

During this study, there was a shortage of chemicals and literatures.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Nematodes and their feeding habit

Nematodes are minute eel-like life forms that survive in soil and water. Nematodes are the most plentiful multicellular organisms on earth. Most soil lodging nematodes are valuable organisms that perform a task in the break down and discharge of nutrients from macrobiotic substance (Celetti & Potter, 2006).

Plant parasitic nematodes vary from nematodes that be nourished by bacteria and fungi in that they have a specific feeding structure, the spear or stylet (Coyne et al., 2007).

The stylet is required into plant cells and enzymes are inserted to decay the cell content. The nematode takes out the partly assimilated cell contents through the stylet. A number of nematodes for instance the root knot and the cyst nematodes set up a specific feeding spot where they stay put for the rest of their life cycle. Other nematodes such as the root lesion nematode dig into the root, feeding and causing destruction as they travel through the root (Celetti & Potter, 2006).

2.2. Life cycle of plant parasitic nematodes

The majority plant parasitic nematodes lay eggs in the soil or roots of host plants or are kept inside the female body or cyst. Subsequent to the egg give forth, the young nematodes bathe to other close by plant roots and nourish them. Injure make happened by nematodes in numerous yields can also offer a contamination place for new disease causing organisms, which additional diminishes harvests. Nematodes finish their life cycle in three to six weeks in the course of the emergent time of year depending upon obtainable dampness and hotness. Tremendous dampness and hotness will destroy a few kinds of nematodes (Celetti & Potter, 2006).

2.3. Classification of plant parasitic nematodes

Plant parasitic nematodes can be catagorized into aerial parasites- those feeding on in-flight parts of plants- and roots and tuber parasites- those feeding on under- ground parts. Moreover, they can be grouped by their feeding behavior and motility into three major groups: Migratory endoparasites, Sedentary Endoparasites and Ectoparasites (Coyne *et al.*, 2007).

Migratory endoparasitic nematodes, in every life stages, are mobile except the egg. The nematodes dig throughout the plant from cell to cell, or may depart the plant tissue in look for of new feeding location. Root rot fungi and bacteria are frequently associated with invasion of migratory endoparasitic nematodes, which penetrate the plant tissues through areas injured by nematodes (Coyne *et al.*, 2007).

Sedentary endoparasitic nematodes attack plant tissue typically as newly hatched second-stage juveniles; the 'infective' worm like stage. At the nourishing site the female matures, lasting enduringly sited for the time of her life. As she matures, her body enlarges to a spherical, lemon, kidney, or ovoid form. a few groups (e.g. cyst and root-knot nematodes) produce 'giant' feeding cells to form in the host plant (Coyne *et al.*, 2007).

Ectoparasitic nematodes feed on the face of the plant, externally, typically on root hairs or cortical tissue. They frequently originate in high densities, but do not constantly cause a trouble. Examples of ectoparasitic nematodes are ring nematodes (*Criconemoides* spp.), spiral nematodes (*Helicotylenchus* spp) and the aerial the aerial rice white-tip nematode (*Aphelenchoides besseyi*) (Coyne *et al.*, 2007).

Root-lesion nematodes (Pratylenchus spp) are among the most frequent and injurious to coffee (Coffea spp) to the side from root-knot nematodes and some other genera. The genus Pratylenchus includes 97 well-founded species of universal dissemination and economic significance, which parasitize an extensive diversity of plant species. Elements of this genus are called root-lesion nematodes because they create lesions on feeder roots and rarely on other underground plant parts as an outcome of their feeding. They are occasionally known as meadow nematodes because of their repeated happening in that location (Ricardo, 2008).

Root lesion nematodes cause small scratch-like lesions on feeder roots providing a road for other root rooting organism to contaminate. Actually, root lesion nematodes have repeatedly been associated with disease complexes involving other soil-borne pathogens resulting in considerably greater disease and reduced yields compared to either the fungal pathogen or nematode infection alone. Some parasitic nematodes such as the dagger nematode are vectors plant viruses (Celetti & Potter, 2006).

Root-Knot nematodes (RKNs) are classified in the genus Meloidogyne, which was established by Gòldi (1887) and includes 17 coffee-parasitic valid species. Meloidogyne species are characterized primarily on morphological features of females, particularly the perineal pattern (Ricardo, 2008).

Since nematodes are hard or not viable to observe in the field, and their symptoms are frequently imprecise, the injure they cause is frequently recognized to other, more noticeable causes. Farmers and researchers similar often undervalue their belongings (Coyne *et al.*, 2007).

Warning signs triggered by nematode invasion diverge depending on the harvest and the kind of nematode nuisance. Nematode injured plants habitually take place in patches or next to a row. Diseased plants may emerge undersized, floppy and unthrifty. Nematode feeding also causes symptoms such as yellowing, stem twisting, crown and bulb bloating, root galls and root forking and deformation. High soil population levels of plant parasitic nematodes can cause death of juvenile plants (Celetti & Potter, 2006).

Generally, plant parasitic nematodes reduce agricultural production by approximately 11% globally (Agrios, 2005). The amount of damage nematodes cause depends on a wide range of factors, such as their population density, the virulence of the species or strain, and the resistance (ability of the plant to reduce the population of the nematode) or tolerance (ability of the plant to yield despite nematode attack) of the host plant. Other factors also contribute to a lesser extent, including climate, water availability, soil conditions, soil fertility, and the presence of other pests and diseases (Coyne *et al.*, 2007).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Jimma town, which is located 353 km southwest of Addis Ababa. It is located at 70^0 39' latitude, 36^o 50' longitude, and altitude ranging 1700-1750 meter above sea level. A field survey was conducted at Jimma University College of Agriculture and Veterinary Medicine Campus, on a single coffee growing field with large ground cover vegetation, to investigate the occurrence, the population density and to identify plant-parasitic nematodes.

3.2. Sample collection

Nematode samples were collected from a single 4×3 coffee field. Eight soil cores were taken from randomly selected 6 coffee trees and were thoroughly mixed in to one composite sample and finally the composite sample was transferred into a labeled polythene bag. During transit, the sample was stored in an insulated cooler (ice bag) to avoid getting them hot and dry. The sample was immediately transported to the Jimma University, Biology department Postgraduate laboratory for further study.

3.3. Extraction method

First, 100 g soil samples were immersed in bucket containing 2 liters of water and hand-stirred gently until all soil aggregates were dispersed. The soil suspension was then poured through sieves with descending aperture sizes of 2 mm, 1.5 mm and 0.25 mm. This step will be repeated five times. Material retained on each of the sieves was backwashed with a tap water delivered as fine stream. Then, the final sample, from 0.25mm sieve, was collected in beakers and the particulate contents were allowed to settle for a few minutes.

3.4. Microscopic examination

First, specimens from the sample were fixed in 4% formalin and mounted on clean slides. Then, counting and identification to the order level was performed under light microscope using standard identification key.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1. Identification of extracted nematodes

Based on microscopically identification, Table 1 shows taxonomic diversity of the extracted nematodes. During this investigation, *Dorylamida* was recovered with maximum population densities of 13 nematodes/ 100 g soil, whereas Mononchida was recovered with maximum population densities of 1 nematode/100 g soil.

Table 1. Taxonomic diversity of the extracted nematodes, 2013

Order	Number of extracted nematodes
Dorylaimida	13
Mononchida	1
Total	**14**

Plant parasitic nematodes were not detected in this study. But, all of the extracted nematodes were free-living and dominated by fungivores and bacterivores. (Table 2.)

Table 2. Tropic diversity of extracted freee-living nematodes, 2013

Order	Trophic (feeding habit) diversity	Abundance	%
Dorylaimida	Bacterivore/Fungivore	13	92.9
Mononchida	Predator	1	7.1
Total		**14**	**100**

The mouth or stoma of a Dorylaimida is a hollow tube, whereas a Mononchida has a "tooth" that it uses to grab prey (Fig1 and 2).

Fig 1. The mouth part of a Dorylaimida

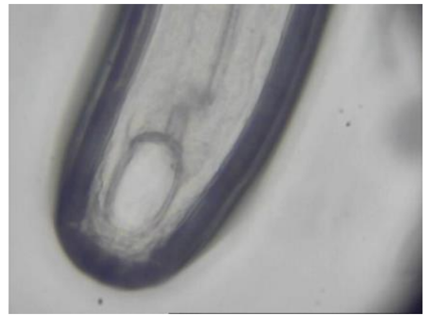

Fig 2. The mouth part of a Mononchida

4.2. DISCUSSION

In this study, plant parasitic nematodes were not recovered. The first reason is that soil sampling was accomplished by using systemic pattern sampling. According to Ganpati (2011) this sampling method is used for both large areas (fields, golf course fairways and rough areas) and small areas (home lawns, recreational parks and golf course greens). He continued to say that trees or shrubs, such as coffee, should be sampled under the canopy drip line. The second reason is that extraction of nematodes was only carried out by using sieving technique. Luc *et al.* (2005) suggested that sieving technique is less laborious but population loss may be higher. Those scholars further mentioned that using sieves simultaneously by stacking them may reduce the efficiency of recovery.

Although the occurrence of plant parasitic nematodes is the main task to be examined in this study, all of the extracted nematodes were free-living and dominated by fungivores and bacterivores. According to James (2011) bacterial-feeders consume bacteria through a stoma, a large open channel. Fungal-feeders feed by puncturing the cell wall of fungi using a small slender stylet to suck out the internal contents. Predatory nematodes eat all types of nematodes and protozoa using a stylet. They eat smaller microorganisms whole or attach themselves to the cuticle of larger nematodes, scraping away until the prey's internal body parts can be extracted.

This finding suggests that free- living nematodes are most abundant in the natural and plantation forests as pointed out by Kimengu *et al.* (2004). Moreover, Dorylaimida, free-living forms, very abundant in both number and species in natural and cultivated soils as well as in freshwater sediments. Because of their abundance and diversity, Dorylaims play important roles in the soil food webs of ecosystems they inhabit (Yeates *et al.,* 2009). In addition, they are regarded as good bio-indicators of soil health (quality) due to their sensitiveness and response (their number significantly decreases) to any change (presence of pesticides, heavy metals, etc.) in their habitat (Ekschmitt and Korthals, 2006).

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

In conclusion, soil nematodes are highly diverse in their habitats and feeding habits. These microscopic organisms may inhabit in aggregates of soil particles. The food sources for different groups of soil nematodes include bacteria, fungi, and nematodes. The finding of the study revealed that the abundance of free-living nematodes particularly Dorylaimida in the soil sample taken from the coffee plantation. Even though the objective of the study was to investigate parasitic nematodes, we were unfortunate to find parasitic nematodes due to sampling and absence of replicates

5.2. RECOMMENDATION

Detailed research on the distribution, identity and damage potential of plant parasitic nematodes in coffee should be studied. This is recommended for the reason that root parasitic nematodes do not produce specific aboveground symptoms and most of the time, remain unnoticed. The symptom they produce (chlorosis, poor growth, tillering) are attributed to other causes such as poor soil conditions or lack of fertilizer. However, because they are omnipresent and permanently present coffee field soils, root parasitic nematodes can be major pests of coffee.

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