Distribution of Citrus Nematode (*Tylenchulus semipenetrans* Cobb, 1913) in Major Citrus (*Citrus sinesis* L.) Growing Areas of Ethiopia

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Abstract

Citrus production in Ethiopia is threatened by a number of biotic and abiotic factors. Among these, citrus nematode (*Tylenchulus semipenetrans*) is one of the most important biotic constraints in the country. The objective of this study was to assess the incidence and distribution of citrus nematodes in major citrus growing areas of Ethiopia. A survey was conducted in five major citrus growing provinces throughout the country. Ten genera of plant-parasitic nematodes were identified. Among all, the citrus nematode, *Tylenchulus semipenetrans* was predominant and was found in 95%, 90% and 20% of the orchards sampled from Hurso, Nura Era and Fetule, and Gota respectively. Other genera of plant-parasitic nematodes identified include *Criconemella* spp., *Hemicriconemoides* spp., *Longidorus* spp., *Pratylenchus* spp., *Radopholus* spp., *Paratrichodorus* spp., *Paratylenchus* spp., *Trichodorus* spp., and *Tylenchulus* spp. This study documented that citrus orchards in Ethiopia are highly infected with plant parasitic nematodes and indicated sound nematode management practices are urgently needed.

Key words: Citrus, Distribution, Slow Decline, *Tylenchulus semipenetrans*

Introduction

Citrus (*Citrus sinesis* L.) fruit has been cultivated over 4000 years (Davies and Albrigo, 1994). It is grown worldwide and the major producing countries include Brazil, the United States, China, Mexico, and Spain (Patrick and Dominique, 2008). Citrus is among the most important fruit crops of Ethiopia. Its cultivation started in Upper Awash Valley and Melkassa areas in central Ethiopia (Kassahun *et al.*, 2006). It is being produced mainly, in Dire Dawa, lower and middle Awash, and Melkassa areas in southeast region. The annual production of citrus and its total area coverage were estimated 77,087 tons and 5, 947 ha, respectively (Central Statistics Authority (CSA), 2011/12). Ethiopia has vast potential of exporting citrus in a fresh and processed form. Moreover, agro-climatic conditions are highly suitable for production of high quality citrus fruits in Ethiopia. The export-oriented horticulture sector has been growing rapidly and forms an important element in the country's efforts to expand and diversify the economy, raise export earnings and create employment (Frank, 2007). This rapid growth is remarkable particularly when placed in a historic context. In the year, 2000/2001, the total foreign currency earnings from the Ethiopian horticulture sector accounted to USD 4.7 million (Frank, 2007).

However, currently, many citrus plantations in Ethiopia are suffering from slow decline disease caused by the citrus nematode, *Tylenchulus semipenetrans*. Some serious diseases are crippling the citrus plantations leading to lower production and even threatening the complete destruction of orchards. In the past, citrus was being exported, but now it is not sufficient even for domestic markets. In Erer Gota State Commercial Farm, 100% trees in some of the orchards have died in the last decade, while others

have varying levels of decline problem (personal observation). As a result, emphasis is being given on cultivating vegetables, where citrus used to be there. In Hurso Military Camp Farm, quit a large number of trees have also showed a decline syndrome. The citrus nematode (*Tylenchulus semipenetrans)* occurs in all citrus-producing regions of the world. This is a major factor in initiating citrus declines since it predisposes citrus roots for secondary infections such as fungal and bacterial pathogens (Chhabra and Sakhuja, 1993). Consequently, it limits production of citrus fruits under a wide range of environmental and edaphic conditions.

In the major citrus-producing regions, various surveys estimated that the citrus nematode infests from 24–60% (Florida and California) to as many as 70–90% (Brazil, Spain, Texas and Arizona) of commercial orchards (Duncan, 2005). However, in Ethiopia, except few reports on the association of nematodes with declining citrus orchards (Godfry *et al.*, 1988; Sakhuja *et al.*, 2005b) there is still a scarcity of information about the problem of nematodes in citrus orchards. Thus, this study was undertaken with the objective of assessing the incidence and distribution of plant-parasitic nematodes in major citrus growing orchards of Ethiopia.

Materials and Methods

Survey areas

A survey was conducted in five major citrus growing provinces of Ethiopia to determine the distribution of citrus nematodes during a period of September to December 2011. Citrus orchards included in the survey were selected from different afro-ecological zones of Ethiopia and are from: Hurso, Erer, Gota, Nura Era, Merti, Tibilla, Melkassa Agricultural Research Center (MARC), and Koka (Fig 1). The GPS coordinates; soil texture, soil organic matter content and altitude of the surveyed areas were also recorded.

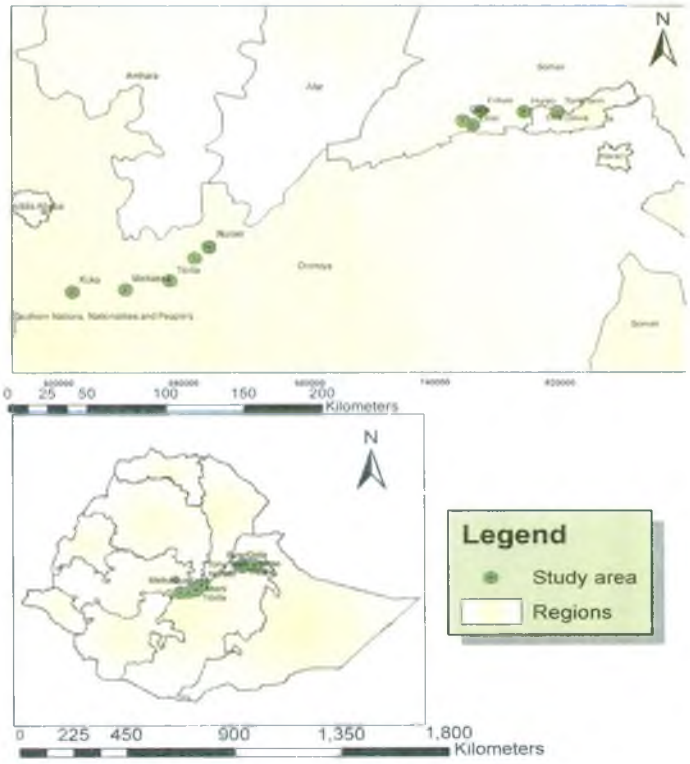

Figure 1. Map of Ethiopia and Citrus Orchards Covered during the survey of 2011 growing season

Soil and root sampling

Sampling was done based on the procedure of sampling from deep-rooted perennial crops (Michel *et al.*, 2005). Soil samples were collected from (25-45cm) soil depths using augers following the drip line of the citrus tree. Roots (100grams each) were also collected from the adventitious roots (actively growing roots) of the stand. The zigzag sampling pattern was used. Orchards with large areas were grouped in small areas by considering the topography, similarity of soil texture and the citrus cultivar grown to reduce bias. A sample was composed of 25-30 cores that weigh around 1 kg. A total of 90 composite root and soil samples (45 each) were collected. The soil cores were collected from the root zone using an auger corer and spade, and then samples were bagged in polyethylene and brought to Nematology Laboratory of Haramaya University, Ethiopia, for further analysis.

Extraction and staining of nematodes

Soil samples were mixed thoroughly and a subsample of 150g from each composite sample was extracted using Baermann funnel method. The soil was placed in a square of muslin cloth, which were folded to enclose the material, and then gently submerged in the funnel filled with water (Michel *et al.*, 2005). After about 24–48hrs, the pinch clamp was gently removed and the suspensions with nematodes were collected using a small beaker. Plant-parasitic nematodes were also extracted from 10 gram of root using the modified Baermann method. Briefly, roots were washed gently to remove the adhered soil and 10g root was chopped to smaller pieces then extracted with a modified Baermann method (Southey, 1970). Five grams of roots were cut into 1-2 cm pieces in a 150ml glass beaker and 1.5% NaOCl was added into the beaker, and left for 4 minutes with frequent stirring. Then NaOCl solution was removed and roots were rinsed with abundant tap water. After removing the excess water from the root tissues 1ml staining solution (3.5g fuchsin acid, 250ml acetic acid, and 750ml distilled water) plus 30ml tap water were brought into boiling for 30 seconds. The roots were then left to cool at room temperature and then rinsed with abundant water. Then after, 25ml glycerol were added on the roots and heated till boiling point. Finally, the stained roots were stored in sealed petridish in a fridge till observation under a compound microscope (Byrd *et al.*, 1983).

Nematode identification and counting

The nematode suspension obtained after extraction of the soil and root samples were analysed immediately. However, in some cases it was kept for a few days at 4°C refrigerator till analysis was made. Number of nematodes was enumerated by taking a sub sample of 10ml of nematode suspensions from 100 ml volume of nematode suspensions in three replicates. The average of the three counts were taken to bring the coefficient of variation to acceptable point (usually <15%). The subsamples were transferred into the counting dish and left for 5 minutes till they settle. Finally, nematodes were counted using stereomicroscope with 25-50x magnification (Michel *et al.*, 2005). Then nematodes were fixed and mounted on a glass slide using a standard procedure (Michel *et al.*, 2005) and examined directly under a compound microscope at 40x and 100x magnification. Plant-parasitic nematodes genera were identified based on the pictorial key for identification of plant-parasitic nematodes by Mai and Mullin, 1996 and Inserra *et al.*, 1988. In most cases, the morphological features of adult females were used for identification and in rare instances male and juveniles were considered when present.

Results and Discussion

Distribution of citrus nematodes

All orchards were infested with a mixture of different plant-parasitic nematode species and magnitude (Table 1). Among identified plant-parasitic nematodes, *Tylenchulus semipenetrans,* was predominant and frequently detected across the surveyed areas. The highest occurrence was at Hurso Camp (95%) and Nura Era and Fetule (90%) and the lowest occurrence was recorded at Gota 20% (Table 1). The prevalence of this genus was 100% in all the surveyed citrus orchards. The present survey confirms the occurrence of citrus nematode in all provinces of citrus growing orchards of Ethiopia. *Tylenchulus*

semipenetrans is broadly adapted to most edaphic conditions common to production of citriculture (Ploetz *et al.*, 2003; Michel *et al.*, 2005). The nematode also survives in any soil whose texture is suitable for citrus, although, unlike many plant-parasitic nematodes parasites, development in pot studies is often less rapid in sandy soils. Moderate amounts of clay and silt and organic matter favor infection and development of the citrus nematodes (Ploetz *et al.*, 2003; Michel *et al.*, 2005). Soil sample analysis showed seven of eight orchards have a clay soil texture and one of eight shows loam with lower PH (7.52) relative to the rest (Table1).

Table 1. List of Plant-parasitic nematode genera, proportion of *T. semipenetrans* to other parasitic nematodes, their feeding habit and physico-chemical properties of the soil in the major citrus orchards of Ethiopia during 2011 growing season

Location	Altitude (masi)	Soil texure	PH	Soil OM	Nematodes	Percentage [a]	Feeding Habit[b]
Hurso	1108	Clay	8.1	2.76	*T.semipenetrans*	95	Semi-endoparasitic
					Paratrichodorus spp	5	Ectoparasite
Erer	1174	Clay	8.2	2.41	*T.semipenetrans*	75	Semi-endoparasitic
					Paratrichodorus spp	20	Ectoparasite
					Criconemella spp	5	Ectoparasite
Gota	1174	Clay	8.00	2.41	*T.semipenetrans*	20	Semi-endoparasitic
					Paratrichodorus spp	70	Ectoparasite
					Longidorus spp	10	Ectoparasites
Fetule	-	-	-		*T. semipenetrans*	90	Semi-endoparasitic
					Longidorus spp	10	Ectoparasites
Nura Era	1126	Clay	7.97	1.93	*T. semipenetrans*	90	Semi-endoparasitic
					Trichodorus spp	10	Ectoparasite
Merti	1175	Clay	7.90	2.76	*T. semipenetrans*	84.9	Semi-endoparasitic
					Paratrichodorus spp	7.9	Ectoparasite
					Paratylenchus spp	5.1	Ectoparasite
					Trichodorus spp	2	Ectoparasite
Tibilla	1296	Clay	7.98	2.41	*T. semipenetrans*	90	Semi-endoparasitic
					Longidorus spp	10	Ectoparasites
Melkassa	1557	Loam	7.52	1.72	*T. semipenetrans*	35	Semi-endoparasitic
					Paratrichodorus spp	60	Ectoparasite
					Longidorus spp	5	Ectoparasites
Koka	1611	Clay	8.17	3.10	*T. semipenetrans*	65	Semi-endoparasitic
					Paratrichodorus spp	27	Ectoparasite
					Hemicriconemoides spp	3	Ectoparasite
					Tylenchulus spp	2	Ectoparasite

[a] percentage proportion of *T. semipentrans* over other plant-parasitic nematodes recovered per 150gram soil
[b]based on Michel et al., 2005

The citrus nematode is aptly named since it occurs in all citrus-producing regions of the world and limits production of citrus fruits under a wide range of environmental and edaphic conditions (Ploetz *et.al.*, 2003; Michel *et al.*, 2005; Agrios, 2005). In the major citrus-producing regions, various surveys estimated that the nematode infests from 24–60% (Florida and California) to as many as 70–90% (Brazil, Spain, Texas and Arizona) of commercial orchards. Similar statistics are reported worldwide (Ploetz *et al.*, 2003; Michel *et al.*, 2005). The result obtained in this survey is supported by Becker and Westerdahl (2009) who has reported as the nematode is observed to be present in most citrus orchards and in all soil types. Michel *et al.* (2005) also reported as the citrus slow decline nematode was distributed to nearly all areas where citrus crops were grown. According to Singh (1999) and Ferris (2011) worldwide dis-

tribution with that of citrus occurs in 95% of citrus in California and is common in East side San Joaquin Valley grapes, particularly in former citrus-growing areas, originally spread with planting stock and further with irrigation water (Ijaz *et al.*, 2003). *Tylenchulus semipenetrans* has been recognized as one of the greatest threats to citrus production throughout the world. It occurs in all citrus producing regions of the world and limits production of citrus fruits under a wide range of environmental and edaphic conditions. Likewise, in this survey, all orchards were infested by the citrus nematode in different magnitude ranged from (20-95%) and supported the efforts of many (Fig. 2).

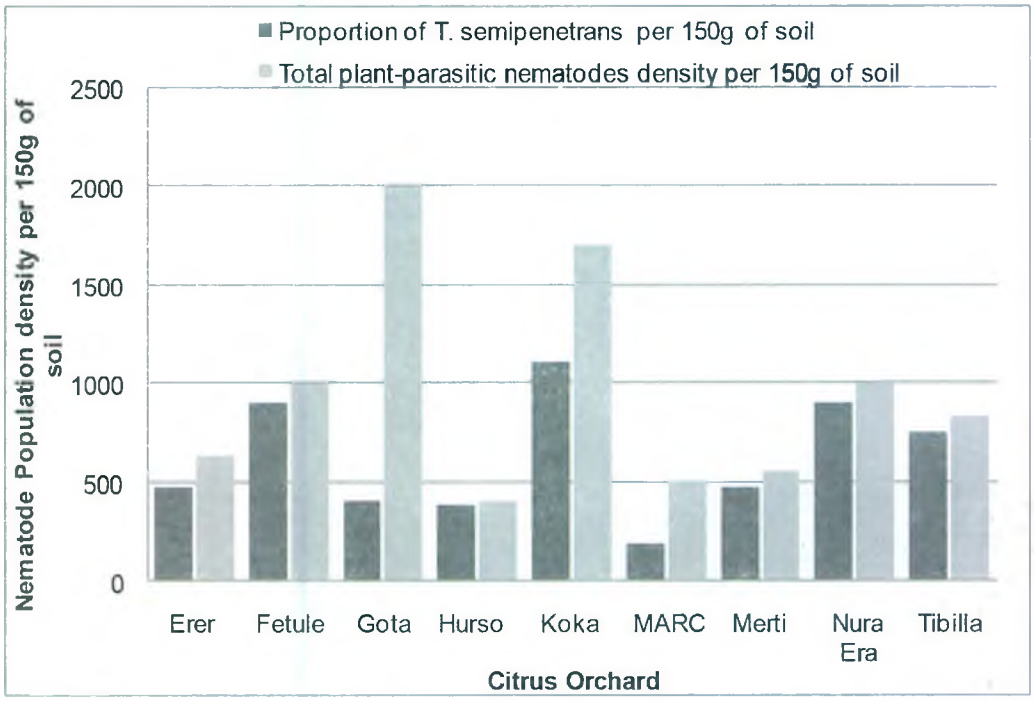

Figure 2. The proportion of *Tylenchulus semipenetrans* population density over the total plant-parasitic nematodes recovered from 150-gram soil and its distribution across the major citrus orchards surveyed during 2011 growing season

The PH of the surveyed orchards was ranged from 7.52 at Melkassa to 8.2 in Erer. The proportion of citrus nematode density over other plant-parasitic nematodes recovered was 35 and 75% at Melkassa and Erer respectively. However, the genus *Paratrichodorus* was nearly double of the *T. semipenetrans* at Melkassa than Erer. The population development was best at soil pH 6.0–8.0 range; however, at less optimum pH, the nematode is also pathogenic to citrus (Bello *et al.*, 1998 & Plotez, 2003).

Types of plant-parasitic nematodes characterized

Nematode population numbers, type and root feeding habit are given in (Table 1). Plant-parasitic nematodes extracted from 150g sub samples were characterized to genus level except *T. semipenetrans* that was characterized to species level based on the key developed by Inserra *et al.*, 1988. *Paratrichodorus* spp., *Paratylenchus* spp., *Pratylenchus* spp., *Radopholus* spp., *Trichodorus* spp., *Longidorus* spp., *Hemicyclophora* spp., *Hemicriconemoides* spp., *Criconemella* spp and *Tylenchulus* spp were identified to genus level (Table 1). From the extracted samples the immature female nematodes were more frequent where as the male was rarely found (Fig 3).

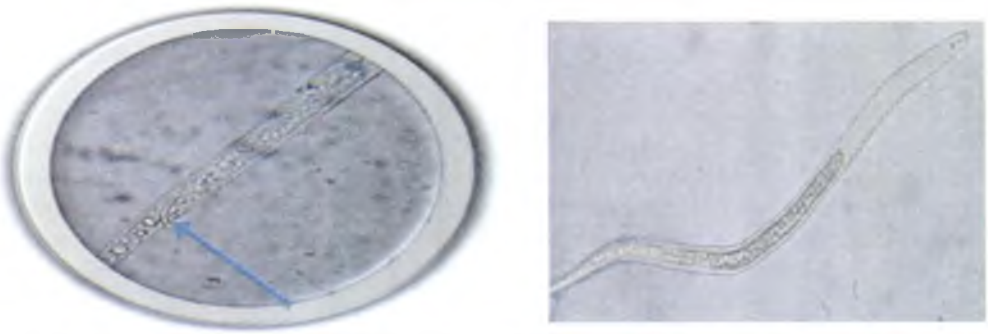

Figure 3. Vermiform second stage juvenile (J2) of the citrus nematode directly hatched from an egg and an adult male nematode with clear spicule at 40x magnification using compound microscope

The plant parasitic nematodes recovered from roots have shown a similar trend in their distribution and frequency of occurrence across the surveyed areas as the soil samples analyzed. The female citrus nematode was also stained partially embedded in the roots in a few samples (Fig. 4). Though juveniles inside the root tissue were not significantly present in higher numbers the root lesions were quite clear with a few J2s stained inside the tissue.

Figure 4. Citrus tree showing symptoms of slow decline, soil sampling, stained females of *Tylenchulus semipenetrans* partially embedded inside the citrus root tissue at 40x magnification under a compound microscope

Conclusions and Recommendations

Tylenchulus semipenetrans highly affected the citrus production in Ethiopia. This survey result is the first extensive report of citrus nematodes from Ethiopia. The result showed the presence of different kinds of nematodes including the major citrus nematode (*T. semipenetrans*) in all surveyed citrus orchards. The present survey confirms the occurrence and distribution of citrus nematode in different citrus orchards of Ethiopia. The genus *Paratrichodorus* was frequently found in the orchards of citrus with loamy soil and relatively highland areas like Melkassa and Gota. Eco-friendly and easily affordable management practices should be employed to reduce the population level of citrus nematodes from citrus orchards of Ethiopia. Awareness creation work packages should also be implemented to appreciate the problem and design different management strategies.

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